eman ta zabal zazu
Universidad del País Vasco
Euskal Herriko Unibertsitatea
PiE
Plentziako Itsas Estazioa
Estación Marina de Plentzia

CONTRIBUTION TO THE DEVELOPMENT OF BIOMONITORING PROGRAMMES FOR THE ASSESSMENT OF CHEMICAL POLLUTION AND ECOSYSTEM HEALTH DISTURBANCE IN MANGROVE-LINED CARIBBEAN COASTAL SYSTEMS USING BIVALVES AS BIOMONITORS AND SENTINELS

JAVIER RAFAEL AGUIRRE RUBÍ

International PhD Tesis

June 2017

eman ta zabal zazu
Universidad del País Vasco
Euskal Herriko Unibertsitatea
PiE
Plentziako Itsas Estazioa
Estación Marina de Plentzia

CONTRIBUTION TO THE DEVELOPMENT OF BIOMONITORING PROGRAMMES FOR THE ASSESSMENT OF CHEMICAL POLLUTION AND ECOSYSTEM HEALTH DISTURBANCE IN MANGROVE-LINED CARIBBEAN COASTAL SYSTEMS USING BIVALVES AS BIOMONITORS AND SENTINELS

International PhD Thesis submitted by

JAVIER RAFAEL AGUIRRE RUBÍ

For the degree of

Philosophiae Doctor

Under the supervision of Prof. Ionan Marigómez

Plentzia, June 2017

This work has been funded by:

ETORTEK research strategic actions K-Egokitzen I & II (Basque Government 2007-2011).

Spanish Agency for International Development Cooperation (CARIBIOPOL-AECID 11-CAP2-1595).

Supported by the Spanish Ministry of Economy and Finance (BMW-CTM2012-40203-C02-01).

University of the Basque Country UPV/EHU Research & Formation Unit (UFI 11/37) and Basque Government through Consolidated Research Groups by mean a pre-doctoral fellowship (IT810-B).

Mobility network between Europe and Mexico/Central America, Erasmus Mundus External Cooperation Window (EMECW; EMundus 13/09 Lote 20b; 2009-5122/001-001 ECW).

Dedication

Este trabajo lo quiero dedicar a mi familia, a mi esposa Ana, quien durante todo este tiempo me ha apoyado en este duro camino de "hacer ciencia". A mis hijas Alaia y Lorea, quienes cada día me dan el amor y la fuerza para seguir adelante.

Como dice Mercedes Soza: *" Todo cambia, lo que cambió ayer tendrá que cambiar mañana. Así como cambio yo en esta tierra lejana".* Cambiar es parte del ciclo de vida de todo los seres vivos y por eso digo, gracias mis "Bellotas" por ayudarme a cambiar.

Acknowledgements

En primer lugar, me gustaría agradecer a mi tutor/director de tesis Ionan Marigómez, por el preciado tiempo que ha dedicado (incontables fines de semana) para guiarme en el campo de la ciencia y por haberme dado la oportunidad de formar parte de su grupo de investigadores. También, me gustaría agradecer a Manu Soto, que en ausencia de mi tutor/director me ayudó a resolver problemas tanto administrativos como de investigación.

Gracias L Garmendia, B Zaldibar, M Ortiz-Zarragoitia por toda la ayuda y disponibilidad que me brindaron en los momentos más difíciles de mi aprendizaje. A U Izagire por iniciarme en el uso del micrótomo, criostato y lisosomas. También quiero agradecer a mis compañeros de doctorado del Laboratorio de Zoología y Biología Celular Animal: Joshua, Cristina, Nerea García, Iratxe, Amaia Irizar, Alberto, Txema y Esther por su amistad y quienes siempre estuvieron disponible para ayudarme en mi trabajo de investigación. A María Múgica quien me ayudo a adquirir los conocimientos básicos para realizar un muestreo de campo dentro de un programa de biomonitoreo. También me gustaría agradecer a Luisma Mendoza (técnico del Laboratorio de Zoología y Biología Celular Animal) por enseñarme el uso y manejo de todos los equipos de laboratorio. A Gorka Eguiguren por su amistad y todas las gestiones administrativas llevada a cabo en el proyecto CARIBIOPOL.

Me gustaría agradecer a los coordinadores del proyecto CARIBIOPOL en Nicaragua a Félix Espinoza y en Colombia a Andrea Luna y Michael Ahrens por todas sus gestiones y coordinación realizada en el proyecto. Estoy en deuda con Larraitz, Maren y Nestor Etxebarria de la UPV/EHU, Luisa Villamil de la UJTL y colegas del Dpto. de Biología (Prog. de Educación Ambiental, UNAN-León) por su apreciado colaboración durante los muestreos. Igualmente, con el grupo de investigadores del Dpto. de Biología de la Bluefields Indian and Caribbean University (BICU, Nicaragua) por su apoyo logístico en los muestreos, así como también al Centro de Investigación en Salud, Trabajo y Ambiente (CISTA, UNAN-León) y al Dpt. de Microbiología de la Facultad de Medicina de la UNAN-León por su colaboración en el pretratamientos de las muestras de química y análisis biológico.

Muchas gracias a todos, Eskerrik asko gustiei.

Index

I. **Introduction** 1

1. Regulatory policies for marine environmental protection 3
2. Chemical pollution and its assessment in coastal ecosystems 10
3. Marine ecosystem health and environmental stressors. 15
4. Biological effects assessment and monitoring 24
5. Caribbean mangrove-lined ecosystems 32

II. **State of the Art, Hypothesis and Objectives** 61

III. **Results and Discussion** 67

1. Chapter 1. Chemical pollution assessment in mangrove-lined Caribbean coastal systems using the oyster *Crassostrea rhizophorae* as biomonitor species 69
2. Chapter 2. Assessment of ecosystem health disturbance in mangrove-lined Caribbean coastal systems using the oyster *Crassostrea rhizophorae* as sentinel species 113
3. Chapter 3. Comparison of potential biomonitor and sentinel bivalve species suitable for biomonitoring of pollutants and ecosystem health disturbance in mangrove–lined Nicaraguan coastal systems 165
4. Chapter 4. Needs analysis and recommendations for biomonitoring chemical pollution and ecosystem health disturbance in mangrove-lined coastal systems using bivalves as biomonitors and sentinels 217

IV. **Conclusions and Thesis** 227

1. Conclusions 229
2. Thesis 231

V. **Annexes** 233

I. Introduction

1. Regulatory policies for marine environmental protection

1.1. The United Nations international regulatory framework

It is widely recognised that the planet faces serious environmental challenges that can only be addressed through international cooperation (Sands et al., 2012). Acid rain, ozone depletion, climate change, loss of biodiversity, toxic and hazardous products and wastes, aquatic pollution and depletion of aquatic resources are amongst the issues that international regulation is being called upon to address (Wilhelmsson et al., 2013). The United Nations Conference on the Human Environment held in Stockholm in 1972 is regarded as the starting point of the development of modern day international environmental law (Kamminga, 1995).

Thus, a series of conventions and regulations deal with the protection of the marine environment. For instance, the approval in the early 1980's of the United Nations Convention on the Law of the Sea (UNCLOS), established a framework to define the responsibilities of the contracting nations in the prevention, reduction and control of marine pollution (UNEP, 1995). As a result, different international cooperation structures were created to manage, in a coordinated way, the actions necessary to promote the sustainable use and conservation of the regional seas (Johnson and Miller, 1971; Teclaff, 1972; Sands et al., 2012). United Nations (1992) defined specific actions at international regulatory level aimed at pursuing the protection and sustainable development of the marine and coastal environment and its resources (Chapter 17 of Agenda 21; United Nations, 1992); thus, new approaches were required for marine and coastal area management and development, at the national, subregional, regional and global levels. Besides, in 1995, the United Nations Environment Programme (UNEP) launched the Global Programme of Action for the Protection of the Marine Environment from Land-based Activities, in the context of UNCLOS (UNEP, 1995; Franckx, 1998). Different international agencies and organisations contribute to this issue: International Maritime Organization (IMO), Food and Agriculture Organization (FAO), World Meteorological Organization (WMO), International Atomic Energy Agency (IAEA), and (World Bank) International Bank for Reconstruction and Development (IBRD). Outside the UN system, regional organisations that have adopted significant legal instruments in the field of the environment include the Organization for Economic Cooperation and Development (OECD), the Conference on Security and Cooperation in Europe (CSCE) and

the Organisation of African Unity (OAU). Non-Governmental Organisations (NGOs) which have had such impact include the International Union for the Conservation of Nature (IUCN), Friends of the Earth (FOE) and Greenpeace International (Kamminga, 1995).

The Regional Seas Programme, launched in 1974, is one of UNEP's most significant achievements in the past five decades. As a result, several worldwide Regional Sea Conventions (RSCs) are used as a guidance framework for the implementation of the most recently developed marine legislation. The Programme aims to address the accelerating degradation of the world's oceans and coastal areas through a "shared seas" approach by engaging neighbouring countries in comprehensive and specific actions to protect their common marine environment. Today, more than 143 countries have joint 18 RSCs and Action Plans for the sustainable management and use of the marine and coastal environment. In most cases, the Action Plan is underpinned by a strong legal framework in the form of a regional Convention and associated Protocols on specific problems. All individual Conventions and Action Plans reflect a similar approach, yet each has been tailored by its own governments and institutions to suit their particular environmental challenges. UNEP coordinates the Regional Seas Programme, based at the Nairobi headquarters (Table 1).

Table 1. Regional Seas programmes. The bold letters are programmes where mangrove-lined ecosystems are present.

FRAMEWORK	PROGRAMMES
UNEP-administered Regional Seas programmes	**Caribbean Region** **East Asian Seas** **Eastern Africa Region** Mediterranean Region North-West Pacific Region Western Africa Region Caspian Sea
Non-UNEP administered Regional Seas programmes	Black Sea Region North-East Pacific Region Red Sea and Gulf of Aden ROPME Sea Area **South Asian Seas** **South-East Pacific Region** **Pacific Region**
Independent Regional Seas programmes	Arctic Region Antarctic Region Baltic Sea North-East Atlantic Region

In Europe, four RSCs deal with the protection of the marine environment of member states and neighbouring countries: (a) the OSPAR Convention in the North-East Atlantic; (b) the Helsinki convention (HELCOM) in the Baltic sea; (c) the Barcelona convention in the Mediterranean sea; and (d) the Bucharest convention in the Black sea (EC, 2006). These conventions fulfil an important role in the implementation of international legal requirements as they provide valuable regional frameworks for (a) assessing the state of the marine environment; (b) addressing key developments that interact with the marine environment (e.g., socio-economic activities, coastal settlements, land-based activities); and (c) agreeing on appropriate responses in terms of strategies, policies, management tools and protocols (UNEP, 2007).

In the Caribbean, UNEP established the Caribbean Environment Programme (CEP) in 1981 as one of its Regional Seas Programmes. The importance and value of the Wider Caribbean Region's fragile and vulnerable coastal and marine ecosystems is recognised. Countries of the Wider Caribbean Region (WCR) adopted an Action Plan that led to the development and adoption of the Cartagena Convention on 24 March 1983. This Convention is the first-and-only regionally binding treaty of its kind. It promotes the protection and development of the marine environment of the Region. It is supported by three technical agreements or protocols on (a) oil spills, (b) specially protected areas and wildlife, and (c) land-based sources of marine pollution. The Caribbean Regional Co-ordinating Unit, located in Kingston (Jamaica), was established in 1986 as the Secretariat to the Cartagena Convention. The Caribbean Environment Programme has three sub-programmes:

- Assessment and Management of Environment Pollution (AMEP)
- Specially Protected Areas and Wildlife (SPAW)
- Communication, Education, Training and Awareness (CETA)

Nevertheless, in the Caribbean region a number of shortcoming and challenges have been identified in relation with regulatory policies for marine environmental protection (Fig. 1); these are the low ratification of various multilateral agreements, the poor implementation, and the inadequate stipulations in conventions to effectively respond to issues such as pollution (Singh, 2008).

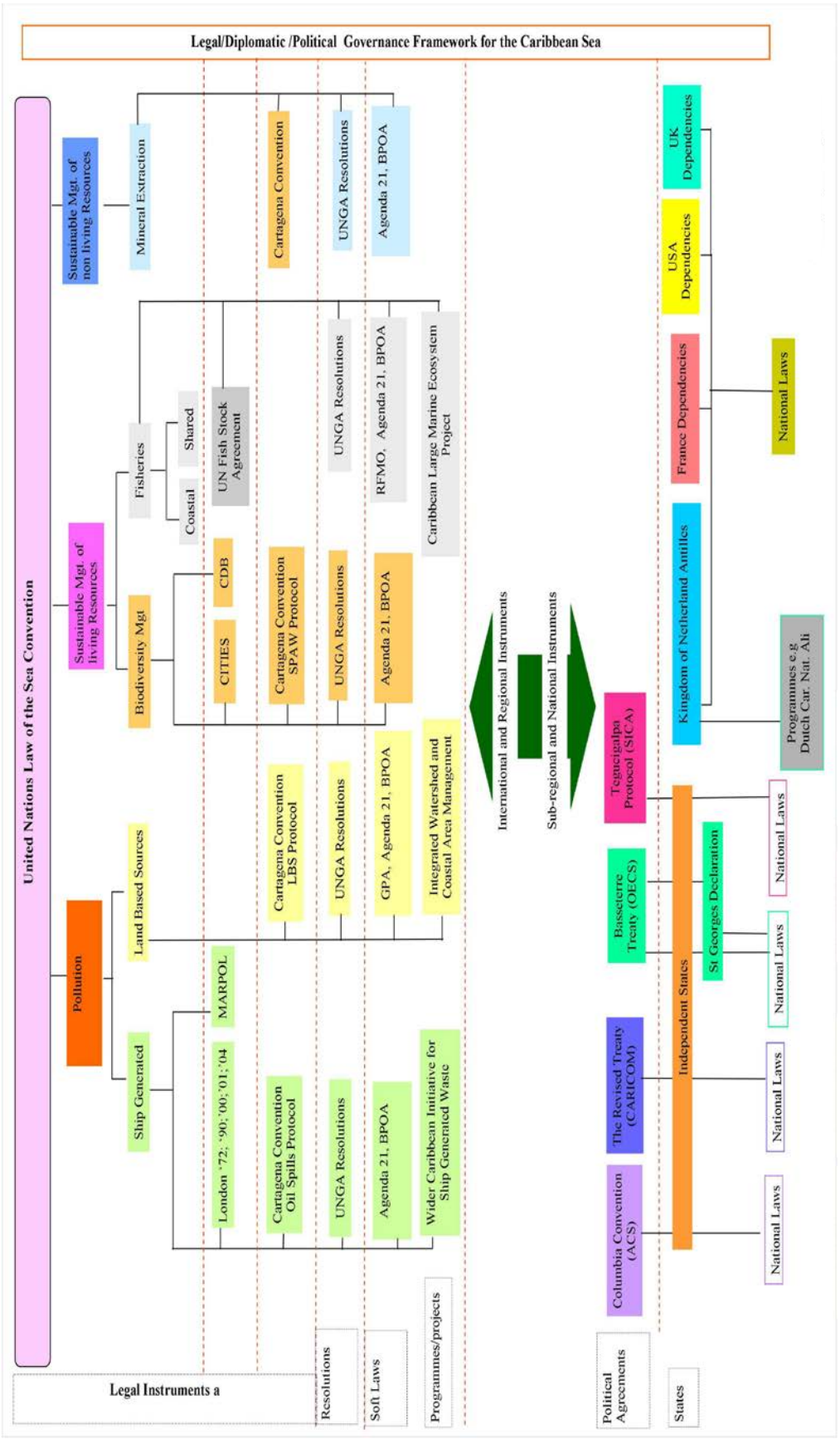


Fig. 1. The legal, political and diplomatic governance mechanisms in the Caribbean Sea with respect to three core sustainable development issues (Singh, 2008).

1.2. European policies for protection of the marine environment

The European Union (EU) goes ahead in the context of protection of coastal marine ecosystems. EU has adopted several environmental directives, strategies, recommendations, and agreements that focus on biological endpoints with ecosystem health at the centre of regulation and management decision making. For example, the Water Framework Directive (WFD; 2000/60/EC; EC, 2000) and the Marine Strategy Framework Directive (MSFD; 2008/56/EC; EC, 2008) deal with the assessment and management of the environmental quality of estuarine and marine water bodies, respectively.

Since its entrance into force in 2000, the WFD has established a framework for the protection of groundwater, inland surface waters, estuarine waters and coastal waters. Its first objective was to achieve the “Good Ecological Status” (GES) for all the European water bodies by 2015 (EC, 2000). The WFD deals with the compliance of environmental quality standards (EQSs), established for chemical substances at European level (Environmental Quality Standards Directive; 2008/105/EC), in the water basins from inland to transitional and coastal waters. Complementarily, the EU adopted the MSFD with the aim of achieving the “Good Environmental Status” (GEnS) for all European seas, by 2020 (Bertram and Rehdanz, 2013). The main objectives of the MSFD are to protect and restore the quality of European seas while ensuring the sustainability of human activities, the conservation of marine ecosystems, and the provision of safe, clean, healthy and productive marine waters to current and future generations (Borja et al., 2010, 2008). The WFD works as a “deconstructing structural approach”; however, the MSFD is conceived as a “holistic functional approach” that defines the quality of the marine ecosystems in an integrative way, considering together biological, physicochemical and pollution parameters, biological elements becoming especially important. Concretely, the MSFD requires criteria and methodological standards to allow consistency in approach in evaluating the extent to which Good Environmental Status (GEnS) is being achieved. MSFD's Descriptor 8 (“Concentrations of contaminants are at levels not giving rise to pollution effects") is closely linked with Descriptor 9 ("Contaminants if fish and other seafood") and Descriptor 10 ("Marine litter").

Therefore, given the adverse impacts that marine pollution events can induce in the biological functioning of estuarine and marine ecosystems, both directives require the identification of the polluting pressures and the spatio-temporal scales in which these are reflected.

They require the integration of the results of chemical monitoring programmes in combination with the assessment of the biological effects at different level of biological complexity; this approach helps in detecting early warning signals of pollution (Law et al., 2010).

1.3. U.S. programmes for protection of the marine environment

Likewise, USA is a pioneering reference for activities pursuing the protection of the marine environment. Thus, the US Environmental Protection Agency (USEPA) has long-lasting experience in protecting and restoring ocean and coastal ecosystems by promoting watershed-based management, preventing aquatic pollution, managing ocean dumping sites, assessing coastal conditions, establishing effective partnerships and facilitating community-led science-based efforts. These programs help to ensure clean and safe waters that sustain human health, the environment and the economy.

For instance, the Coastal Nonpoint Pollution Control Program addresses nonpoint pollution problems in coastal waters. This program is administered jointly with the National Oceanic and Atmospheric Administration (NOAA) and National Centres for Coastal Ocean Science (NCCOS) in order to study and monitor the effects of coastal pollution. These results provide communities with the information and tools they need to identify pollution "hotspots" and to develop practices and policies that reduce pollution and improve coastal health. NCCOS Coastal Pollution research is focused on chemical contaminants, hypoxia and eutrophication, invasive species and pathogens and microbes. NCCOS identifies coastal areas that are contaminated, the pollutants and their sources and impacts. In parallel, the Bioeffects Program has undertaken since 1991 a series of regional environmental assessments designed to describe the magnitude and extent of toxic contaminant impacts in estuarine and coastal areas. The Bioeffects Programme aims at assessing the spatial distribution and effects of chemical contamination, and at developing indicators of environmental contaminant exposure in water bodies, ranging from small estuaries to large bays and coastal areas. This information is integrated to develop a comprehensive assessment of the health of the marine habitat.

The National Estuary Program (NEP) constitutes another example (USEPA, 2014). In 1987, Congress established the NEP as a non-regulatory, community-based program to protect and restore the water quality of estuaries (Section 320 of the Clean Water Act of 1987). The NEP identifies nationally-significant estuaries threatened by pollution, development or overuse, and

requires the preparation of comprehensive conservation and management plans to ensure the long-term ecological integrity of those estuaries. These plans include (a) assessing water quality, natural resources and human use trends, (b) characterisation and identification of the causes of environmental problems, identifying pollutants and its sources and impact, (c) recommending priority corrective actions and compliance schedules, and (d) monitoring effectiveness of actions taken.

1.4. Marine environment protection programmes in the Caribbean

Within the UNEP CEP framework, several tools (e.g. the protocol for Pollution from Land-based Sources and Activities) were agreed in order to classify water bodies, establish legally binding standards, identify major sources of pollutants and prevent pollution (Siung-Chang, 1997). Further on, institutions such as the International Oceanographic Commission (IOC) collaborated with the UNEP and the NOAA in order to support the creation of the International Mussel Watch Project in the Caribbean since the 1990's. The program was directed by the International Mussel Watch Committee and coordinated and administered by Coastal Research Center of the Woods Hole Oceanographic Institution (Farrington and Tripp, 1995). Like the initial US Mussel Watch programme, the Caribbean Mussel Watch program was also focused on chemical approaches and mainly targeted to organochlorine contaminants (Fig. 2; Farrington and Tripp, 1995; Siung-Chang, 1997; UNEP, 2006). Following the same guidelines, the UNEP Global Environmental Facility project for the assessment of environmental and health effects of persistent toxic substances (PTSs) was launched in 2000. It incorporated Central American and Caribbean Regions (denoted Region X), which included 23 countries (Colombia and Nicaragua amongst others) and 136 million inhabitants. The regional component addressed the 12 persistent organic pollutants (POPs) defined by the Stockholm Convention on Persistent Organic Pollutants held in 2001 (aldrin, endrin, dieldrin, chlordane, DDT, toxaphene, mirex, heptachlor, hexachlorobenzene, polychlorinated biphenyls, polychlorinated dibenzodioxins, and polychlorinated dibenzofurans), and additional regionally important compounds (atrazine, endosulfan, pentachlorophenol, polybrominated diphenyl ethers, lindane, organic lead, organic mercury, organic tin, polychlorinated phenols, polycyclic aromatic hydrocarbons, short chain paraffins, phthalates, octylphenols, and nonyl phenols) (UNEP, 2002). Other relevant monitoring program was carried out in the Caribbean by the Caribbean Regional Coordinating Unit for the UNEP (UNEP-CAR/RCU), with the support of the Global Environment Facility (GEF), embarked on efforts to reduce agricultural pesticide runoff

to the Caribbean Sea through a joint project with the Governments of Colombia, Costa Rica and Nicaragua (GEF-REPCar); which began in 2006 and ended in 2011.

Exceptionally, biomonitoring programmes have been run at national level, as in the case of the REDCAM programme in Colombia (Vivas-Aguas et al., 2014). Likewise, specific pollution assessment studies have been carried out locally in a few cases (Ebanks-Mongalo et al., 2013).

Fig. 2. Sampling sites of the International Mussel Watch Programme (Farrington and Tripp, 1995).

2. Chemical pollution and its assessment in coastal ecosystems

2.1. Marine and coastal chemical pollution

According to OSPAR Commission (2000a) "Marine pollution" is defined as *the introduction by humans, directly or indirectly, of substances or energy into the marine area which results, or*

is likely to result, in hazards to human health, harm to living resources and marine ecosystems, damage to amenities or interference with other legitimate uses of the sea.

Metals. Metals are persistent and toxic, tend to bioaccumulate, and pose a risk to humans and marine organisms and ecosystems (Rainbow, 2002; Ponizovsky, 2003; Funes et al., 2006). There is an increasing metal input to the coastal zone from both rivers and non-point sources, especially in developing countries. Thus, heavy metal contamination at local, regional and global scales has been intensively studied in recent years,

Natural metal inputs such as erosion, wind-blown dust, volcanic activity, and forest fires account for the main sources of some metals like Al and Hg (Mahboob, 2013). Today's industrial pollution still includes Zn, Cu, Ni, Cr and Pb (Shi et al., 2010; Simpson et al., 2004). Metals such as Pb, Zn, Cd and Cu, among others resulting from anthropogenic activity, are released trough atmospheric and river inputs, mining activities, dredging spoil, direct discharges, industrial dumping and sewage sludge, contributing to metal pollution leading to the marine environment (Schindler, 1991; Audry et al., 2004; ICES, 2006). Once into aquatic systems metals will undergo several processes such as dissolution, precipitation, sorption and complexation, which can further affect their biological effects and environmental consequences, as the form of the metal (chemical species, compound, matrix and particle size) may influence its bioavailability, fate, and effects on the biota (Looi et al., 2013). Environmental properties, such as pH, particle size, moisture, redox potential, organic matter, cation exchange capacity, among others are also relevant to determine the biological effects of the metal pollutants (Belabed et al., 2013; Fairbrother et al., 2007; Ivanina and Sokolova, 2013).

The mechanisms for uptake, distribution/storage and excretion of metals are well known in aquatic organisms (Fairbrother et al., 2007; Marigómez et al., 2002). Metals may form complexes with proteins such as metallothioneins (Amiard et al., 2006; Pellerin and Amiard, 2009), metalloproteins and other carrier molecules such as chaperons (Amiard et al., 2006; Hédouin et al., 2010; Kozlowski et al., 2013); thus they are distributed to target cell compartments for sequestration and excretion, their bioaccumulation usually being tissue and cell type specific (Marigómez et al., 2002); which depends on the affinity of the metals for different electron donors: metals such as Cs, K, Na, Li, Ba, Sr, Ca, Mg, Be, La, Gd, Y, Lu, Sc, and Al bind oxygen donors such as carbonate, oxalate, phosphate and sulphate, whilst Au, Cu, Hg, Se, Pd, Pt, Bi, Ti, Pb and Ag bind sulphur donors such as sulphur and nitrogen; other metals such as Cd, Sn, Pb, Ti, Mn, Fe,

V, Co, Zn, Ni, Cr, In, Ga, Sb, and As are classified as borderline and may bind both oxygen and sulphur donors (Nieboer and Richardson, 1980).

Polycyclic aromatic hydrocarbons (PAHs). PAHs are composed of hydrogen and carbon arranged in the form of two or more fused benzene rings (in linear, angular, or cluster arrangements) that may or may not have substituted groups attached (Sims and Overcash, 1983; Webster et al., 2010). These can be naturally occurring, but their presence in the marine environment is mainly due to anthropogenic activities; especially those related to the combustion of any kind of organic matter and to the transport and use of fossil fuels (Galgani et al., 2011; Soclo et al., 2000). PAHs arrive at marine and coastal ecosystem mainly through long range atmospheric transport and waterborne inputs (OSPAR Commission, 2009a). As a consequence of their hydrophobic nature, PAHs in aquatic environments rapidly tend to become associated with sediments, which represent the most important reservoir of PAHs in the marine environment. The background levels of PAHs in the marine environment are also present as a result of biosynthesis and natural oil seeps (Cortazar et al., 2008; OSPAR Commission, 2000a).

Most PAHs are toxic and persistent and may be metabolised or bioaccumulated, the latter especially in marine invertebrates such as mussels; and some of them such as naphthalene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and dibenzo[a,h]anthracene have a recognised mutagenic and carcinogenic potential (Eisler, 1987; IARC, 1989; Rocher et al., 2006; OSPAR Commission, 2009a). Physical and chemical characteristics of PAHs generally vary with molecular weight. For instance, solubility in water varies from 0.3 ng/l for low molecular weight PAHs (two and three ring compounds) to <0.001 ng/l for the high molecular weight PAHs (five or more ring compounds). As a general rule, high molecular weight PAHs exhibit low aqueous solubility and high melting point, boiling point, and the $\log K_{ow}$ (octanol/water partition coefficient), which reveals a high fat solubility and a low resistance to redox reactions and vapour pressure (Eisler, 1987; Marigómez et al., 2013a; OSPAR Commission, 2000b). Consequently, depending on their molecular weight, the PAHs present different behaviour and distribution in the environment and different toxicity on marine organisms (Bustamante et al., 2010; Orbea and Cajaraville, 2006).

Persistent organic pollutants (POPs). POPs include polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs), which are anthropogenic synthetic compounds that have been used extensively in agriculture and industry in

the past (OSPAR Commission, 2009a; Sturludottir et al., 2013). Their high chemical stability, lipophilicity, and persistence, make these chemicals tend to bioconcentrate and biomagnify in the food chains and persist in the environment for many years, representing a definite health hazard for both marine organisms and humans (Naso et al., 2005; Perugini et al., 2004). POPs can induce a series adverse effects at different biological complexity levels in marine organisms; these include oxidative stress, DNA damage, membrane destabilisation, suppressed filtration rate, and reproductive and growth impairment (Islam and Tanaka, 2004; Höher et al., 2012; Bizarro et al., 2014; Ortiz-Zarragoitia et al., 2014; Valencia et al., 2017). The major route of POPs intake for small organisms at lower trophic levels (e.g., plankton, bivalves and crustaceans) is the direct uptake from water or sediments by passive diffusion through body surfaces; which is positively correlated with the $\log K_{ow}$ of the chemicals and the amounts of lipids within the organisms (Gray, 2002; MacKay and Fraser, 2000). As the body mass and the trophic level in the food chain of marine organisms increase, the biomagnification process plays an increasing role in the accumulation of organic pollutants, especially for the most hydrophobic ones (Binelli and Provini, 2003; Loizeau et al., 2001). Elimination rates for lipophilic contaminants generally decrease with increasing $\log K_{ow}$ (Gray, 2002). Passive diffusion also represents the main mechanism for the elimination of these chemicals. Interestingly, bivalves show a very limited capacity to metabolise PCBs through the cytochrome P450 system (Zanette et al., 2013), and hence the concentrations of each of the PCB congeners, as well as the total congener pattern, are scarcely modified by biotransformation. For these reasons, the levels of organochlorines and the accumulation profile of PCBs detected in mussels reflect the state of pollution of the coastal waters (Naso et al., 2005).

Special interest is increasing in PBDEs as they are widely used as brominated flame retardants in many domestic and industrial products (television sets, computers, radios, textiles, new synthetic building materials, and automobiles) and data on their biological effects are lacking or scarce (Darnerud et al., 2001; OSPAR commission 2009b; Llorca et al., 2016). PBDEs are persistent man-made aromatic chemicals composed of two phenyl rings linked by an oxygen bridge (ether linkage). Bromine atoms can replace up to ten hydrogen atoms on the phenyl rings. Theoretically, 209 unique PBDE structures (congeners) are possible and are classified into 10 congener groups (mono- to decabromodiphenyl ethers). Congeners with the same number of bromine atoms are referred to as a homologue, and thus, there are ten homologues ranging from mono- to decaBDE. The chemical structure of PBDEs is similar to that of PCBs, being referred to as the new PCBs (Darnerud et al., 2001; Kimbrough et al., 2009). Commercial PBDEs are quite

resistant to physical, chemical, and biological degradation. The boiling point of PBDEs is between 310 and 425°C and their vapour pressure is low at room temperature. PBDEs are lipophilic, and their solubility in water is low, especially for the higher brominated compounds (Darnerud et al., 2001; Kimbrough et al., 2009). Two PBDE transformation pathways are found in the environment: incineration and photodegradation, both resulting in formation of PBDDs and PBDFs (Thomas et al., 1987; Dumler et al., 1989); which are structurally similar to the corresponding chlorinated compounds, more toxic and very persistent in the environment (Watanabe and Satsukawa, 1987; Öberg and Bergström, 1990). Toxicological studies are not well documented (OSPAR commission, 2009b). Available data in human suggest that the acute toxicity of PBDEs is low (Darnerud et al., 2001). In Latin America, PBDE concentrations in the range of 86 ng/g lipid wt have been reported in coastal molluscs such as clams and razor clams (Barón et al., 2013; Pozo et al., 2015; Llorca et al., 2016)

2.2. Pollution monitoring and assessment

"Monitoring" is *a systematic and orderly gathering of data to ensure that previously established quality control conditions are being met*. Monitoring can be regarded as a series of observations in time, carried out to show the extent of compliance with a set of environmental objectives. To be effective, monitoring needs to be carried out at the appropriate time and space scales; for which a range of strategies need to be adopted making use of a variety of monitoring tools. Thus, the goal of environmental biomonitoring is to provide an early warning that unacceptable levels of environmental stress have occurred (Cairns, 2013).

Marine organisms have been commonly used as core tools in biomonitoring programs (Shugart et al., 1992; George et al., 2013; Brenner et al., 2014; Melwani et al., 2014), referred to as sentinel species (Basu et al., 2007; George et al., 2013; Viarengo et al., 2007), bioindicator (Corsi et al., 2002; Fattorini et al., 2008; Storelli and Marcotrigiano, 2005; Zhou et al., 2008), biomonitors (Conti et al., 2012; Luoma and Rainbow, 2005), or bioaccumulators (Beeby, 2001; Conti et al., 2006). Amongst them, due to their widespread geographic distribution, reasonable size and availability throughout the year, bivalves (mussels, oysters, clams and cockles) have been used most commonly (Farrington and Tripp, 1995; Cajaraville et al., 2000; Zorita et al., 2007; ICES, 2012; Garmendia et al., 2011a, b; George et al., 2013; Marigómez et al., 2013b; Zuykov et al., 2013). When used to quantify chemical bioavailability they are designated as bioindicators or

biomonitors, whilst when used in biological effect monitoring programs to assess ecosystem and environmental integrity they are referred to as sentinel organisms (Morgano and Bebiano, 2005).

3. Marine ecosystem health and environmental stressors

3.1. Healthy vs stressed ecosystems

An "ecosystem" may be defined as *any unit that includes all of the organisms in a given area interacting with the physical environment so that a flow of energy leads to a clearly defined trophic structure, biotic diversity, and material cycles within the system* (Odum, 1963). Ecosystems are complex entities that change on daily and seasonal bases as well as in the long-term due to natural environmental variations and in response to stressors such as global climate change and anthropogenic contaminants (Denslow et al., 2007).

The concept of ecosystem health can be used to generate holistic methodologies for the monitoring and management of marine ecosystems, but it is itself a subject of debate (Tett et al., 2013). An ecosystem is healthy and free from “distress syndrome” if it is stable, sustainable and resilient to stress (Costanza et al., 1992; Costanza, 2012). An excellent review on marine ecosystem health has been published as a position paper by Tett et al., (2013). According to these authors, a "good health for marine ecosystems" can be understood as *the condition of a system that is self-maintaining, vigorous, resilient to externally imposed pressures, and able to sustain services to humans. It contains healthy organisms and populations, and adequate functional diversity and functional response diversity. All expected trophic levels are present and well interconnected, and there is good spatial connectivity amongst subsystems.* Overall, the various levels of biological organisation (cell, tissue, organism, population, community and ecosystem) can be regarded as healthy or unhealthy (Elliott, 2011). Thus, ecosystem health upends on (a) the physiological health of the constituent organisms; (b) the characteristic properties and interactions of the species present; and (c) the emergent properties of the system comprising the biota and their environment (Tett et al., 2013). Healthy ecosystems contain organisms and populations that are free of stress-induced pathologies. Interestingly, at the level of organism, the meaning of ‘health’ seems unambiguous and identical with that of human physical well-being whilst at the next levels the argument becomes more complex; for instance, "community health" can be described as that

of an assemblage of organisms that can continue to function in terms of inter-species relationships, and "ecosystem health" as providing protection against ‘ecosystem pathologies’ (Elliott, 2011).

"Environmental stress" is *any action, agent, or condition that impairs the structure or function of a biological system* (Cairns, 2013). However, the environmental stress can only be defined in reference to its interaction with some biological system (environmental stress receptor). Moreover, there must be an adverse response; say, a particular structure or function of the receptor is changed by exposure to the environmental stress towards the detriment of that system. However, if the survival of that biological system (or another) is not threatened by the change, then there is no environmental stress (Cairns, 2013). Yet, environmental stress appears to play a substantial role in the evolution of life (Fink, 2009; Steinberg, 2012). Environmental stress can be either natural or anthropogenic. Natural stressors, such as most hurricanes, droughts, floods, and fires are a periodic feature of life on Earth; in contrast, anthropogenic stressors such as the production and release of new chemical compounds and large-scale land-use changes can be eventual, periodic or chronic (Evans and Cohen, 1987; Cairns, 2013). Both natural and anthropogenic stressors can be characterised on the basis of their spatial distribution, temporal distribution, intensity and novelty; implying unequal sizes of effects upon species responses, species diversity, as well as ecosystem functioning and recovery (Kelly and Harwell, 1989; Segner et al., 2014). These differences between stressors in terms of intensity, frequency, temporal, and spatial scales (Fig. 3) is one of the complicating factors in the assessment of the combined effects of stressors in multiple stress scenarios naturally occurring (Segner et al., 2014). Many stressors such as increasing temperature, ocean acidification, hypoxia, increasing eutrophication, overfishing, diseases and pollution co-occur in time and space, resulting in almost all marine organisms and ecosystems becoming subject to the impacts of multiple stressors (Mackenzie and Schiedek, 2007; Schiedek et al., 2007; Wilhelmsson et al., 2013).

To diagnostic and predict multiple stressor impacts, assessment strategies should focus on properties of the biological receptors rather than on stressor properties. This change of paradigm is required because (a) multiple stressors affect multiple biological targets at multiple organizational levels, (b) biological receptors differ in their sensitivities, vulnerabilities, and response dynamics to the individual stressors, and (c) biological receptors function as networks, so that actions of stressors at disparate sites within the network can lead via indirect or cascading effects, to unexpected outcomes (Segner et al., 2014). In addition for the potential effect of these

multiple stress sources on marine ecosystems will be further exacerbated in the context of global environment and climate change (Fig. 4; Steffen, 2006; Hoegh-Guldberg and Bruno, 2010; IPCC, 2013).

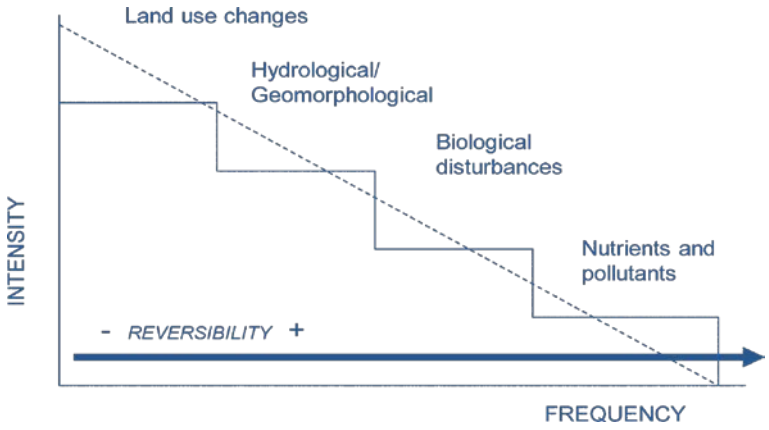


Fig. 3. Chemical, biological, and physical stressors differ in their inherent properties which are relevant for the biological receptors. The intensity and frequency of each type of stressors influence the reversibility or irreversibility of their effects (Segner et al., 2014).

3.2. Ecosystem health assessment

Ecosystem health assessment is one of the hotspots in the fields of ecology and ecosystem management (Peng et al., 2007); this integrates environmental conditions with the impact of anthropogenic activities in order to give information for a sustainable use and management of natural resources (Burkhad et al., 2008). The key objectives in the assessment of marine ecosystem health are to provide information necessary to ensure maintenance of biodiversity and the integrity of marine communities, to limit human influences on living resources, to protect critical habitats and to safeguard human health (Marigomez et al., 2013b). In Canada, the Marine Environmental Quality (MEQ) program is charged with the development of methods for marine quality assessment, which are based on the concept of ecosystem health (Mark et al., 2003). In a European context, a condition of good health in estuarine and coastal marine ecosystems is implied by the 'Good Ecological Status' of the WFD and the 'Good Environmental Status' of the MSFD (Table 2; Tett et al., 2013).

Table 2. Components of (good) ecosystem health, according to the empirical approach, and as interpreted by EU Directives. Column 3 gives corresponding specifications from Annex V of the EU Water Framework Directive (WFD) for 'high quality status' (which we equate with good health) in 'transitional' and 'coastal' waters. Column 4 refers to the relevant 'qualitative descriptors for determining good environmental status' in Annex I of the Marine Strategy Framework Directive (MSFD), which are expanded by COM (2010) (after Tett et al., 2013).

1. Generic component of ecosystem health	2. Ecological norm	3. WFD 'high quality status'	4. MSFD 'qualitative descriptors'
Autochthonous **primary photosynthetic production** plus import of organic matter is roughly in balance with consumption, so that there is no large excess of respiration that might lead to de- oxygenation nor substantial export of unconsumed material	Life-form of primary producer is typical of ecohydrodynamic type and production is within characteristic range for undisturbed example of this type	• Phytoplankton biomass to be consistent with the type specific physico-chemical condition. • Macro-algal cover, and angiosperm abundance to be consistent with undisturbed conditions. • Oxygen balance… remain[s] within the range … normally associated with undisturbed conditions.	• Human-induced eutrophication is minimised, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algae blooms and oxygen deficiency in bottom waters.
Nutrient supply, cycling rates and elemental ratios are adequate to support community functioning and structure; communities make efficient use of these resources	Nutrient seasonal cycles, amounts, and elemental ratios are similar to those under undisturbed conditions	• Nutrient concentrations remain within the range normally associated with undisturbed conditions.	Not explicitly mentioned
Sufficient **biodiversity** to fulfil all the necessary bio-geochemical roles, to support species at higher trophic levels, and to provide a reserve in case of loss of species; keystone species flourishing where essential for community functioning; there is a mixture of r- and k-adapted species, and a mixture of reproductive and young individuals within populations	All aspects of diversity are appropriate for undisturbed example of ecosystem type as determined by climate and local (eco) hydrodynamic conditions	• The composition and abundance of phytoplanktonic taxa are consistent with undisturbed conditions. • All disturbance sensitive macroalgal and angiosperm taxa associated with undisturbed conditions are present. • The level of diversity and abundance of invertebrate taxa is within the range normally associated with undisturbed conditions.	• Biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions. • Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems.
Community structure includes multiple trophic levels and a variety of trophic links between levels; in cases where autogenic or responsive successions are important, then either a sub- stantial proportion of the eco- system is in the mature state, or there are no impediments to reaching such a state	Structure is that characteristic of this ecosystem type under undisturbed conditions	(Not explicitly dealt with by this Directive)	• All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity.
Individual organisms are healthy and reproductively fit, not showing widespread pathologies, nor substantially contaminated with pollutants, nor exhibiting reduced resistance to disease or stress or reduced ability to detoxify	Body burden of contaminants below defined threshold; no substantial differences in performance com- pared with individuals at unpolluted station	• Pollutant concentrations remain within the range normally associated with undisturbed conditions.	• Concentrations of contaminants are at levels not giving rise to pollution effects.

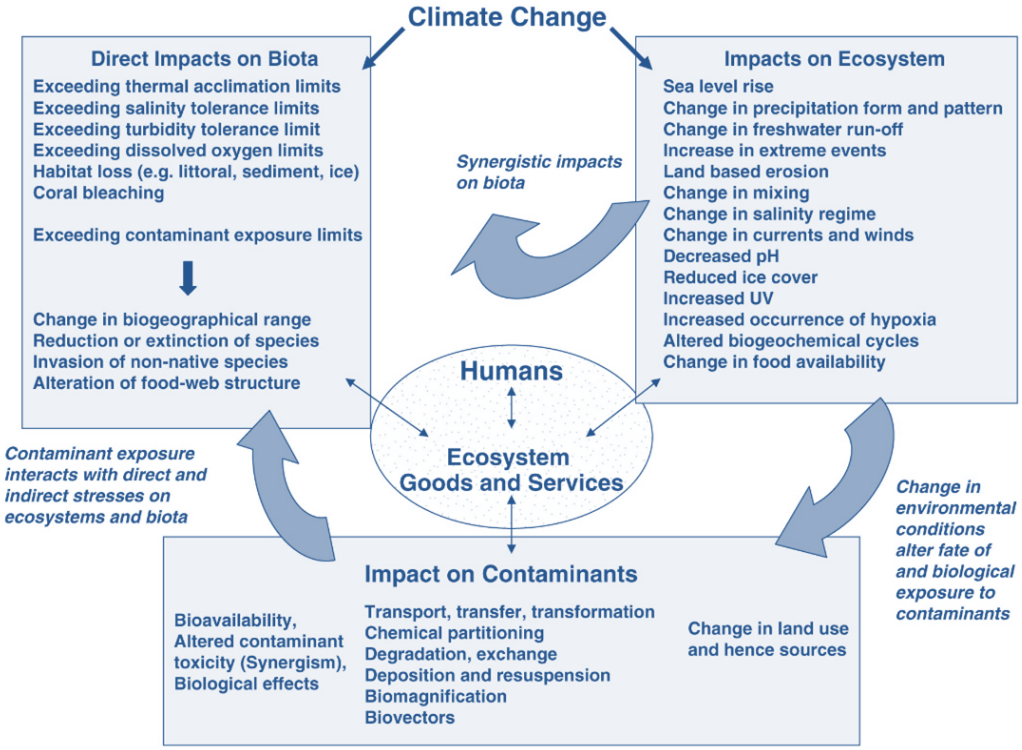


Fig. 4 Overview of climate change impacts on ecosystem and biota, and how they interact with contaminants, and their fate and effects (Schiedek et al., 2007).

Another approach is the so-called empirical ecosystem health, which sees health not as a single property of an ecosystem, but as an aggregate of contributions from organisms, species and processes within a defined area (Tett et al., 2013). At the level of communities and ecosystems, monitoring allows 'detection of things going wrong' against a background of system variability (Elliott, 2011); however, early signals of ecosystem health disturbance can be used preventively anticipating the occurrence of such undesired wrong events, as below discussed (Marigomez et al., 2013b).

To evaluate the "empirical health status" (say, the aggregated health status; or simply the general status) of marine ecosystems a science-based and integrated Ecosystem Approach[1] is

[1] *Ecosystem Approach:* The Ecosystem Approach [defined in CBD (2000)] is a management and resource planning procedure that integrates the management of human activities and their institutions with the knowledge of the functioning of ecosystems. In the management of marine ecosystems and resources, it requires to "identify and take action on influences that are critical to the health of marine ecosystems, thereby achieving sustainable use of ecosystem goods and services and maintenance of ecosystem integrity" (cf., Farmer et al., 2012, for a review of the concept of ecosystem approach in marine management). The Ecosystem Approach can be defined as the ability to fulfil the major aim of protecting and maintaining the natural structure and functioning while at the same time ensuring the creation of ecosystem services from which societal benefits can be obtained (Elliott, 2011).

highly valuable (Burkhad et al., 2008; Borja et al., 2016). The environmental status assessment is an important component of the integrated ecosystem approach for marine management; this assessment describes the health of marine ecosystems in an integrative way, and hence it requires that a considerable amount of data are available (Borja and Elliott, 2013; Borja et al., 2013; 2016). The evaluation of the "empirical health status" can be achieved by different methods (Borja et al., 2016): e.g., the HELCOM HOLAS Tool; the Ocean Health Index; and the MARMONI Tool. The HELCOM HOLAS Tool for the Assessment of ecosystem health of the Baltic Sea (Baltic Marine Environment Protection Commission-Helsinki Commission; Baltic Sea Action Plan; HELCOM, 2007) is based on the Ecosystem Approach for establishing a region-wide baseline and an indicator-based assessment of ecosystem health; the assessment main endpoints include eutrophication, biodiversity and chemical status (HELCOM, 2009a; 2009b; 2010b; Andersen et al., 2010; 2011; 2014; 2016). The MARMONI Tool for MSFD Marine Biodiversity Assessment (www.sea.ee/marmoni/) uses various indicators for the assessment area with several options for GEnS determination (Auninš and Martin, 2015). A global scale initiative in the field of evaluation of the "empirical health status" of marine systems is the Ocean Health Index (Halpern et al., 2008; 2012); this includes not only the negative impacts exerted on the oceans but also captures the tangible and less-tangible benefits derived from the oceans. The OHI framework scores a suite of benefits ("goals") that are delivered to people by assessing the current status and likely future state (including pressures and resilience measures) of each goal for each region that together comprise the whole assessment area (Fig. 5).

Fig. 5. The Ocean Health Index is a global scale initiative in the field of evaluation of the l health status of marine systems (Halpern et al., 2008; 2012).

The OHI is a way to measure how healthy oceans are. Understanding the state of our oceans is a first step towards ensuring they can continue providing humans benefits now and in the future. Determining how healthy oceans are and managing for the future requires an assessment approach that evaluates current conditions comprehensively from social, economic, and environmental perspectives. The OHI is an assessment framework that measures progress towards a suite of key societal 'goals' representing the benefits and services people expect healthy oceans to provide. By analyzing these goals together and scoring them from 0-100, OHI assessments provide an integrated picture of the state of the ecosystem and can be communicated to a wide range of audiences. Originally developed by an interdisciplinary team of scientists (Halpern et al., 2012), the OHI framework is standardized yet tailorable to different contexts and spatial scales. This is possible because the core framework of how goals are scored does not change while the goal models themselves are developed with local information and local decisions specific to the context.

Changes in community structure are often used to indicate ecosystem health status but, regrettably, these responses are manifestations of damage rather than prognostic indices (Knap et al., 2002). Changes at molecular, cellular and tissue-level levels, which underlie effects at complex biological levels and for which causality can be established (Knap et al., 2002), may provide early warning of ecosystem health deterioration (Beliaeff and Burgeot, 2002). "Biomarkers" are those *responses at simple levels of biological complexity that indicate the presence of pollutants (exposure biomarkers) or the magnitude of the biological response to pollutant exposure* (*effect biomarkers;* McCarthy and Shugart, 1990). Effect biomarkers give a general picture of the health status of the environment whereas exposure biomarkers have specificity of reaction (McCarthy and Shugart, 1990). Recently, biomarkers have been integrated in ecosystem health indices (Table 3) that provide comprehensive information about the biological effects of pollution in marine organisms and may therefore serve as useful tools for environmental managers (Broeg and Lehtonen, 2006).

The bioeffects assessment index (BAI; Broeg et al., 2005), was designed for the assessment of multifactorial contamination in coastal areas using fishes as sentinels (Broeg et al., 2005). BAI has been applied for long-term studies of the biological effects of pollution in Europe using fish and mussels as sentinels (Broeg et al., 2005; Broeg and Lehtonen, 2006; Marigómez et al., 2013ab). The Health Status Index (HSI) is computed by an expert system designed and developed to evaluate and integrate biomarkers recorded at different levels of biological organisation in

mussels (Viarengo et al., 2000; Dondero et al., 2006; Dagnino et al., 2007). The Integrated Biomarker Response (IBR; Beliaeff and Burgeot, 2002) has been applied in sentinel fish and mussels from the Baltic Sea, the Mediterranean Sea, the Bay of Biscay (e.g. example in Fig. 6) and the North Sea (Beliaeff and Burgeot, 2002; Bocquene et al., 2004; Broeg and Lehtonen, 2006; Damiens et al., 2007; Brooks et al., 2011; Marigómez et al., 2013a). The Ecological Health Condition Chart (EHCC) was designed to integrate biomarker and chemical data in the Urdaibai Reserve of the Biosphere and was recently adapted to sentinel mussels to monitor the biological consequences of the Prestige oil spill (POS; Marigómez et al., 2013b). The Integrative Biomarker Index (IBI) was also recently developed in order to integrate biomarker data recorded within the framework of the Mussel Watch monitoring program carried out after the POS in Galicia and the Bay of Biscay (Marigómez et al., 2013b). IBI was based on the calculation of five specific indices of deleterious effects at different levels of biological complexity (Molecular/Metabolic Response Index; Cellular Response Index; Tissue Response Index; Systemic Response Index; and Disease Response Index) and existing reference and critical values are taken into consideration (Marigomez et al., 2013b). Overall, the integrative biomarker indices provide comparable information (Broeg and Lehtonen, 2006; Dagnino et al., 2007; Marigómez et al., 2013ab).

The selection of biomarkers is a crucial issue (Marigómez et al., 2013b). For instance, BAI and IBI are only based on biomarkers of general stress while HSI, IBR and EHCC can be constructed using both effect and exposure biomarkers. Besides, whereas BAI, HSI, IBI and EHCC require a more or less extensive knowledge of mechanisms of the biological response and the existence of reference/critical values, IBR is a simple mathematical transformation which does not need such knowledge. On the other hand, EHCC allows describing each scenario using pure biomarkers without any kind of transformation. Finally, whereas BAI and HSI provide a basic indication of the ecosystem health status, IBR, IBI and EHCC provide complementary information concerning the mechanisms of biological response to environmental insult. The selection of the indices and the biomarkers used for their calculation depends on (a) the researchers' expertise and technical capability as regards biomarkers; (b) the existence of reference/critical values or previous studies in the impacted area; and (c) the available resources.

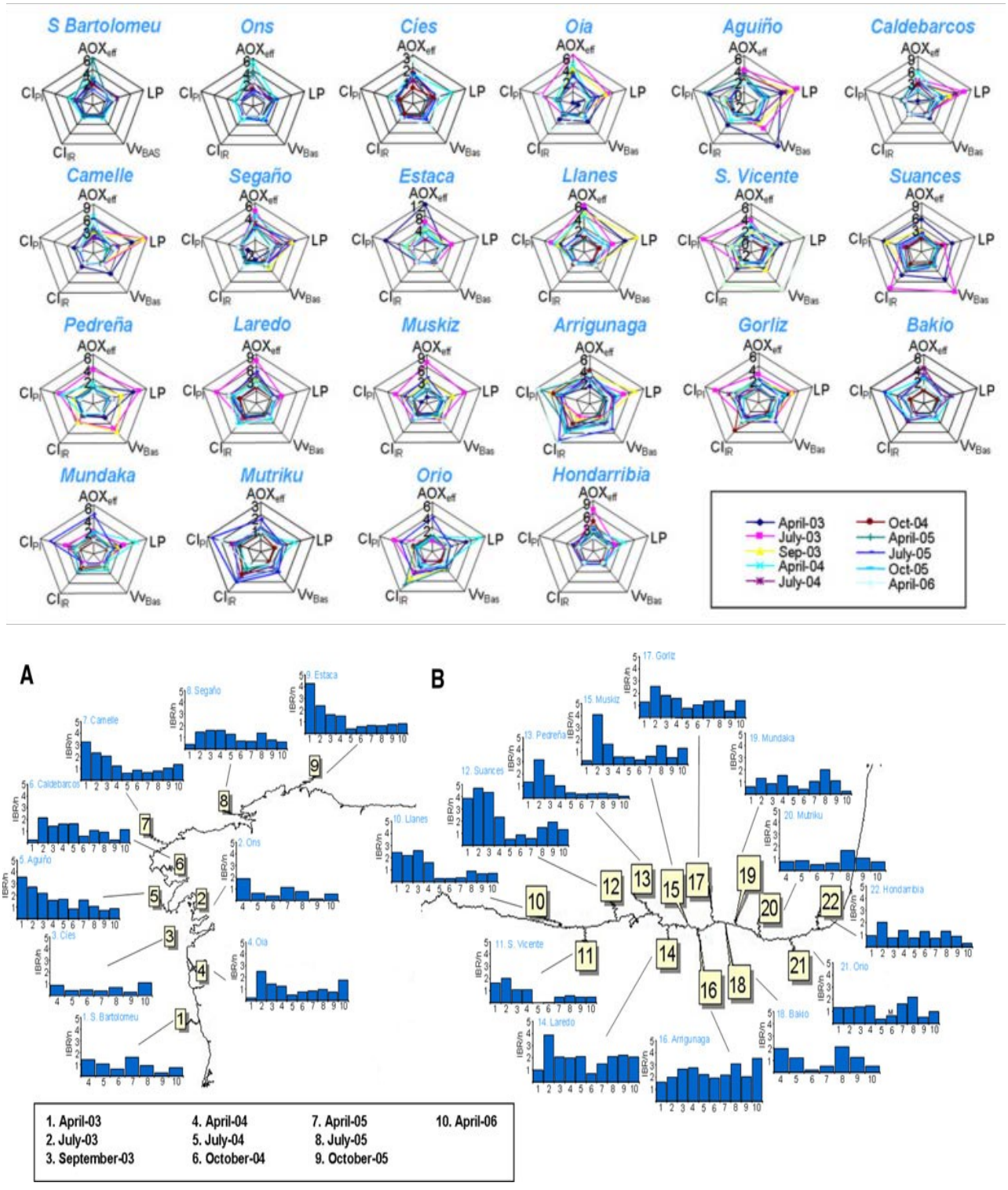


Fig. 6 Top: Star plots representing the five biomarkers (AOX_{eff}, LP, Vv_{BAS}, CI_{IR} and CI_{PI}) used to compute the IBR/n index that were measured in localities studied during the biological Mussel Watch programme carried out to monitor ecosystem health after POS in Galicia and Biscay Bay (2003–2006). Each of the five axes of the star plots represents the relative degree of response of one biomarker. Colour lines represent different samplings (legend). Bottom: IBR/n index (based on the same 5 biomarkers) in mussels *M. galloprovincialis* from Galicia (A) and the Bay of Biscay (B) after POS (Marigómez et al., 2013b)

Table 3. Quantitative biomarker indices (after Schettino et al., 2012**;** and Marigómez et al., 2013b).

Index	Reference	Selected Biomarkers	Species
Health Assessment Index (HAI)	Adams et al. (1993)	Histopathology, hematocrit, plasma proteins, parasitisation.	Fish
Bioeffect Assessment Index (BAI)	Broeg et al. (2005) Broeg and Lehtonen (2006)	LMS, INLA, MMCs, AcP, MFO, parasitisation.	Fish Mussel
Integrated Biomarker Response (IBR)	Beliaeff and Burgeot (2002)	AChE, GST, CAT, MT, LMS, MN, INLA.	Fish Mussel
Ecosystem health condition chart (EHCC)	RBU-Rep (1994) Díez (1996)	AOX_{eff}, AOX_{exp}, LMS, lysosomal enlargement, cell type replacement, MET, inflammatory responses, parasitisation.	Mussel
Health Status Index (HSI) Expert System	Dagnino et al. (2007)	LMS, LPF, INLA, CAT, Ca-ATPase, MFO, SOD, AChE, B[a]P-MO, MT, MN, SoS, peroxisome proliferation.	Mussel
Biomarker Response Index (BRI)	Hagger et al. (2008)	LMS, AChE, MT, MN, antioxidant status, cell viability, heart rate, feeding rate, immune function.	Mussel
Integrative Biomarker Index (IBI)	Marigomez et al. (2013a)	AOX_{eff}, LMS, lysosomal enlargement, cell type replacement, inflammatory responses, parasitisation.	Mussel

4. Biological effects assessment and monitoring

Conventional monitoring studies were essentially focused upon chemical data, whilst the biological effects were often used just as a complement. During the last years, however, there has been an increasing trend to focus upon the biological effects with chemical data accompanying. Thus, the OSPAR Co-ordinated Environmental Monitoring Programme CEMP (OSPAR Commission, 2013) focuses upon the concentrations of contaminants in sediment and biota, and upon their biological effects. This shift has been due to several reasons:

a) measured contaminants are limited to some few 30 compounds whilst 1000's are released by human activities into the environment, including new emerging pollutants for which chemical analytical methods are not available;

b) the concentrations of contaminants give no or limited information on their consequences for the ecosystem health status;

c) biological effects may occur below the detection limits of chemistry;

d) measurement of contaminants in sediment, water and biota can be transient and does not necessarily reveal a biologically effective exposure;

e) organisms' response will give indication of contaminant exposure and bioavailability;

f) unlike chemical data, the biological responses integrate the effects of mixtures, time-trends and seasonal variations and the influence of confounding factors (temperature, salinity); and

g) an increasing variety of cost-effective and reliable tools is available to detect alterations in the health status of marine organisms.

Multidisciplinary approaches are required in pollution biomonitoring programme to assess the chemical, biological and toxicological impact of complex mixtures of stressors in different environmental matrices, i.e., water, sediments and biota (Viarengo et al., 2007). The OSPAR guidelines for contaminant specific or general monitoring (JAMP 1998a) contain advice on the appropriate combinations of chemical and biological effects measurements in integrated monitoring programmes of using fish and shellfish (mussels and oysters) as sentinel organisms. These sentinel species can provide integrated and relevant information on the types, amounts, availability, and effects of environmental contaminants (Basu et al., 2007). For an organism to be considered a key sentinel species, it must satisfy certain requirements: (a) widespread distribution, (b) high trophic status, (c) ability to bioaccumulate pollutants, (d) suitability to be maintained and studied in captivity, (e) easy to be captured in sufficient numbers, (f) restricted home range, (g) well known biology, and (h) sensitivity to pollutants (Basu et al., 2007; Beeby, 2001; Fox, 2001; Masson et al., 2010). However, in sentinel species assimilation and excretion rates of pollutants and their biological effects are subjected to biological determinants (e.g.: age/size, season, gender, physiological and nutritional status, and population/genetic traits) as well as to the duration and/or level of exposure (Beeby, 2001; Phillips and Rainbow, 1994; Phillips and Segar, 1986).

4.1. Biomarkers in biological effects assessment

The OSPAR guidelines for biological effects techniques is since its commencements reviewed based on ICES advice (i.e., Fig. 7; Table 4; ICES, 2011; 2012; 2013; OSPAR Commission, 2013). These documents standardise reference methods for biomarkers and include assessment criteria/levels for various techniques (e.g., fish disease index; OSPAR Commission, 2013). Some biomarkers (PAH metabolites in bile, and Cyt P4501A activity or metallothionein levels) indicate contaminant linked sublethal responses but cannot with current knowledge be used to predict adverse effects on individual health (Law et al., 2010). Other biomarkers (lysosomal

membrane stability, oxidative stress, DNA damage and histopathology) constitute sublethal responses that are not necessarily linked to the action of pollutants; adverse health effects, however, can be predicted because the biological mechanisms underneath are known (Table 2).

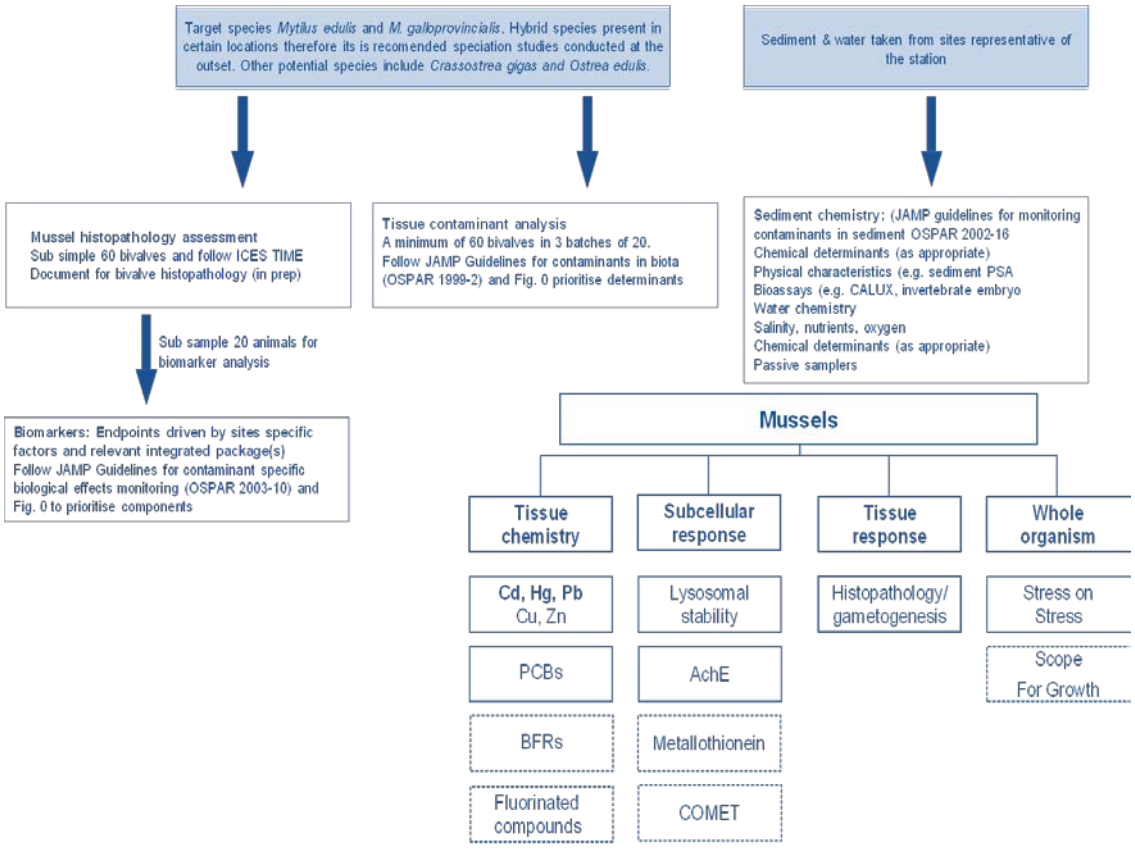


Fig.7. Overview of methods to be included in an integrated biomonitoring programme for selected bivalve species: solid lines - core methods; broken lines - additional methods (ICES, 2011).

On the other hand, biological effects monitoring based on the biomarker approach is not commonly carried out in developing countries, as the monitoring capacities and facilities can be far away from those required to conduct sophisticated and most-advanced biomarker-based monitoring using fish and molluscs as sentinels (Van Lavieren et al., 2011). Nevertheless, the application of biomarkers is not necessarily inhibited by monetary and logistic constraints because effortless biomarkers can provide basic knowledge on animal health and water quality and are technically achievable everywhere; the toolbox may include histopathological analyses and responses easy to measure at population and individual level (Blaise et al., 2016). For instance, Blaise et al., (2016) proposed three simple physiological biomarkers, quantifiable in bivalves (SoS response, condition index and growth index), which are reliable indicators of ecosystem health disturbance.

Table 4. Biomarkers recommended by ICES (2006; 2011) for international biomonitoring programs and their biological significance (after Garrigues et al., 2003). OJ: OSPAR JAMP; H: HELCOM; M: MED POL. F: fish, B: bivalve, G: Gastropod, MXR/MDR: multi xenobiotic resistance, multi drug resistance, AchE: acetylcholinesterase, MT: matallothionein, PAH: polycyclic aromatic hydrocarbon, PCB: polychlorinated biphenyl, EROD: ethoxyresorufin-O-deethylase, ALA-D: δ-amino levulinic acid dehydratase, VTG: vitellogenin.

Biomarkers	Issues addressed	Biological significance	Organism	Recommended by
Lysosomal membrane stability	Reduced lysosomal membrane stability due to a wide variety of xenobiotic contaminants and metals leading to a release of hydrolytic enzymes into the cytosol	Index of cellular damage, predictor of pathology	F, B	OJ, H, M
MXR/MDR	Activation of MXR pump to expel small hydrophobic xenobiotic from the cell	Index of organic xenobiotic exposure	B	OJ
AchE activity	Inhibition of the nervous transmission due to the inactivation of AchE by organophosphorous and carbamate pesticides	Index of neurotoxicity	F, B	OJ, H, M
MT determination	Induction of MT by certain metals (Zn, Cu, Cd, Hg) to bind metal in the cytosol	Index of metal exposure, disturbance of Cu and Zn metabolism and oxidative stress	F, B	OJ, H, M
Mussel digestive gland histopathology	Perturbation in the digestive system produced by contaminants and other stressors	Index of general stress	B	OJ
Imposex	Alteration of reproduction induced by hormone disruption	Index of exposure to organotin compounds	G	OJ
PAH bile metabolites	Measure of PAH metabolites	Index of exposure and metabolism of PAHs	F	OJ, H
EROD activity	Induction of enzymes detoxifying PAHs, planar PCBs and dioxins	Index of organic xenobiotic exposure	F	OJ, H, M
ALA-D	Inhibition of the enzyme for the synthesis of haemoglobin	Index of organic xenobiotic exposure	F	OJ
DNA adducts	DNA damage induced by genotoxic compounds	Index of genotoxic effects, predictor of pathology	F	OJ, H, M
External visible fish diseases	External alterations produced by pathogens and a wide variety of organic contaminants and metals	Index of general stress	F	OJ, H
Liver histopathology	Alteration of liver function produced by exposure to a wide variety of organic contaminants and metals	Index of general stress	F	OJ, H
Intersex	Alteration of reproduction induced by hormone disruption	Index of exposure to endocrine disruptors	F	OJ, H
VTG induction	Feminization of male fish and reproductive impairment	Index of exposure to estrogenic substances	F	OJ

4.2. Stress-on-Stress (SoS) response

It well known that contaminant exposure may alter the ability of organisms to survive environmental stress. (Viarengo et al., 1995; Pampanin et al., 2005; Nesto et al., 2007; Davies and Vethaak, 2012). The reduction of survival in air, or stress on stress (SoS), is a simple, low cost, organism level response and can show pollutant induced alterations in an organism's physiology that render the animal more sensitive to further environmental changes (Hellou and Law, 2003; Davies and Vethaak, 2012;).

SOS is defined as a test and it measures how long mussels at different conditions survive when are removed from water and exposed to air (Eertman et al., 1993; Smaal et al., 1991; Viarengo et al., 1995). According to Viarengo et al., (1995) mortality in air would presumably occur more rapidly in pollutant pre-stressed animals than in control animals.

This method has been applied routinely to pollutants exposed mussels in laboratory studies (Eertman et al., 1993; Viarengo et al., 1995) and mussels collected in national monitoring from polluted environments and along pollution gradients (Hellou and Law, 2003; Davies and Vethaak, 2012) . Showing their applicability of anoxic/aerial survival as an early warning indicator of contaminant induced stress. The effects of xenobiotics, including heavy metals, organometals, organics, and contaminated field sediments, on survival in air in invertebrates have been demonstrated (de Zwaan and Eertman, 1996). However, there are several research works in where this test did not demonstrate a strong correlation between stressed mussels and their survival to air (Eertman et al., 1996; Nicholson, 2003; Nesto et al., 2007).

In general, several factors can be involved in the alteration of SoS; these can be abiotic such as temperature, humidity, intertidal and subtidal zone, acclimatization, seasonal variation and pollutants (Eertman et al., 1993; Sokolova et al., 2012) or biotic such as the body size, age, reproductive cycle, sexual maturity, sex, food availability and pathologies (Eertman et al., 1993; de Zwaan and Eertman, 1996; Hellou and Law, 2003; Sokolova et al., 2012).

4.3. Biometry and flesh condition

The changes in normal condition patterns are generally regarded as useful measurements of the nutritive status of bivalves (Crosby and Gale, 1990). These condition indices may be used to describe physiological state of mollusc, a parameter of economic relevance reflecting the ecophysiological conditions and the health of animals (Orban et al., 2002; Strogyloudi et al., 2012).

Biometrical parameters and Flesh Condition Index (FCI) are employed in the field of toxicology to monitoring the environmental stress of pollutants and disease in marine organism (Soto et al., 2000; Andral et al., 2004; Strogyloudi et al., 2012; Gomiero et al., 2015). The condition index also provides some clues as to the levels of chemical contamination, especially in the case of trace metals (Andral et al., 2004). Mussels exposed to polluted areas spend part of their energy budget in detoxification processes to maintain homeostasis to the detriment of body growth and gonadal production (Gomiero et al., 2015). Andral et al., (2004) proposed a linear model explaining the relationship of FCI and metal concentrations in mussel whole soft tissue: tissue concentrations are inversely proportional to the condition index (Ivanković et al., 2005; Gorbi et al., 2008) and the other hand for organic compounds, tissue concentration is proportional to the condition index although stations located in contaminated areas did not obey this rule due to their consistently higher results (Andral et al., 2004).

The decrease of biometry and FCI values, also are correlated to bioavailability of nutrients, occurrence of phytoplankton blooms and seawater temperature which are known to influence, directly or indirectly, bivalves physiology (Acarli et al., 2011; Brenner et al., 2014). These alterations are most pronounced during or after periods of reproduction when energy reserves are exhausted, easily leading to misinterpretation of the biomarkers for nutritional status such as the condition index or glycogen.

High concentrations of metabolic end products, such as lipofuscins and neutral lipids have been observed in the submerged mussels due the permanent feeding mode (leading to higher ingestion of food and associated chemical contaminants) (Brenner et al., 2014), but also when bivalves are exposed to pollutants (Kagley et al., 2003).

Seasonal pattern of FCI and lipid content in mussels were observed by Strogyloudi et al., (2012): elevated values during the warm period of the year followed by an additional peak in

autumn to minimum levels in January and a subsequent gradual increase through spring, presenting their best physiological condition from April to July.

4.4. Sex and reproduction

The gonad development analysis of bivalves play an essential role in marine ecosystem health assessment which face to exposure to environmental stress (Ortiz-Zarragoitia et al., 2011; Garmendia et al., 2011a; Cuevas et al., 2015). The collection of these data provide additional information on the health and physiology of the mussel (Cajaraville et al., 2006; ICES, 2011).

Reproductive condition parameters include reproductive markers such as adipogranular cells, gonadal apoptosis, atresia, hermaphroditism and intersex, it have shown the effect to exposure to organic contaminants and metal (Bignell et al., 2008, 2011; Ortiz-Zarragoitia and Cajaraville, 2010; Ortiz-Zarragoitia et al., 2011) . An increased levels of DNA damage has been detected in indigenous mussels after oil spills and in mussels exposed to oil in laboratory experiments (Laffon et al., 2006). Disruption of gonad development at early stage may affect gamete quality, causing alteration as deformities and growth in bivalves, threatening the survival of future generations (Baussant et al., 2011). According to Ortiz-Zarragoitia and Cajaraville, (2010) the oocyte atresia is more frequent within advanced maturation stages. However, high prevalence of oocyte atresia and necrosis in mussels were associated to pollutant exposure (Ortiz-Zarragoitia and Cajaraville, 2010; Puy-Azurmendi et al., 2010).

Some authors have found incidence of hermaphroditism in bivalves, which suggest associations with endocrine disruptors as organotins, estrogenic hormones, bisphenol, alkylphenol and phthalates from urban and industrial sources (Montenegro et al., 2010; Puy-Azurmendi et al., 2010). So, one of the main drawbacks that face an environmental pollution biomonitoring is the presence of potential confounding factors that may lead to misinterpretation of the toxicological findings (Garmendia et al., 2010; Costa et al., 2013). Parasites burden (Cuevas et al., 2015) and temperature can alter the timing of reproduction as well as in reproductive success, recruitment and growth performance (Pörtner, 2002; Suárez et al., 2005; Lemaire et al., 2006; Okaniwa et al., 2010). Moreover, elevated temperatures can induce earlier start of spawning (Philippart et al., 2003). Hence thermal stress has serious implications on the total reproductive effort and mortality of individuals (Fearman and Moltschaniwskyj, 2010; Múgica et al., 2015) altering the ‘good’ health status of bivalves and marine ecosystem.

4.5. Histopathology and tissue-level biomarkers

Histopathology is essential in understanding the role of sublethal effects of environmental stressors at population level (Stentiford and Feist, 2005). Histopathology of digestive gland in bivalves provides valuable information concerning changes in cellular and sub-cellular structures of an organ or tissue much earlier than the external manifestations (Kim and Powell, 2006; Aarab et al., 2008; Kim et al., 2008; Bignell et al., 2011; Garmendia et al., 2011b).

High prevalence of tubular dilation or atrophy, brown cell aggregates, lipid vacuoles in basophilic cells and eosinophilic bodies in the digestive cells has been related to the presence of PAHs compounds (Moore and Clarke, 1982; Aarab et al., 2008). Augmented incidence of granulocytomas and vacuolisation have been reported in the digestive gland of clams and mussels exposed to heavy metals, pesticides and PAHs (Wolfe, 1992, Wedderburn et al., 2000; Kim et al., 2008; Khan et al., 2015).

Inflammatory responses as hemocytes are responsible of immune system in molluscs their raised incidence represent a response to detoxification and removes xenobiotics from the hemolymph or tissues and thereby keeps the concentrations in the blood and tissues below toxic levels (Marigómez et al., 1990; Soto et al., 1996). Also hemocytic infiltration constitutes a repair process following tissue damage (DesVoigne and Sparks, 1968), also pathological effects may be responsible for this immune response (Couch, 1984; Lee et al., 2001). Severe inflammations accompanied by an increased prevalence of granulocytomas within the connective tissues were observed in mussels with infestation by *Marteilia sp.*, *Steinhausia mytilovum* or digenean metacercariae (Bignell et al., 2008), provoking an extensive destruction of digestive diverticula structure (Villalba et al., 1997).

Different kind of stressors, such as food availability, water temperature, salinity, tide ranges and pollutants can increase the susceptibility to parasites and diseases (Laird, 1961; Sabry et al., 2011; Costa and Costa, 2012; Höher et al., 2012). It has been observed that a decrease in water quality can affect the immunological response in aquatic organisms thus making them more susceptible to parasitic infection (Svärdh and Johannesson, 2002; Bignell et al., 2008; Lynch et al., 2014).

5. Caribbean mangrove-lined ecosystems

5.1. The Caribbean Sea region

The Caribbean Sea is a semi-enclosed basin of the western Atlantic Ocean, bounded by the coasts of Central and South America on two sides and by the Antilles island chain on the other two. The WCR has an area of about 2754000 km^2 and over 13500 km of coastline, and is home to 28 countries as well as 12 dependent territories (Table 4; Launche, 2008).

Table 4. States and Dependent Territories of the Wider Caribbean Region (after Launche, 2008).

States	Dependent Territories or Associated States
Parties to the Cartagena Convention : Antigua and Barbuda Barbados Belize Colombia Costa Rica Cuba Dominica Dominican Republic France Grenada Guatemala Jamaica Mexico Netherlands Nicaragua Panama Saint Kitts and Nevis Saint Lucia Saint Vincent and the Grenadines Trinidad and Tobago United Kingdom United States of America Venezuela **Out of the Cartagena Convention :** Bahamas Guyana Haiti Honduras Suriname	**Overseas territories of the United Kingdom:** Anguilla British Virgin Islands Cayman Islands Montserrat Turks and Caicos Islands **Three French overseas regions (departments):** Guadeloupe Martinique French Guiana (which includes 2 French overseas collectives—Saint Barthélemy and Saint Martin) **Two self-governing units of The Netherlands:** Aruba The Netherlands Antilles **One organized unincorporated U.S. Territory:** Virgin Islands **One U.S. Territory with Commonwealth Status:** Puerto Rico

The regional atmospheric seasonality is dominated by the position of the Inter-Tropical Convergence Zone (ITCZ) which controls wind and precipitation (Andrade, 2000; Poveda et al., 2006; Torres and Tsimplis, 2012). The surface wind field in the Caribbean is dominated by the Northern Trade Winds. A permanent and intense westward wind jet, known as Caribbean Low Level Jet (CLLJ), has its core in the center of the basin (~15ºN, 75ºW) and exhibits large spatial and temporal variability (Wang and Lee, 2007; Amador, 2008). Precipitation in the Caribbean is seasonal with a rainy season (May–November) and a dry one (December–April). A diminution in rainfall (July–August) is found during the middle of the rainy season and described as the mid-summer drought (Magaña et al., 1999). The midsummer drought modulates the precipitation over the entire Caribbean Sea with significant spatial variability in timing and strength, and has been attributed mainly to the intensification and expansion of the North Atlantic Subtropical High (Gamble and Curtis, 2008). Freshwater runoff is significant in the South Colombia Basin, where the two largest rivers which discharge directly into the Caribbean Sea have a mean annual water discharge of 7200±2000 m^3/s (Magdalena River) and 2700±700 m^3/s (Atrato River; Restrepo and Kjerfve, 2000a; 2000b). However, studies of freshwater budgets show water loss over the entire Caribbean basin caused by an excess of evaporation over precipitation (74 cm/year) not compensated by river discharge (Etter et al., 1987; Yoo and Carton, 1990).

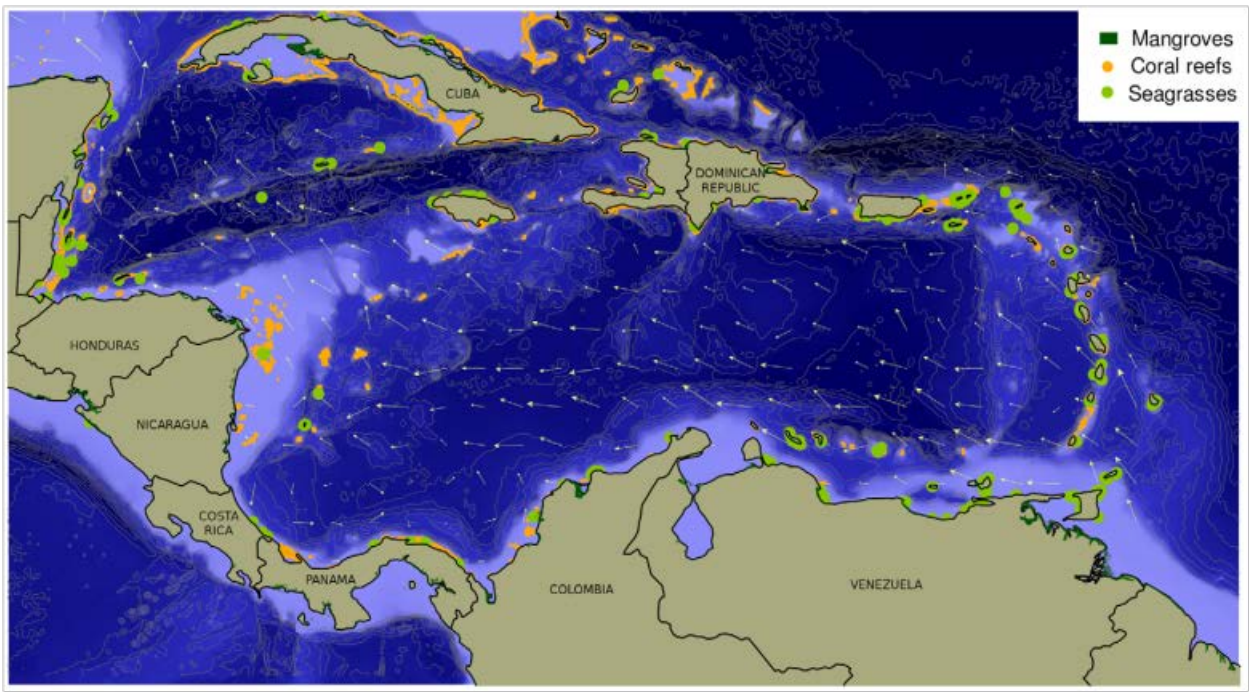


Fig. 8. Bathymetry, main currents, and ecosystems of the Caribbean Sea (Miloslavich et al., 2010).

The Caribbean Sea has an overall counterclockwise circulation (Fig. 8). The Caribbean Current enters the southeast corner of the basin through several passages of variable sill depth between the Lesser Antilles and, to a lesser extent, the Windward Passage, and slightly increases its velocity as it flows west-northwesterly into the Gulf of Mexico through the Yucatan Channel, where it forms the Gulf Stream (Gyory et al., 2008). Caribbean waters are mostly clear and warm (22–29 ºC), and the tidal range <0.4 m (Kinder et al., 1985). The water column is highly stratified in the upper 1200 m because of the sill depths of the Antilles Islands arc, which prevents the flow of deep water into the Caribbean Basin (Gordon, 1967).

The most characteristic ecosystems present in this region are mangroves (~11560 km^2), coral reefs (~26000 km^2) and seagrass beds (~66000 km^2; Fig. 8; Jackson, 1997; FAO, 2003; Burke and Maidens, 2004; Miloslavich et al., 2010). As discussed by Harborne et al., (2006) Caribbean coral reef habitats, seagrass beds and mangrove stands provide many important ecosystem goods and services such as coastal defence, sediment production, primary production, fisheries and the maintenance of high species diversity. Furthermore, all three systems often occur in close proximity and many physical and ecological processes transcend individual habitats. For example, estuarine mangroves trap riverine sediments that might otherwise discharge onto reefs and cause mortality through sedimentation.

5.2. Environmental quality status from WCR

In the Caribbean Sea and its watersheds (WCR), degradation of water and land resources is related to major declines in biodiversity, species extinction, mass mortality of organisms, and reduction of marine resource productivity (Rivera-Monroy et al. 2004). The WCR has fragile coastal ecosystems that are susceptible to environmental impacts, in part because of their oligotrophic conditions (Richards and Bohnsack 1990, Lapointe 1997) and their critical support of economic development.

According to Ocean Health Index the WCR have an overall index score of 65 in a scale from 0 to 100[2] (OHI, 2015). Nicaragua showed the lowest overall score (47) and Aruba, Antigua and

[2] - “100” means that the evaluated system has achieved its defined target (reference point), is sustainably delivering all of the specified benefits that it can; and appears likely to be able to continue doing so in the near Future.
- Intermediate scores mean that the optimal benefit is not being obtained and/or is not being obtained in a sustainable way.
- “0” means that global data were available, but the region either did not achieve any of the potential benefits or that the benefits it did obtain were not gained in a sustainable manner.

Barbuda the highest overall score (>80) in relation with the rest of localities from WCR (Table 5). Overall, more than 80% of countries from WCR are achieving optimal benefit from marine ecosystems and go on sustainable way (Table 5 and Fig. 9). Regarding the Clean Water goal (contamination by chemicals, eutrophication, human pathogens and trash) also a similar average score (64) was recorded at the WCR as a whole. Nevertheless, Guatemala, Haiti and Jamaica score for this goal was <50 whilst in the French Guiana it was >80 (Fig. 9).

In both cases indicates that we should improve polices of use and management, reducing the inflow of pressures toward natural resources.

Table 5. Global score of Ocean Health Index from Wider Caribbean Region calculated for 2016.

Territories from WCR	Rank	Overall score	Territories from WCR	Rank	Overall score
Anguilla	96	69	Guyana	155	63
Antigua and Barbuda	18	81	Haiti	193	56
Aruba	17	81	Honduras	147	65
Bahamas	39	76	Islands Montserrat	54	73
Barbados	198	56	Jamaica	188	58
Belize	114	68	Mexico	112	68
British Virgin Islands	83	70	Nicaragua	216	47
Cayman Island	32	77	Panama	150	64
Colombia	179	60	Puerto Rico and Virgin Islands	69	72
Costa Rica	178	60	Saint Kitts and Nevis	140	66
Cuba	161	62	Saint Lucia	182	60
Dominica	206	52	Saint Vincent and the Grenadines	194	56
Dominican Republic	79	70	Suriname	38	76
French Guiana	76	71	Trinidad and Tobago	63	72
Grenada	176	60	Turks and Caicos Islands	78	71
Guadeloupe and Martinique	73	71	Venezuela	167	61
Guatemala	173	61			

- The higher the score, the closer a region is to obtaining the maximal sustainable benefits possible with the given reference points.

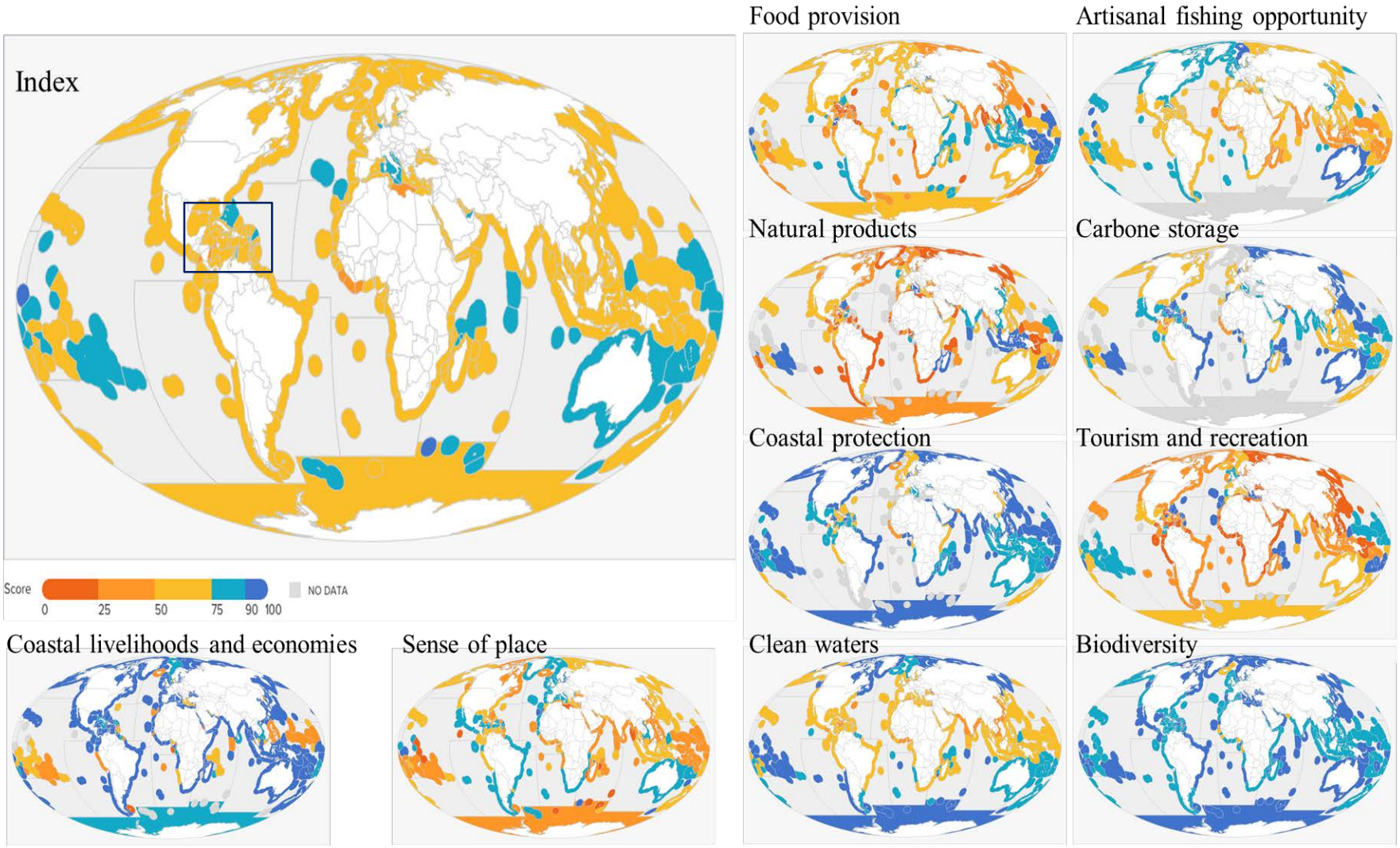


Fig. 13. Map of Ocean Health Index 2016 and individual goal scores per country. All waters within 171 exclusive economic zones (EEZs), that is, up to 200 nautical miles, were assessed and are represented on the map (Modified by http://www.oceanhealthindex.org). NA, not available.

Focal sources of metal contamination in the WCR are mining, industrial processes (smelters, oil refineries, chemical industry, shipyards), untreated sewage sludge and diffuse sources such as metal piping, traffic and combustion by-products from coal-burning power stations; being Cd, Hg, and Pb being the metallic pollutants of major concern (UNEP, 2006). At regional level, PAHs, are the result of burning of waste, burning of vegetation for clearing land, and crude oil production and transportation (UNEP, 2002; Fernandez et al., 2007). In the case of POPs such as OCPs, PCBs and PBDEs, the improper use and/or disposal of agrochemicals and industrial chemicals, combustion processes and release of by-products contributes to environmentally relevant levels of POPs (UNEP, 2006). It known that the use of agricultural pesticides has greatly increased during the last 30 years and DDT residues have become ubiquitous and constitute de major threatened for the ecosystem from WCR (Rawlins et al., 1998; Fernandez et al., 2007). PCBs have been used extensively since the 1930s in the WCR (e.g. in electrical transformers). Correspondingly, they have been detected in water, sediment, seafood and biota samples (Fernandez et al., 2007), although data and monitoring capacity are limited at regional scale for these POPs. Certainly, PCBs and PBDEs are contaminants distinctive of urban and industrialised areas; however, they have been found at increasing levels in developing countries, as a result of poor waste management practices and improper burning (UNEP, 2006). Nevertheless, with the exception of some pesticides, the monitoring of most of them relies on occasional analyses for research purposes or for impact assessment e.g. after accidental spills (UNEP, 2004; GEF-REPCar, 2011).

5.3. Mangrove ecosystems: concepts, services and threats

Ecologically, "mangroves" are defined as *an assemblage of tropical and semi-tropical trees and shrubs that inhabit the coastal intertidal zone* (Duke, 1992). Mangroves are highly complex transitional coastal ecosystems that include 0.7% of world's tropical and subtropical forests and provide a unique ecosystem that represent complex food webs with a strong relationship with neighbouring habitats (Giri et al., 2011). A mangrove community is composed of plant species whose special adaptations allow them to survive the variable flooding and salinity stress conditions imposed by the coastal environment. From a total of approximately 20 plant families containing mangrove species worldwide, only two, Pellicieraceae and Avicenniaceae, are comprised exclusively of mangroves. In the family Rhizphoraceae, for example, only four of its sixteen genera live in mangrove ecosystems (Duke, 1992).

According to Twilley, (1998) there are two types of classification systems for the mangrove forest, geomorphological and ecological (Fig. 9). Coastal settings can be catalogued as river deltas, river-dominated estuaries, lagoons, and oceanic islands, with varying sources of sediments from terrigenous runoff to carbonate deposits. These geomorphologically distinct landforms have local variations in topography and hydrology that result in the evolution of distinct ecological types of mangroves referred to as riverine, fringe, or interior mangroves (Lugo and Snedaker, 1974). Fringe forest borders protected shorelines, canals, and lagoons, and is inundated by daily tides. A riverine forest flanks the estuarine reaches of a river channel and is periodically flooded by nutrient-rich fresh and brackish water. Drainage depressions in the interior of mangrove areas harbor basin forests, characterized by stagnant or slow-flowing water. Scrub (syn.; dwarf) forests grow in areas where hydrology is restricted, resulting in conditions of high evaporation, high salinity, or low nutrient status (Twilley et al., 1998, Twilley and Rivera-Monroy, 2005, Twilley and Day, 2012). Mangrove forests are transitional coastal ecosystems among land and marine ecosystems and provide a unique ecosystem that represents complex food webs with a strong relationship with neighbouring habitats (Bosire et al., 2008; Nagelkerken et al., 2008; Giri et al., 2011).

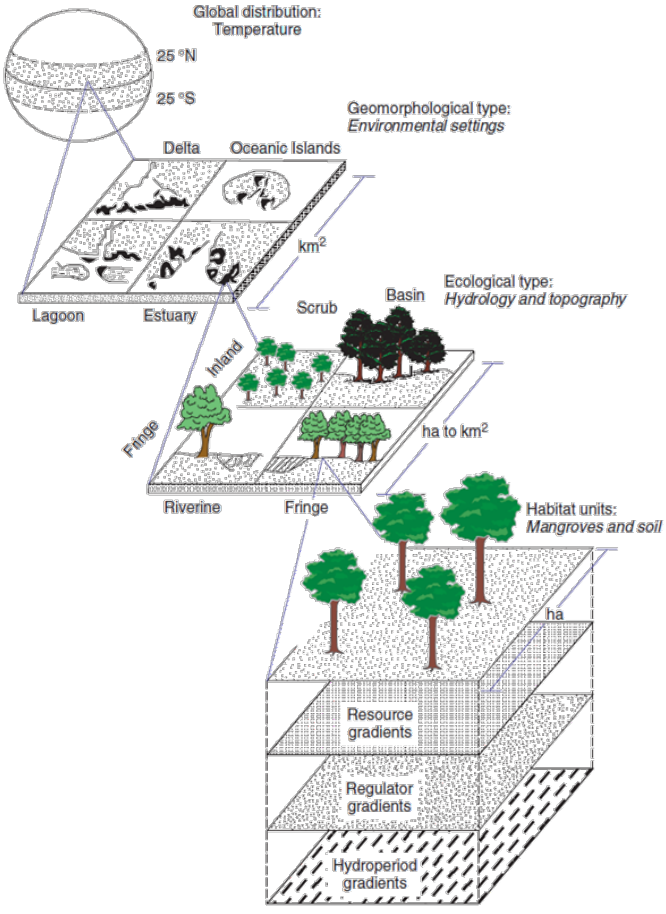


Fig. 9. Hierarchical classification system to describe patterns of mangrove structure and function based on global, geomorphological (regional), and ecological (local) factors that control the concentration of nutrient resources and regulators in soil along gradients from fringe to more interior locations from shore (Twilley and Day, 2012).

Mangrove-lined coastal systems are important because they are a natural resource for tourism, support fisheries (e.g., by providing fish nurseries), provide food supply, contribute to conservation of biological diversity, protect shoreline of storm, and purify wastes that enter coastal zones from land-based sources of pollution. Mangroves also act as bio-shields, important for protecting against coastal erosion (UNEP, 1994, Nagelkerken et al., 2008, Walter et al., 2008). Collectively, the ecosystem services provided by mangroves worldwide is estimated at 1.65 trillion dollars annually (Barner and Clevenger, 2014). These mangrove ecosystems and the services they provide are being severely threatened by anthropogenic changes.

Mangrove ecosystems and the services they provide are being severely threatened by anthropogenic changes. Some of the anthropogenic threats to mangroves include extraction for uses as timber, coastal pollution, reclamation of land for human use, and the effects of climate change on these ecosystems. Deforestation due to reclamation of coastal land is one of most pressing threats to mangroves, and recently, this reclamation has been attributed to development associated with tourism (Ellison and Farnsworth, 1996). In the last five decades, mangrove ecosystems worldwide have been severely impacted by a combination of natural and anthropogenic stressors (Lewis et al., 2011; Bayen, 2012). Trace metals, PAHs, PCBs, OCPs and other organic pollutants (anthropogenic origin) have been detected in mangrove ecosystems around the world (Bayen, 2012)

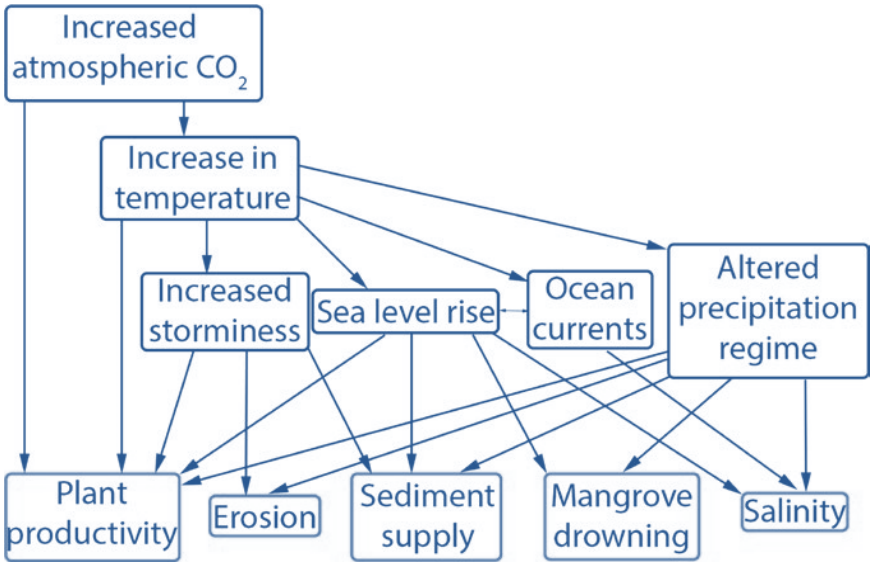


Fig. 10. Conceptual framework principal impacting factors of climate change and how they are likely to negatively influence mangrove communities (Ward et al., 2016).

5.3.1. Threats for mangrove ecosystems in the WCR

Mangrove-lined coastal systems, which are an important natural resource for tourism, fisheries and storm protection in the WCR, are vulnerable to land-based human activities (UNEP, 1994).

Oil pollution resulting from off-shore oil exploration and production, pipelines, tanker accidents, and intentional clearing of ship's ballast tanks affects mangroves throughout the WCR (Rodriguez, 1981; Ellison and Farnsworth, 1996; UNEP, 2002; Fernandez et al., 2007). In 1979, the Atlantic Empress oil spill released 280000 tonnes of oil 10 miles off Tobago. Most of the oil was burned towards open seas and no significant shore pollution was recorded on the nearest islands, albeit no impact study was carried out. In order to give an idea of the relevance of oil pollution in the WCR it is worth noting that in the 1980's there were around 30 oil spills that impacted mangroves in the region (i.e., Refineria Panamá spill, Galeta island, 1986). Moreover, spills had occured much before since the early 1960's: i.e., Argea Prima (Puerto Rico, 1962), Santa Augusta (St Corix US Virgin islands, 1971); Zoe Colocotronis spill (Puerto Rico, 1973). In the majority of the cases, oil accumulated among mangrove roots, killing large numbers of invertebrates, fish, and turtles and hydrocarbon residues were still detectable in the mangrove soil and sediments two decades after the spills (Ellison and Farnsworth, 1996). The best available data on effects of oil spills on mangrove flora, fauna, and ecosystem processes come from studies conducted at the Smithsonian Tropical Research Institute following the 1986 spill at Galeta, Panama. Continued dependency of most Caribbean states on imported oil for energy needs, the existence of large refinery and trans-shipment complexes throughout the region, and the huge amounts of oil transported annually through the Panama's Canal strongly suggest that petroleum will continue to be the major pollutant impacting Caribbean mangroves and mangrove-lined systems (Ellison and Farnsworth, 1996).

Mining, industrial processes, untreated sewage sludge and diffuse sources (metal piping, traffic and combustion by-products) are the main anthopogenic sources of metal pollution in the WCR; and Cd, Hg, and Pb are major metallic pollutants (UNEP, 2006). In addition to these sources, the disposal of agrochemicals and industrial chemical also contributes with POPs (UNEP, 2006). Thus, persistent pesticides constitute a major environmental concern in the WCR, (Rawlins et al., 1998; Fernandez et al., 2007). PCBs have been detected in water, sediment, seafood and biota samples (Fernandez et al., 2007), although data and monitoring capacity are limited at

regional scale. In view of their environmental relevance at regional scale, POPs ranked second in the priority rankings of Caribbean contaminant categories (GESAMP, 2001). Rodriguez (1981) identified four locally or regionally important pollutants: Hg (Colombia, Cuba); mine tailings and compounds (e.g., sodium hydroxide) associated with bauxite mines (Cuba, Jamaica, and the US Virgin Islands); sewage (Cuba, Haiti, Jamaica, Puerto Rico, Venezuela); and urban runoff (Cuba, Dominican Republic). Later on, pesticide runoff, heavy metals from industrial activities, and domestic sewage in Belize, Colombia, Cuba, Jamaica, Nicaragua, Puerto Rico, and Trinidad & Tobago; solid waste dumping throughout the Lesser Antilles, Jamaica, and Trinidad & Tobago, and dredge spoil dumping in Antigua again were identified as pollutant hazards in Caribbean mangrove ecosystems (Ellison and Farnsworth, 1996). The increasing pace of industrial and tourist development, and the continued lack of sewage treatment throughout the Caribbean, can be related to the increasing impact of chemical pollutants into mangrove ecosystems in the WCR.

5.4. Mangrove-lined coastal ecosystems in Nicaragua and Colombia

Nicaragua. The mangrove forest from Nicaragua extends throughout 69050 ha and represents one of the main coastal ecosystems, which is approximately equally distributed between the Pacific and the Caribbean coasts (Jiménez, 1999; FAO, 2007). Nicaraguan mangroves are influenced by 21 watersheds, 13 of them drain in the Caribbean and 8 in the Pacific Coast (González, 1997). Thus, the Caribbean coastal area is a major sink for contaminants transported along the watersheds as it receives the 90% of the Nicaragua's territory catchment (UNEP, 2002). Moreover, maritime traffic and ports, urban settlements, shellfish farming and intensive agriculture are also potential sources of pollutants in the Pacific coast (Spongberg and Davies, 1998; Gener, 2009; Spongberg and Witter, 2008, Vargas et al., 2015).

Mangrove-lined coastal ecosystems are basically lagoons in the Caribbean Sea and estuaries in the Pacific coast. Thus, intertidal mangrove swamps are characteristics of the Pacific coast estuaries such as e.g. Corinto, and the Natural Reserve Padre Ramos (UICN/ORMA, 2001). These mangroves[3], which are dominated by seawater, are structurally less developed than that further south as a result of its orientation within the dry tropical climate zone. Also there is lower annual rainfall, than further south of the transition line, ranging from 1300 mm in Nicaragua to 2000 mm

[3] https://www.worldwildlife.org/ecoregions/nt1434

in Costa Rica, but mostly falling during the short wet season resulting in a longer dry season from December to April. Annual temperature fluctuates between 25°C and 27°C (Polanía and Mainardi, 1993). In general, the river basins that drain into this ecoregion are of high relief, therefore seasonally intense rainfall and highly erodable soils make them prone to erosion caused the removal of vegetation for agricultural practices, timber, fuelwood and livestock grazing. Major threats include the pressure to convert mangroves to agricultural uses, shrimp culture and salt production, as well as urban encroachment, runoff of pesticide residues and eutrophication (Polanía, 1993; Ramsar, 1999).

In the Nicaraguan Caribbean coast, mangroves are sparse are primarily found in estuarine lagoons and small patches at river mouths and are dominated by freshwater (Tomlinson, 1986). The sparseness of mangroves is a result of the high inflow of freshwaters to the coastline ocean zone. Among the highest rates of rainfall in the world, this region receives over 6 m a year. Peak rainfall occurs in the warmest months between May and September. A relatively dry season occurs from January to April, which coincides with stronger trade winds. Tides are semi-diurnal and have a range of less than 0.5 m. SST is on average around 29-30ºC and salinity can be as low as <2‰ in the rainy season. The habitats include mixed rainforest, wooded swamps, coastal wetlands, estuarine lagoons, sandy beaches, sea grasses and coral reefs. This coastal area generally consists of low alluvial floodplain, in which there is a network of black-water canals and creeks. In between are beaches that are important nesting areas for endangered sea turtles that feed in the sea grass beds and visit mangrove areas[4]. Deforestation in the upper watershed has resulted in drainage and sedimentation problems. Other threats include gold mining, sewage contamination, runoff of agricultural chemicals, and erosion (Dumailo, 2003; MARENA, 2010). The most important lagoons in the Nicaraguan Caribbean coast are Bluefields and Pearl Lagoon. Bluefields receives water load from the Escondido River basin and where corn, banana, sugarcane, oil palm and coconut intensive agriculture has promoted the elevated application of pesticides for the last 50 years (GEF-REPCar, 2011; Ebanks-Mongalo et al., 2013). Thus, dieldrin, HCHs, DDTs, chlorpyrifos, and terbufos were reported in water, sediment and mangrove cupped oysters from Bluefields (GEF-REPCar, 2011; Ebanks-Mongalo et al., 2013). Pollution monitoring in Nicaragua has been sporadically carried out by academics and research groups (GEF-REPCar, 2011; Ebanks-Mongalo et al., 2013), as well as by international organisations such as the International Mussel

[4] https://www.worldwildlife.org/ecoregions/nt1431

Watch and the UNEP Caribbean Environment Programme (Farrington and Tripp, 1995; Siung-Chang, 1997; UNEP, 2006).

Colombia. The Colombian Caribbean coast is located in the Department of Magdalena encompassing the Gulfo de Urabá then east to just past the Sierra Nevada de Santa Marta at the base of the Guayjira Peninsula. The region is particularly arid with an average isomegathermic climate of 27°C, a notably seasonal pattern of precipitation of 400-760 mm and high annual evapotranspiration of 1400 mm; three times higher than precipitation (Sánchez-Páez et al., 1997). The latitudinal movement of the intertropical conversion front defines the two rainy seasons, which are from March to May and from October to November, representing 70% of total annual volume. Two dry seasons lie between these rainy season months[5]. SST ranges 29-33ºC and salinity 15-37‰. Thus, mangrove species in general have developed special adaptations to tolerate tidal flooding and high salinity. Hydrodynamic processes are governed by the Magdalena River, which has an effect on the western section of the region and by the rivers on the western slope of the Sierra Nevada de Santa Marta, which exclusively feed the Ciénaga Grande. From an ecological and socioeconomic perspective, this region holds the most degraded estuarine complex in Colombia. Decline in mangrove forest during the period between 1956-1987 was 40.6%; and in 1995 reached 63.8% of the total alive in 1956 (Gónima et al., 1998), which has created effects such as the loss of habitats for a large number of species of fish, birds and bentonic organisms. The diversion of water by building channels and dikes to prevent flooding on the major rivers as well as the resulting silting of water flow due to the high loads of sediment from erosion (Gónima et al., 1998). The enrichment of masses of water with organic matter from the rivers, from nearby human population centers and from chemical fertilizers (eutrophication), creating anoxic conditions leading to the death of massive numbers of fish and other organisms (Hernández and Márquez, 1991; Mancera and Vidal, 1994). There are also locally relevant environmental problems. In Cartagena Bay, mangle islet and islands are offshore the Cartagena Port and the industrial zone of Mamonal (oil refineries, petrochemicals, and asphalt, cement and smelting plants); and receives the direct impact of the Dique Channel; and dissolved and dispersed hydrocarbons have been reported in seawater, especially in the rainy season (Vivas-Aguas et al., 2014). This channel was open 5 centuries ago to connect the Magdalena River with the Cartagena Bay for navigation and constitutes a major source of sediments and chemical pollution in the

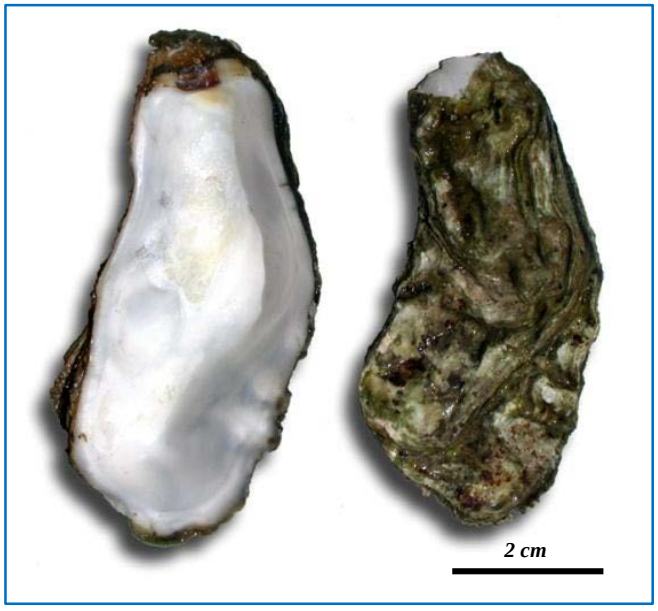
[5] https://www.worldwildlife.org/ecoregions/nt1417

Cartagena Bay (Vivas-Aguas et al., 2014). Isla Barú in Barbacoas Bay is also influenced by the Magdalena River to which it communicates by smaller channels via the Dique Channel since the 1950's (Gómez-Giraldo et al., 2009). In Santa Marta Bay, the Marina Santa Marta is subject to strong anthropogenic influence (Garcia et al., 2012). In the early 2000's, DDT and its metabolites, heptachlor, aldrin and HCHs were widespread pollutants in surface waters in Santa Marta Bay as the result of runoff from upstream plantations of coffee and banana, but in the recent decade these chemicals have been below detection limits in tributary rivers as result of regulatory restrictions for the use of these OCPs (Vivas-Aguas et al., 2014). Similarly, a marked reduction in the OCP levels in seawater and sediments have been recorded in Cartagena Bay and Barbacoas Bay during the last decade (Vivas-Aguas et al., 2010).

5.4.1. Relevant candidate species for pollution biomonitoring programmes

Four species of bivalves were proposed for pollution biomonitoring in mangrove-lined coastal ecosystems, two for Caribbean coast (*Crassostrea rhizophorae* and *Polymesoda arctata*) and other two for Pacific coast from Nicaragua (*Anadara tuberculosa* and *Larkinia grandis*). Three of them (*C. rhizophorae, A. tuberculosa* and *L.* grandis) were already used in the International Mussel Watch Programme carried out in Nicaragua in the early 1990's (Farrington and Tripp, 1995).

Mangrove cupped oysters, *C. rhizophorae*, were selected because they had been previously suggested as potential biomonitor and sentinel species for pollution assessment in mangrove-lined coastal ecosystems (Farrington and Tripp, 1995; Nascimento et al., 1998; Wallner-Kersanach et al., 2000; Rebelo et al., 2005; Silva et al., 2003; 2006; Valdez Domingos et al., 2007).



These oysters are (a) filter-feeding sessile organisms inhabiting both clean and polluted sites attached either to mangrove roots or to coastal rocks; (b) bioaccumulate pollutants such as metals, PAHs and POPs in their tissues (Farrington and Tripp, 1995; Rainbow, 2006; Van

Lavieren et al., 2011; Torres et al., 2012; Kanhai et al., 2014); (c) respond to environmental insult (Silva et al., 2003; Rebelo et al., 2005; Torres et al., 2012); (d) are widely distributed in tropical regions, e.g. from Caribbean to Southern Brazil; and (e) are easy to collect including a large range of size classes (Beeby, 2001; Fox, 2001; Basu et al., 2007; Valdez Domingos et al., 2007; Masson et al., 2010).

C. rhizophorae (Guilding, 1828) is distributed all over the Caribbean Sea, including the islands of the West Indies Archipelago such as Puerto Rico, Cuba, etc. The mangrove cupped oyster inhabits areas of relatively high salinity and commonly attaches itself to the roots of the *Rhizophora mangle* but also to rocks and/or forming oyster reefs in coastal lagoon. The shell is white, foliated and rough. The left valve is deeply indented and the right valve is flat, smooth and fits into the left valve. The shell interior is smooth and somewhat strongly purple coloured, the left shell margin being especially strongly coloured. The umbo is bent to the back. Mature individuals grow to more than 10 cm (Arakawa, 1990).

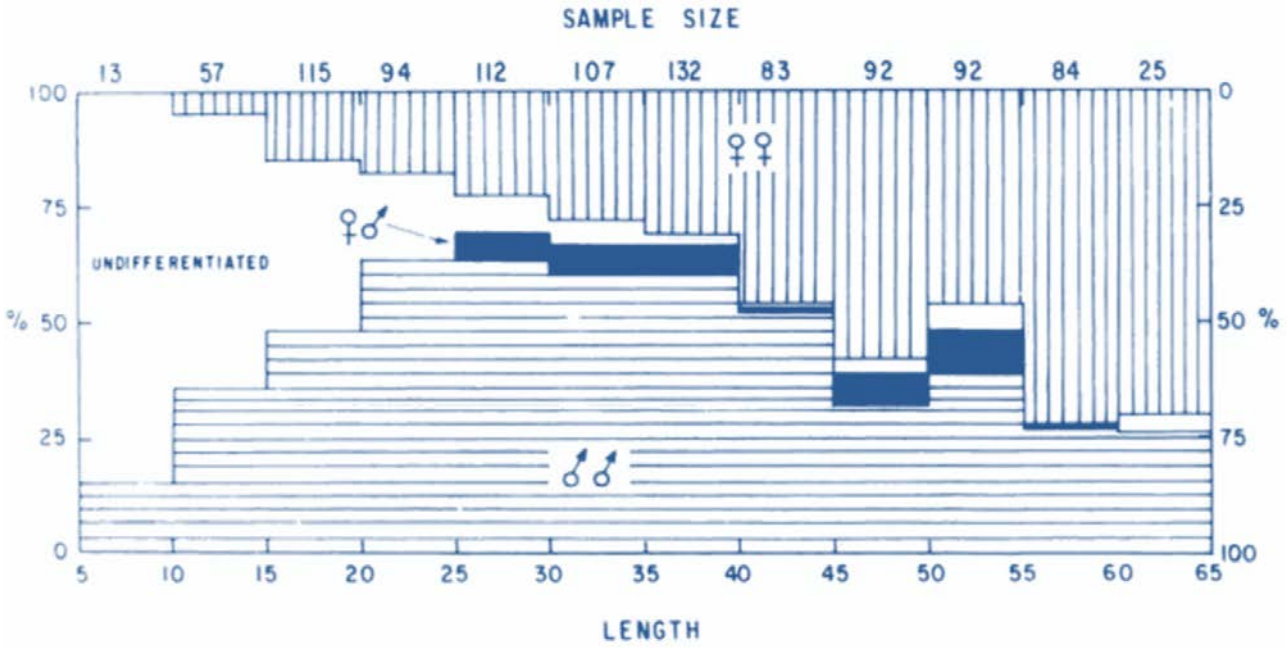
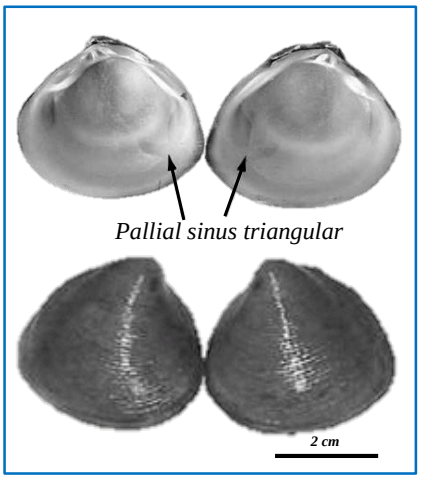


Fig. 11. Sex ratio of *C. rhizophorae* in each size class. The number of oyster in each size class es shown at the top of the diagram (Vélez, 1982). ♀, female; ♂, male.

Mangrove cupped oyster can reproduce continuously throughout the year. At least 2-3 spawning peaks per reproductive cycle have been reported in different regions, but the main one seems to be in the rainy season (Villarroel et al., 2004; Lenz and Boehs, 2011). This seasonal spawning may be associated with increase of temperature and salinity. In areas where water

temperature fluctuates between 23 to 29 ºC, there are bimodal peaks, lower from January to June and higher from July to December. However, in lagoons with temperature higher than 25 ºC, the spawning are continuous. In *C. rhizophorae*, females predominate and can reach up to 90% of the population although their prevalence may vary with size and season (Fig. 11; Vélez, 1982). Overall, *Crassostrea* oysters are protandrous hermaphrodites and sex ratio values can vary depending on the species, age, size and local conditions (Dove and O'Connor, 2012; Park et al., 2012). Sex appears to be determined by a single gene with a dominant male allele M and a recessive protandrous allele F, such that FF animals are protandrous and MF animals are permanent males (Powell et al., 2011).



Slender marsh clam, *P. arctata*, as above mentioned for the case of *C. rhizophorae*, also has the requisites to be a candidate biomonitor/sentinel species for pollution monitoring in mangrove-lined coastal systems in the Caribbean. They are filter-feeding, sessile organisms that bioaccumulate pollutants (Pérez-cruz et al., 2013; Leal et al., 2014; Vargas et al., 2015), respond to environmental insult, and present a wide distribution in the Caribbean. Thus it can be an alternative to mangrove cupped oysters in those sites where these are not sufficiently present, such as sandy areas of low salinity. However, data on pollutant bioaccumulation and its general biological (growth, reproductive cycle, affection parasitic, range of size, seasonality and biological responses to pollutants) are quite limited.

P. artacta (Deshayes, 1854; syn., *P. solida* (Deshayes, 1854)[6], according to Bouchet, 2015) is a benthic mollusc with a biogeographical range extending from Belize to the Orinoco River in Venezuela. It inhabits fine, sandy sediments of the low-to-medium salinity estuarine zone (high organic content, salinity: 3-20 ppt). It is identified by having V-shaped pallial sinus, with a long curved muscle insertion area, departing from its anterior side completely separate from the anterior adductor scar (Severeyn et al., 1994). According to Rueda and Urban (1998) *P. arctata* is monoecious combined with protogyny, and the sex-ratio changes with individual size-classes (Fig. 12). Juveniles start becoming males when they reach 10 mm shell height so that above 16 mm

[6] According to the World Register of Marine Species (http://www.marinespecies.org/index.php), the currently accepted name of *P. solida* is *Polymesoda arctata* (Deshayes, 1854), Bivalvia: Cyrenidae (Bouchet, 2015).

shell length no juvenile is observed; and at 21 mm shell height males start changing to hermaphrodite.

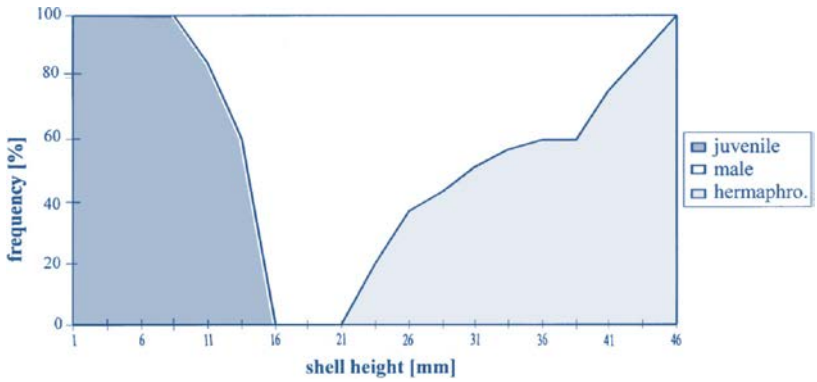


Fig.12. Gender distribution by shell height intervals in *P. arctata* (Rueda and Urban, 1998).

Mangrove cockles *Anadara tuberculosa* (pustulose ark) and *Larkinia grandis* (shell clam ark) from the Pacific Ocean, also have been used as biomonitor species to measure the tissues concentration of metals, PAHs and POPs in mangrove ecosystems (Farrington and Tripp, 1995; Ziarrusta et al., 2015; Vargas et al., 2015). Like *P. arctata*, these species are a potential biomonitor/sentinel in mangrove-lined ecosystems; however, study about bioaccumulation and pollutant biological effects are practically lacking. In contrast some general biology data are available because these species constitte a regionally relevant fishery that demands sustainable management (Gener et al., 2009; Lucero-Rincón et al., 2013; Silva-Benavides and Bonilla, 2015).

Pustulose arks, *A. tuberculosa* (GB Sowerby I, 1833) have a geographic distribution range from Laguna Ballena (Baja California Sur, Mexico) to Bahia de Tumbes (Perú; Mora-Sanchez, 1990; Cruz and Jimenez, 1994). *A. tuberculosa* inhabits muddy sediments (15 cm deep in the mud) among the aerial prop roots and under the canopies of the mangrove trees in mangrove swamps along the main-lands and islands of lagoons (Mackenzie, 2001; Stern-Pirlot and Wolff, 2006). This species has two valves of equal shape that are obliquely oval. Each valve has 34–37 radial ribs and a dark brown periostracum with bristles between the ribs (Mackenzie, 2001). The dorsal margin of the valves is angular. Reproduction is continuous throughout the year with two peaks of seasonal spawning (García-Domínguez et al., 2008; Lucero-Rincón et al., 2013).

The sex ratio reported is 1:1 (female:male) and no intersex was reported in localities from Costa Rica and Nicaragua (Gener et al., 2009; Silva-Benavides and Bonilla, 2015). However, in the Pacific coast from El Salvador, Colombia and Ecuador the sex ratio may vary throughout the year between 1.2:1 to 5.9:1 (female:male). Postulose ark is considered a protandric in which intersex cases have been reported (Flores and Lincadeo, 2010; Lucero-Rincón et al., 2013).

Shell clam arks, *L. grandis*, (Broderip & GB Sowerby I, 1829; syn., *Anadara grandis* (Broderip & GB Sowerby I, 1829)[7], according to Huber 2015) are found along an ample biogeographical distribution range from Bahia Magdalena (Baja California, Mexico) to Bahia de Tumbes (Perú; Mora Sanchez, 1990; Cruz and Jimenez, 1994). *L. grandis* lives in intertidal mudflats and some subtidal areas beyond the edges of mangrove swamps (Mackenzie, 2001). It grows to a size as large as >12 cm shell length. Its valves are almost square, and their length almost equals their height. The valves are convex with high umbos, and they have 26 ribs separated by deep interspaces. The shell has a dark periostracum. The blood is red. Unlike in neighbouring countries such as Honduras and El Salvador where it is commercially exploited (Galdámez et al., 2007), in Nicaragua this mangrove cockle (commonly known as "*casco de zburro*" -donkey's hoof-) is endangered by overharvest (Gener et al., 2009), except in the Padre Ramos reserve, where it is under community management for recovery (Manzanares L, personal comm.). The species show the same reproductive pattern than *A. tuberculosa*, with a continuous reproduction throughout the year with two peaks of seasonal spawning, being the highest prevalence of spawning (60-80%) from December to February and June, associated to the highest salinities of the year. Sex ratio has been reported to be 1:1.25 (female:male) (Fournier and de la Cruz, 1987; Galdámez et al., 2007).

[7] On the other hand, taxonomic name of *Anadara grandis* is not accepted according to the World Register of Marine Species (http://www.marinespecies.org/index.php), the currently accepted name is *Larkinia grandis* (Broderip & GB Sowerby I, 1829), Bivalvia: Arcidae (Huber, 2015).

6. References

Aarab N, Pampanin DM, Naevdal A, Oysaed KB, Gastaldi L, Bechmann RK (2008) Histopathology alterations and histochemistry measurements in mussel, *Mytilus edulis* collected offshore from an aluminium smelter industry (Norway). Mar Pollut Bull 57: 569–74.

Acarli S, Lok A, küçükdermenci A, Yildiz H, Serdar S (2011) Comparative growth, survival and condition index of flat oyster, *Ostrea edulis* (Linnaeus 1758) in Mersin Bay, Aegean Sea, Turkey. J Fac Vet Medicene, Kafkas Univ 17: 203–210.

Adams SM, Brown AM, Goede RW (1993) A quantitative health assessment index for rapid evaluation of fish condition in the field. Trans Am Fish Soc 122: 63–73.

Amador JA (2008) The Intra-Americas Sea low-level jet, Ann NY. Acad Sci 1146(1): 153–188.

Amiard JC, Amiard-Triquet C, Barka S, Pellerin J, Rainbow PS (2006) Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. Aquat Toxicol 76: 160–202.

Andersen JH, Axe P, Backer H, Carstensen J, Claussen U, Fleming-Lehtinen V, Järvinen M, Kaartokallio H, Knuuttila S, Korpinen S, Laamanen M, Lysiak-Pastuszak E, Martin G, Møhlenberg F, Murray C, Nausch G, Norkko A, Villnäs A (2011) Getting the measure of eutrophication in the Baltic Sea: towards improved assessment principles and methods. Biogeochemistry 106: 137–156.

Andersen JH, Murray C, Kaartokallio H, Axe P, Molvær J (2010) A simple method for confidence rating of eutrophication status assessments. Mar Pollut Bull 60: 919–924.

Andersen JH, Murray C, Larsen MM, Green N, Høgåsen T, Dahlgren E, Garnaga-Budrė G, Gustavson K, Haarich M, Kallenbach EMF, Mannio J, Strand J, Korpinen S (2016) Development and testing of a prototype tool for integrated assessment of chemical status in marine environments. Environ Monit Assess 188: 115.

Andersen JH, Dahl K, Göke C, Hartvig M, Murray C, Rindorf A, Skov H, Vinther M, Korpinen S (2014) Integrated assessment of marine biodiversity status using a prototype indicator-based assessment tool. Front Mar Sci. DOI org/10.3389/fmars.2014.00055.

Andrade C, Barton E (2000) Eddy development and motion in the Caribbean. J Geophys Res 105: 191–201.

Andral B, Stanisiere JY, Sauzade D, Damier E, Thebault H, Galgani F, Boissery P (2004) Monitoring chemical contamination levels in the Mediterranean based on the use of mussel caging. Mar Pollut Bull 49: 704–712.

Arakawa K (1990) Commercially important species of oysters in the world. Mar Behav Physiol 17: (1) 1-13.

Audry S, Schäfer J, Blanc G, Bossy C, Lavaux G (2004) Anthropogenic components of heavy metal (Cd, Zn, Cu, Pb) budgets in the Lot-Garonne fluvial system (France). Appl Geochemistry 19: 769–786.

Auninš A, Martin G (eds.) (2015). Biodiversity Assessment of MARMONI Project Areas. Project report, 175. Available online at: http://marmoni.balticseaportal.net/wp/project-outcomes/.

Barner C, Clevenger T (2014) Examining Anthropogenic Threats to Caribbean Mangrove Habitats. Available online at: http://digitalcommons.colby.edu/clas/2014/program/107/.

Barón E, Rudolph I, Chiang G, Barra R, Eljarrat E, Barceló D (2013) Occurrence and behavior of natural and anthropogenic (emerging and historical) halogenated compounds in marine biota from the Coast of Concepcion (Chile). Sci Total Environ 461: 258-264.

Basu N, Scheuhammer AM, Bursian SJ, Elliott J, Rouvinen-Watt K, Chan HM (2007) Mink as a sentinel species in environmental health. Environ Res 103: 130–44.

Baussant T, Ortiz-Zarragoitia M, Cajaraville MP, Bechmann RK, Taban IC, Sanni S (2011) Effects of chronic exposure to dispersed oil on selected reproductive processes in adult blue mussels (*Mytilus edulis*) and the consequences for the early life stages of their larvae. Mar Pollut Bull 62: 1437–45.

Bayen S (2012) Occurrence, bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems: A review. Environ Int 48: 84–101.

Beeby A (2001) What do sentinels stand for?. Environ Pollut 112: 285–298.

Belabed BE, Laffray X, Dhib A, Fertouna-Belakhal M, Turki S, Aleya L (2013) Factors contributing to heavy metal accumulation in sediments and in the intertidal mussel *Perna perna* in the Gulf of Annaba (Algeria). Mar Pollut Bull 74: 477–489.

Beliaeff B, Burgeot T (2002) Integrated biomarker response: a useful tool for ecological risk assessment. Environ Toxicol Chem 21: 1316–1322.

Bertram C, Rehdanz K (2013) On the environmental effectiveness of the EU Marine Strategy Framework Directive. Mar Policy 38: 25–40.

Bignell JP, Dodge MJ, Feist SW, Lyons B, Martin PD, Taylor NGH, Stone D, Travalent L, Stentiford GD (2008) Mussel histopathology : effects of season, disease and species. Aquat Biol 2: 1–15.

Bignell JP, Stentiford GD, Taylor NGH, Lyons BP (2011) Histopathology of mussels (*Mytilus* sp) from the Tamar estuary, UK. Mar Environ Res 72: 25–32.

Binelli A, Provini A, (2003) POPs in edible clams from different Italian and European markets and possible human health risk. Mar Pollut Bull 46: 879–886.

Bizarro C, Ros O, Vallejo A, Prieto A, Etxebarria N, Cajaraville MP, Ortiz-Zarragoitia M (2014) Intersex condition and molecular markers of endocrine disruption in relation with burdens of emerging pollutants in thicklip grey mullets (Chelon labrosus) from Basque estuaries (South-East Bay of Biscay). Mar Environ Res 96: 19–28.

Blaise C, Gagné F, Burgeot T (2016) Three simple biomarkers useful in conducting water quality assessments with bivalve

mollusks. Environ Sci Pollut R. DOI 10.1007/s11356-016-6908-6.

Bocquené G, Chantereau S, Clérendeau C, Beausir E, Ménard D, Raffin B, Minier C, Burgeot T, Leszkowicz AP, Narbonne J-F (2004) Biological effects of the ''Erika'' oil spill on the common mussel (*Mytilus edulis*). Aquat Living Resour 17: 309–316.

Borja A, Bricker SB, Dauer DM, Demetriades NT, Ferreira JG, Forbes AT, Hutchings P, Jia X, Kenchington R, Marques JC, Zhu C, (2008) Overview of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems worldwide. Mar Pollut Bull 56: 1519–1537.

Borja A, Elliott M (2013) Marine monitoring during an economic crisis: the cure is worse than the disease. Mar Pollut Bull 68: 1–3.

Borja Á, Elliott M, Andersen JH, Berg T, Carstensen J, Halpern BS, Heiskanen A-S, Korpinen S, Lowndes JSS, Martin G, Rodriguez-Ezpeleta N (2016) Overview of Integrative Assessment of Marine Systems: The Ecosystem Approach in Practice. Front Mar Sci 3: 68.

Borja A, Elliott M, Andersen JH, Cardoso AC, Carstensen J, Ferreira JG, Heiskanen AS, Marques JC, Neto JM, Teixeira H, Uusitalo L, Uyarra MC, Zampoukas N (2013) Good Environmental Status of marine ecosystems: What is it and how do we know when we have attained it?. Mar Pollut Bull 76: 16–27.

Borja A, Elliott M, Carstensen J, Heiskanen AS, van de Bund W (2010) Marine management - Towards an integrated implementation of the European Marine Strategy Framework and the Water Framework Directives. Mar Pollut Bull 60: 2175–2186.

Bosire JO, Dahdouh-Guebas F, Walton M, Crona BI, Lewis RR, Field C, Kairo JG, Koedam N (2008) Functionality of restored mangroves: A review. Aquat Bot 89: 251–259.

Bouchet P (2015) *Polymesoda arctata*. In, Mollusca Base. Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=596338.

Brenner M, Broeg K, Frickenhaus S, Buck BH, Koehler A (2014) Multi-biomarker approach using the blue mussel (*Mytilus edulis* L) to assess the quality of marine environments: Season and habitat-related impacts. Mar Environ Res 95: 13–27.

Broeg K, Lehtonen KK (2006) Indices for the assessment of environmental pollution of the Baltic Sea coasts: integrated assessment of a multi-biomarker approach. Mar Pollut Bull 53: 508–22.

Broeg K, Westernhagen H V, Zander S, Körting W, Koehler A, (2005) The "bioeffect assessment index" (BAI) A concept for the quantification of effects of marine pollution by an integrated biomarker approach. Mar Pollut Bull 50: 495–503.

Burke L, Maidens J (2004) Reefs at risk in the Caribbean Washington: World Resurces Institute 80 p.

Burkhard B, Müller F, Lill A (2008) Ecosystem Health Indicators. Encycl Ecol 1132–1138.

Bustamante J, Albisu A, Bartolomé L, Prieto A, Atutxa A, Arrasate S, Anakabe E, De Diego A, Usobiaga A, Zuloaga O (2010) Levels of polycyclic aromatic hydrocarbons, polychlorinated byphenyls, methylmercury and butyltins in the natural UNESCO reserve of the biosphere of Urdaibai (Bay of Biscay, Spain). Int J Environ Anal Chem 90: 722–736.

Cairns JJ (2013) Stress, Environmental In: Levin, SA (Ed), Encyclopedia of Biodiversity Watham, MA: Academic Press, pp 39–44.

Cajaraville MP, Bebianno MJ, Blasco J, Porte C, Sarasquete C, Viarengo A (2000) The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. Sci Total Environ 247: 295–311.

Cajaraville MP, Garmendia L, Orbea A, Werding R, Gómez-Mendikute A, Izagirre U, Soto M, Marigómez I (2006) Signs of recovery of mussels health two years after the Prestige oil spill. Mar Environ Res 62 Suppl: S337–341.

CBD (2000) Conventionon Biological Diversity United Nations. Available online at: www.cbdint/doc/legal/cbd-en.pdf.

COM (European Commission) (2010) Commission Decision 2010/477/EU of 1 September 2010 on criteria and methodological standards on good environmental status of marine waters. Off J Eur Union L232: 14−24

Conti ME, Stripeikis J, Finoia MG, Tudino MB (2012) Baseline trace metals in gastropod mollusks from the Beagle Channel, Tierra del Fuego (Patagonia, Argentina). Ecotoxicology 21: 1112–1125.

Conti ME, Stripeikis J, Iacobucci M, Cucina D, Cecchetti G, Tudino MB (2006) Trace metals in molluscs from the Beagle Channel (Argentina): A preliminary study In: Brebbia, CA, Conti, ME, Tiezzi, E (eds), Management of Natural Resources, Sustainable Development and Ecological Hazards WIT Transactions on Ecology and the Environment Series, 99 WIT Press, Bariloche, pp 473–483.

Corsi I, Mariottin, M, Mench, V, Sensin, C, Balocch, C, Focard, S (2002) Monitoring a marine coastal area: Use of *Mytilus galloprovincialis* and *Mullus barbatus* as bioindicators. Mar Ecol 23: 138–153.

Cortazar E, Bartolomé L, Arrasate S, Usobiaga A, Raposo JC, Zuloaga O, Etxebarria N (2008) Distribution and bioaccumulation of PAHs in the UNESCO protected natural reserve of Urdaibai, Bay of Biscay. Chemosphere 72: 1467–1474.

Costa PM, Carreira S, Costa MH, Caeiro S (2013) Development of histopathological indices in a commercial marine bivalve (*Ruditapes decussatus*) to determine environmental quality. Aquat Toxicol 126: 442–54.

Costa PM, Costa MH (2012) Development and application of a novel histological multichrome technique for clam histopathology. J Invertebr Pathol 110: 411–414.

Costanza R (2012) Ecosystem health and ecological engineering. Ecol Eng 45: 24–29.

Costanza R, Norton BG, Haskell BD (1992) Ecosystem health: new goals for environmental management Island Press, Washinton, DC.

Couch JA (1984) Atrophy of diverticular epithelium as an indicator of environmental irritants in the oyster, *Crassostrea virginica*. Mar Environ Res 14: 525–526.

Crosby M, Gale L (1990) A review and evaluation of bivalve condition index methodologies with a suggested standard method. J Shellfish Res 9: 233–237.

Cruz RA, Jimenez JA (1994) Moluscos asociados a las áreas de manglar de la Costa Pacífica de América Central: guía. Heredia, C. R. Efuna, p 18–20.

Cuevas N, Zorita I, Costa PM, Franco J, Larreta J (2015) Development of histopathological indices in the digestive gland and gonad of mussels: integration with contamination levels and effects of confounding factors. Aquat Toxicol 162: 152–164.

Dagnino A, Allen JI, Moore MN, Broeg K, Canesi L, Viarengo, A (2007) Development of an expert system for the integration of biomarker responses in mussels into an animal health index. Biomarkers 12: 155–172.

Damiens G, Gnassia-Barelli M, Loquès F, Roméo M, Salbert V (2007) Integrated biomarker response index as a useful tool for environmental assessment evaluated using transplanted mussels. Chemosphere 66: 574–583.

Darnerud PO, Eriksen GS, Jo´hannesson T, Larsen PB, Viluksela M (2001) Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. Environ Health Perspect 109: 49–68.

Davies IM, Vethaak AD (2012) Integrated marine environmental monitoring of chemicals and their effects. ICES Coop Res Rep No 315 277 pp.

De Zwaan A, Eertman R (1996) Anoxic or aerial survival of bivalves and other euryoxic invertebrates as a useful response to environmental stress—a comprehensive review. Comp Biochem Physiol Part C 113C: 299–312.

Denslow, N, Garcia-Reyero, N, Barber, D, 2007 Fish "n"chips: the use of microarrays for aquatic toxicology Mol BioSyst 3, 172–177

DesVoigne DM, Sparks AK (1968) The process of wound healing in the Pacific oyster, *Crassostrea gigas*. J Invertebr Pathol 12: 53–65.

Díez G (1996) Correlación multiespecífica entre biomarcadores celulares de estrés ambiental y niveles biodisponibles de contaminantes orgánicos y metálicos: un estudio de campo. University of the Basque Country 268 pp.

Domingos FXV, Azevedo M, Silva MD, Randi MAF, Freire CA, Silva de Assis HC, Oliveira Ribeiro CA (2007) Multibiomarker assessment of three Brazilian estuaries using oysters as bioindicators. Environ Res 105: 350–363.

Dondero F, Dagnino A, Jonsson H, Caprì F, Gastaldi L, Viarengo A (2006) Assessing the occurrence of a stress syndrome in mussels (*Mytilus edulis*) using a combined biomarker/gene expression approach. Aquat Toxicol 78 Suppl 1: 13–24.

Dove MC, O'Connor WA (2012) Reproductive cycle of Sydney rock oysters, *Saccostrea glomerata* (Gould 1850) selectively bred for faster growth. Aquaculture 324–325: 218–225.

Duke NC (1992) Mangrove floristics and biogeography In: Robertson AI, Alongi DM (eds), Coastal and Estuarine Studies Tropical Mangrove Ecosystems Washington, DC: American Geophysical Union, pp 63-100.

Dumailo S (2003) Evaluación de la problemática ambiental por medio del estudio de algunos aspectos de sedimentación y contaminación en la laguna de Bluefields, RAAS, Nicaragua. MSc Thesis. CIRA-UNAN, 242 pp.

Dumler R, Thoma H, Lenoir D, Hutzinger O (1989) Thermal formation of polybrominated dibenzodioxins (PBDD) and dibenzofurans (PBDF) from bromine containing flame retardants. Chemosphere 19: 305–308.

Ebanks-Mongalo BF, Suárez-Sánchez J, Siu-Estrada E, Montoya-Arguello JJ, Mairena-Valdivia DA, Flores-Pacheco A, Van der Wal JC, Valencia-Quintana P (2013) Concentración de plaguicidas en agua, sedimentos y ostiones (*Crassostrea rhizophorae*) de la Laguna de Bluefields, RAAS, Nicaragua. WANI: 49-54.

EC (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Off J Eur Communities L327, 1–72.

EC (2008) Directive 2008/56/EC of the European Parliament and of the Council, of 17 June 2008, establising a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). Off J Eur Union L 164: 19–40.

EC (2006) EU Marine Strategy: The story behind the strategy. Luxembourg: Office for Official Publications of the European Communities, 30 pp.

Eertman RHM, Wagenvoort AJ, Hummel H, Smaal AC (1993) "Survival in air" of the blue mussel *Mytilus edulis* L as a sensitive response to pollution-induced environmental stress. J Exp Mar Bio Ecol 170: 179–195.

Eertman RHM, Zurbur, W, Schipper CA, Sandee B, Smaal AC (1996) Effects of PCB 126 and cadmium on the anaerobic metabolism of the mussel *Mytilus edulis* L. Comp Biochem Physiol - C 113: 267–272.

Eisler R (1987) Polycyclic aromatic hydrocarbon hazards to fish, wildlife, and invertebrates: a synoptic review. US Fish Wildl Serv Biol Rep 85: 1–55.

Elliott M (2011) Marine science and management means tackling exogenic unmanaged pressures and endogenic managed pressures—a numbered guide. Mar Pollut Bull 62: 651−655.

Ellison AM, Farnsworth EJ (1996) Anthropogenic disturbance of Caribbean mangrove ecosystems: past impacts, present trends, and future predictions. Biotropica 28(4a): 549-565.

Etter PC, Lamb PJ, Portis DH (1987) Heat and freshwater budgets of the Caribbean Sea with revised estimates for the Central American Seas. J Phys Oceanogr 17(8): 1232–1248.

European Communities (2006) EU Marine Strategy: The story behind the strategy European Communities.

Evans GW, Cohen S (1987) Environmental stress In: Stokols, D, Altman, I (eds), Handbook of Environmental Psychology. John Wiley & Sons, New York, pp 571–610.

Fairbrother A, Wenstel R, Sappington K, Wood W (2007) Framework for Metals Risk Assessment. Ecotoxicol Environ Saf 68: 145–227.

FAO (2003) Status and trends in mangrove area extent worldwide Forest Resources Assessment Working aper-63 Wilkie M, Fortuna S (eds), Roma: Forest Resources Division, FAO Available online at: http://wwwfaoorg/docrep/007/j1533e/j1533e00htm Accessed 2009 Jun 18.

FAO (2007) The world's mangroves 1980 – 2005. FAO Forestry Paper, 153.

Farmer A, Mee L, Langmead O, Cooper P, Kannen A, Kershaw P, Cherrier V (2012) The Ecosystem Approach in Marine Management. EU FP7 KNOWSEAS Project. Available online at: http://www.msfd.eu/knowseas/library/PB2.pdf.

Farrington JW, Tripp BW (1995) International Mussel Watch Proyect: inicial implementation phase/Final report. NOAA Technical memorandum NOS ORCA 95, pp 188.

Fattorini D, Notti A, Di Mento R, Cicero AM, Gabellini M, Russo A, Regoli F (2008) Seasonal, spatial and inter-annual variations of trace metals in mussels from the Adriatic sea: A regional gradient for arsenic and implications for monitoring the impact of off-shore activities. Chemosphere 72: 1524–1533.

Fearman J, Moltschaniwskyj NA (2010) Warmer temperatures reduce rates of gametogenesis in temperate mussels, *Mytilus galloprovincialis*. Aquaculture 305: 20–25.

Fernandez A, Singh A, Jaffé R (2007) A literature review on trace metals and organic compounds of anthropogenic origin in the Wider Caribbean Region. Mar Pollut Bull 54: 1681–1691.

Fink G (2009) Stress: definition and history In Stress Science: Neuroendocrinology (ed G Fink). San Diego, CA. Academic Press, pp 549-555.

Flores L, Lincadeo R (2010) Size composition and sex ratio of *Anadara tuberculosa* and *Anadara similis* in a mangrove reserve from the northwest of Ecuador. Rev Biol Mar Oceanogr 45: 541-546.

Fournier ML, De la Cruz E (1987) Reproduction of the cockle *Anadara grandis* in Costa Rica. Naga 10(1).

Fox GA (2001) Wildlife as sentinels of human health effects in the Great Lakes--St Lawrence basin. Environ Health Perspect 109: 853–861.

Franckx E (1998) Regional Marine Environmenta Protection Regimens in the Context of UNCLOS. Int J Mar Coast Law 13: 307–324.

Funes V, Alham, J, Nava, JI, López-Bare, J, Peinado J (2006) Ecotoxicological effects of metal pollution in two mollusc species from the Spanish South Atlantic littoral. Environ Pollut 139: 214–223.

Galdámez A, Pacheco S, Pérez I, Kino S (2007) Guía para la producción de *Anadara* spp. 2006 – 2007. Producción artificial de semillas, cultivo intermedio y cultivo de *Anadara tuberculosa* y *A. grandis*. Proyecto de Moluscos. CENDEPESCA – JICA. San Salvador. El Salvador,42 pp.

Galgani F, Martínez-Gómez C, Giovanardi F, Romanelli G, Caixach J, Cento A, Scarpato A, Benbrahim S, Messaoudi S, Deudero S, Boulahdid M, Benedicto J, Andral B (2011) Assessment of polycyclic aromatic hydrocarbon concentrations in mussels (*Mytilus galloprovincialis*) from the Western basin of the Mediterranean Sea. Environ Monit Assess 172: 301–317.

Gamble DW, Curtis S (2008) Caribbean precipitation: Review, model and prospect. Prog Phys Geogr 32(3): 265–276.

García F, Palacio C, Garcia U (2012) Water quality at Santa Marta coastal area (Colombia). Dyna 79: 85–94.

García-Domínguez FA, Haro-Hernández D, García-Cuellar Á, Villalejo-Fuerte M, Rodríguez-Astudillo S (2008) Ciclo reproductivo de *Anadara tuberculosa* (Sowerby, 1833) (Arcidae) en Bahía Magdalena, México. Rev Biol Mar Oceanogr 43(1): 143-152.

Garmendia L, Soto M, Cajaraville M, Marigómez I (2010) Seasonality in cell and tissue-level biomarkers in *Mytilus galloprovincialis*: relevance for long-term pollution monitoring. Aquat Biol 9: 203–219.

Garmendia L, Soto M, Ortiz-Zarragoitia M, Orbea A, Cajaraville MP, Marigómez I (2011a) Application of a battery of biomarkers in mussel digestive gland to assess long-term effects of the Prestige oil spill in Galicia and Bay of Biscay: correlation and multivariate analysis. J Environ Monit 13: 933–42.

Garmendia L, Soto M, Vicario U, Kim Y, Cajaraville M, Marigómez I (2011b) Application of a battery of biomarkers in mussel digestive gland to assess long-term effects of the Prestige oil spill in Galicia and Bay of Biscay: tissue-level biomarkers and histopathology. J Environ Monit 13: 915–32.

Garrigues P, Viarengo A, Lehtonen K, Bocquene G, Anderson OK, Peters L, Cajaraville MP, Cotou E, Dimitriadis V, Minier C, Porte C, Auffret M, Devillers J, Hansen PD, et al., (2003) Biological effects of environmental pollution in the marine coastal ecosystems (BEEP). In: Caumette P, Eccles C, Garrigues P, Krom M, Lebaron P (eds). The impact of human activities on the marine environment quality and health: the EC impacts cluster. EC Directorate-General for Research, Publications de Universite de Pau, BU Sciences, Pau, pp 165-174.

GEF-REPCar (2011) Agricultural pesticide residues in Caribbean coastal waters Colombia, Costa Rica and Nicaragua 2008-2011. GEF-REPCar Rpt UNEP, 63 pp.

Gener RL, Rivas F, Argüello G (2009) Estudio de mercado de la concha negra (*Anadara similis* y *Anadara tuberculosa*) en Nicaragua: Comercialización con garantía de inocuidad, 55p.

George R, Martin GD, Nair SM, Chandramohanakumar N (2013) Biomonitoring of trace metal pollution using the bivalve molluscs, Villorita cyprinoides, from the Cochin backwaters. Environ Monit Assess 185: 10317–31

GESAMP (2001) Protecting the oceans from land based activities: land-based sources and activities affecting the quality and uses of the marine, coastal and associated freshwater environment, GESAMP. 162 pp.

Giri C, Ochieng E, Tieszen LL, Zhu Z, Singh A, Loveland T, Masek J, Duke N (2011) Status and distribution of mangrove

forests of the world using earth observation satellite data. Glob Ecol Biogeogr 20: 154–159.

Gómez-Giraldo A, Osorio A, Toro FM, Osorio JD, Alvarez OA, Arrieta A (2009) Circulation pattern in the "Barbacoas" bay and its influence on sediment transport to the "Rosario" islands. Avances Rec Hidraul 20: 21-40.

Gomiero A, Volpato E, Nasci C, Perra G, Viarengo A, Dagnino A, Spagnolo A, Fabi G (2015) Use of multiple cell and tissue-level biomarkers in mussels collected along two gas fields in the northern Adriatic Sea as a tool for long term environmental monitoring. Mar Pollut Bull 93: 228–244.

Gónima L, Mancera-Pineda JE, Botero L (1998) Aplicación de imágenes de satélite al diagnóstico ambiental de un complejo lagunar estuarino tropical: Ciénaga Grande de Santa Marta, Caribe colombiano. INVEMAR, Serie de Publicaciones Especiales, Santa Marta. 4: 1-56.

González LI (1997) Diagnostico ecológico de las zonas costeras de Nicaragua: MAIZCo (Programa de manejo integrado de zonas costeras) Ministerio del Ambiente y Recursos Naturales, Dirección General del Ambiente Managua-Nicaragua, 69 pp.

Gorbi S, Virno Lamberti C, Notti A, Benedetti M, Fattorini D, Moltedo G, Regoli F (2008) An ecotoxicological protocol with caged mussels, *Mytilus galloprovincialis*, for monitoring the impact of an offshore platform in the Adriatic sea. Mar Environ Res 65: 34–49.

Gordon AL (1967) Circulation of the Caribbean Sea. J Geophys Res 72: 6207–6223.

Gray JS (2002) Biomagnification in marine systems: the perspective of an ecologist. Mar Pollut Bull 45: 46–52.

Gyory J, Mariano A, Ryan E (2008) ‘‘The Caribbean Current.’’ Ocean Surface Currents. Available online at: http://oceancurrents.rsmas.miami.edu/caribbean/caribbean.

Hagger JA, Jones MB, Lowe D, Leonard DRP, Owen R, Galloway TS (2008) Application of biomarkers for improving risk assessments of chemicals under the Water Framework Directive: a case study. Mar Pollut Bull 56. 1111–1118.

Halpern BS, Lbridge S, Selkoe KA, Kappel C V, Micheli F, D’Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R (2008) A global map of human impact on marine ecosystems. Science 319: 948–52.

Halpern BS, Longo C, Hardy D, McLeod KL, Samhouri JF, Katona SK, Kleisner K, Lester SE, O’Leary J, Ranelletti M, Rosenberg AA, Scarborough C, Selig ER, Best BD, Brumbaugh DR, Chapin FS, Crowder LB, Daly KL, Doney SC, Elfes C, Fogarty MJ, Gaines SD, Jacobsen KI, Karrer LB, Leslie HM, Neeley E, Pauly D, Polasky S, Ris B, St Martin K, Stone GS, Sumaila UR, Zeller D (2012) An index to assess the health and benefits of the global ocean. Nature 488: 615–620.

Harborne AR, Mumby PJ, Micheli F, Perry CT, Dahlgren CP, Holmes KE, Brumbaugh DR (2006) The functional value of Caribbean coral reef, seagrass and mangrove habitats to ecosystem processes. Adv Mar Biol 50: 57-189.

Hédouin L, Batista MG, Metian M, Buschiazzo E, Warnau M, (2010) Metal and metalloid bioconcentration capacity of two tropical bivalves for monitoring the impact of land-based mining activities in the New Caledonia lagoon. Mar Pollut Bull 61: 554–567.

HELCOM (2007). HELCOM Baltic Sea Action Plan. Helsinki: HELCOM.

HELCOM (2009a) “Eutrophication in the Baltic Sea. An integrated thematic assessment of eutrophication in the Baltic Sea region”, in Baltic Sea Environmental Proceedings, 115B. Helsinki Commission, 148. Available online at: http://www.helcom.fi/Lists/Publications/BSEP115B.pdf.

HELCOM (2009b). “Biodiversity in the Baltic Sea. An integrated thematic assessment of biodiversity and nature conservation in the Baltic Sea”, in Baltic Sea Environmental Proceedings, 116b. Helsinki Commission, 188. Available online at: http://www.helcom.fi/Lists/Publications/BSEP116B.pdf.

HELCOM (2010) “Hazardous substances in the Baltic Sea. An integrated thematic assessment of hazardous substances in the Baltic Sea”, in Baltic Sea Environmental Proceedings, 120B. Helsinki Commission, 116. Available online at: http://www.helcom.fi/Lists/Publications/BSEP120B.pdf.

Hellou J, Law RJ (2003) Stress on stress response of wild mussels, *Mytilus edulis* and *Mytilus trossulus*, as an indicator of ecosystem health. Environ Pollut 126: 407–416.

Hernández CA, Marquez GE (1991) Producción Primaria en el Ecosistema Ciénaga Grande de Santa Marta, Laguna costera del Caribe colombiano. Trianea 4: 385-407.

Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world’s marine ecosystems. Science 328: 1523–1528.

Höher N, Köhler A, Strand J, Broeg K (2012) Effects of various pollutant mixtures on immune responses of the blue mussel (*Mytilus edulis*) collected at a salinity gradient in Danish coastal waters. Mar Environ Res 75: 35–44.

Huber M (2015) Compendium of bivalves 2. Harxheim: ConchBooks. 907 pp.

IARC (1989) Diesel and Gasoline Engine Exhausts and Some Nitroarenes In: IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. Vol 46 International Agency for Research on Cancer, Lyon, p 458 pp.

ICES (2006) Report of the Working Group on Biological Effects of Contaminants (WGBEC), 27–31 March 2006 ICES Headquarters, Copenhagen, Denmark ICES CM 2006/MHC:04.

ICES (2011) ICES / OSPAR SGIMC REPORT 2011 Monitoring of Contaminants and Biological Report of the Study Group on Integrated Effects (SGIMC) International Council for the Exploration of the Sea.

ICES (2012) Report of the Working Group on Biological Effects of Contaminants (WGBEC), 12-16 March 2012, Porto, Portugal. ICES CM 2012/SSGHIE:04, 131 pp.

ICES (2013) Report of the Working Group on the Biological Effects of Contaminants (WGBEC), 10-15 March 2013, San Pedro del Pinatar, Spain. ICES CM 2013/SSGHIE:04, 37 pp.

IPCC (2013) Climate Change 2013: The Physical Science Basis Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change In: Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds), Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, p 1535.

Islam MS, Tanaka M (2004) Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: A review and synthesis. Mar Pollut Bull 48: 624–649.

Ivanina A V, Sokolova IM (2013) Interactive effects of pH and metals on mitochondrial functions of intertidal bivalves Crassostrea virginica and *Mercenaria mercenaria*. Aquat Toxicol 144-145: 303–309.

Ivanković D, Pavičić J, Erk M, Filipović-Marijić V, Raspor B (2005) Evaluation of the *Mytilus galloprovincialis* Lam digestive gland metallothionein as a biomarker in a long-term field study: Seasonal and spatial variability. Mar Pollut Bull 50: 1303–1313.

Jackson JB (1997) Reefs since Columbus Coral Reefs. 16: S23–S32.

JAMP (Joint Assessment and Monitoring Programme) (1998) JAMP Guidelines for contaminant-specific biological effects monitoring. Oslo and Paris Commissions, 38 pp.

Jiménez JA (1999) Ambiente, distribución y características estructurales en los Manglares del Pacífico de Centro América: Contrastes climáticos. In: Yáñez–Arancibia A, Lara–Domínguez AL (eds.). Ecosistemas de Manglar en América Tropical. Instituto de Ecología AC México, UICN/ORMA, Costa Rica, NOAA/NMFS. pp 51-70.

Johnson RH, Miller JG (1971) Chapter 8: Environmental Law Annu Surv Masschusetts Law 1971, 149–181.

Kagley AN, Snider RG, Krishnakumar PK, Casillas E (2003) Assessment of seasonal variability of cytochemical responses to contaminant exposure in the blue mussel *Mytilus edulis* (complex). Arch Environ Contam Toxicol 44: 43–52.

Kamminga MT (1995) Principles of International Environmental Law. Environ Policy An Int Context Conflicts 1: 111–131.

Kanhai LDK, Gobin JF, Beckles DM, Lauckner B, Mohammed A (2014) Metals in sediments and mangrove oysters (*Crassostrea rhizophorae*) from the Caroni Swamp, Trinidad. Environ Monit Assess 186: 1961–1976.

Kelly JR and Harwell MA (1989) Indicators of ecosystem response and recovery In: Levin SA, Harwell MA, Kelly JR, and Kimball KD (eds), Ecotoxicology: Problems and Approaches, New York: Springer, pp 9–35.

Khan MI, Ayub Z, Siddiqui G (2015) Impact of marine pollution in green mussel *Perna viridis* from four coastal sites in Karachi, Pakistan, North Arabian Sea : Histopathological observations. Indian J Exp Biol 53: 222–227.

Kim Y, Powell EN (2006) Relationships among parasites and pathologies in sentinel bivalves: NOAA Status and Trends“ Mussel Watch” Program. Bull Mar Sci 79: 83–111.

Kim Y, Powell EN, Wade TL, Presley BJ (2008) Relationship of parasites and pathologies to contaminant body burden in sentinel bivalves: NOAA Status and Trends “Mussel Watch”Program. Mar Environ Res 65: 101–127.

Kimbrough KL, Johnson WE, Lauenstein GG, Christensen JD, Apeti DA (2009) An Assessment of Polybrominated Diphenyl Ethers (PBDEs) in Sediments and Bivalves of the US Coastal Zone Silver Spring, MD NOAA Technical Memorandum NOS NCCOS 94, 87 pp.

Kinder TH, Heburn GW, Green AW (1985) Some aspects of the Caribbean circulation. Mar Geol 68: 25–52.

Knap A, Dewailly E, Furgal C, Galvin J, Baden D, Bowen RE, Depledge M, Duguay L, Fleming LE, Ford T, Moser F, Owen R, Suk WA, Unluata U (2002) Indicators of ocean health and human health: developing a research and monitoring framework. Environ Health Perspect 110: 839–845.

Kozlowski H, Potocki S, Remelli M, Rowinska-Zyrek M, Valensin D (2013) Specific metal ion binding sites in unstructured regions of proteins. Coord Chem Rev 257: 2625–2638.

Laffon B, Rábade T, Pásaro E, Méndez J (2006) Monitoring of the impact of Prestige oil spill on *Mytilus galloprovincialis* from Galician coast. Environ Int 32: 342–348.

Laird M (1961) Microecological factors in oyster epizootics. Can J Zool 39. 449–485.

Lapointe BE (1997) Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. Limnology and Oceanography 42: 1119–1131.

Launche B (2008) Wider Caribbean Region—A Pivotal Time to Strengthen Regional Instruments For Biodiversity Conservation. Int J Mar Coast Law 23(3): 499-530.

Law R, Hanke G, Angelidis M, Batty J, Bignert A, Dachs J, Davies I, Denga Y, Duffek A, Herut B, Hylland K, Lepom P, Leonards P, Mehtonen J, Piha H, Roose P, Tronczynski J, Velikova V, Vethaak D (2010) Marine Strategy Framework Directive Task Group 8 Report Contaminants and pollution effects. Jt Res Centre, Off Off Publ Eur Communities 171.

Leal JCM, Polo C, Behling E, Colina G, Rincón N, Carrasquero S (2014) Distribución espacial de Cd y Pb en *Polymesoda solida* y sedimentos costeros del Lago de Maracaibo. Multiciencias 14(1): 7-15.

Lee MK, Cho BY, Lee SJ, Kang JY, Jeong HDo, Huh SH, Huh MDo (2001) Histopathological lesions of Manila clam, *Tapes philippinarum*, from Hadong and Namhae coastal areas of Korea. Aquaculture 201: 199–209.

Lemaire N, Pellerin J, Fournier M, Girault L, Tamigneaux E, Cartier S, Pelletier E (2006) Seasonal variations of physiological parameters in the blue mussel *Mytilus* spp from farm sites of eastern Quebec. Aquaculture 261: 729–751.

Lenz T, Boehs G (2011) (Bivalvia : Ostreidae) en la Bahía de Camamu, Bahia, Brasil. Rev Biol Trop 59: 137–149.

Lewis M, Pryor R, Wilking L (2011) Fate and effect of anthropogenic chemicals in mangrove ecosystems: a review. Envir Pollut 159: 2328–2346.

Llorca M, Farré M, Eljarrat E, Díaz-Cruz S, Rodríguez-Mozaz S, Wunderlin D, Barcelo D (2016) Review of emerging contaminants in aquatic biota from Latin America: 2002–2016. Environ Toxicol Chem. DOI 10.1002/etc.3626.

Loizeau V, Abarnou A, Cugier P, Jaouen-Madoulet A, Le Guellec AM, Menesguen A (2001) A model of PCB bioaccumulation in the sea bass food web from the Seine Estuary (Eastern English Channel). Mar Pollut Bull 43: 242–255.

Looi LJ, Aris AZ, Wan Johari WL, Md Yusoff F, Hashim Z, (2013) Baseline metals pollution profile of tropical estuaries and coastal waters of the Straits of Malacca. Mar Pollut Bull 74: 471–476.

Lucero-Rincón CH, Cantera JRK, Gil-Agudelo DL, Muñoz O, Zapata LA, Cortes N, Gualteros WO, Manjarres A (2013) Análisis espacio temporal de la biología reproductiva y el reclutamiento del molusco bivalvo *Anadara tuberculosa* en la costa del Pacífico colombiano. Rev Biol Mar Oceanogr 48: 321–334.

Lugo AE, Snedaker SC (1974) The ecology of mangroves. Annu Rev Ecol System 5: 39-64.

Luoma SN, Rainbow PS (2005) why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environ Sci Technol 39: 1921–1931.

Lynch SA, Morgan E, Carlsson J, Mackenzie C, Wooton EC, Rowley AF, Malham S, Culloty SC (2014) The health status of mussels, *Mytilus* spp, in Ireland and Wales with the molecular identification of a previously undescribed haplosporidian. J Invertebr Pathol 118: 59–65.

MacKay D, Fraser A (2000) Bioaccumulation of persistent organic chemicals: Mechanisms and models. Environ Pollut 110: 375–391.

Mackenzie BR, Schiedek D (2007) Daily Ocean monitoring since the 1860s shows record warming of northern European seas. Glob Chang Biol 13: 1335–1347.

Mackenzie CL (2001) The Fisheries for Mangrove Cockles, *Anadara* spp., from Mexico to Peru, With Descriptions of Their Habitats and Biology, the Fishermen's Lives, and the Effects of Shrimp Farming. Mar Fish Rev 63(1): 1-39.

Magaña V, Amador JA, Medina S (1999) The midsummer drought over Mexico and Central America. J Clim 12(6). 1577–1588.

Mahboob S (2013) Environmental pollution of heavy metals as a cause of oxidative stress in fish: A review. Life Sci J 10: 336–347.

Mancera JE, Vidal LA (1994) Florecimiento de microalgas relacionado con mortandad masiva de peces en el complejo lagunar Cienaga Grande de Santa Marta, Caribe Colombiano. Bol Investig Mar Cost 23: 103 – 117.

MARENA (2010) Caracterización de la cuenca No. 61. Río Escondido. Ministerio del Ambiente y los Recursos Naturales de Nicaragua, DGPN & DRHCH. 38 pp.

Marigómez I, Cajaraville MP, Angulo E (1990) Histopathology of the digestive gland-gonad complex of the marine prosobranch *Littorina littorea* exposed to cadmium. Dis Aquat Organ 9: 229–238.

Marigómez I, Garmendia L, Soto M, Orbea A, Izagirre U, Cajaraville MP (2013b) Marine ecosystem health status assessment through integrative biomarker indices: a comparative study after the Prestige oil spill "Mussel Watch". Ecotoxicology 22. 486–505.

Marigómez I, Soto M, Cajaraville MP, Angulo E, Giamberini L (2002) Cellular and subcellular distribution of metals in molluscs. Microsc Res Tech 56: 358–92.

Marigómez I, Zorita I, Izagirre U, Ortiz-Zarragoitia M, Navarro P, Etxebarria N, Orbea A, Soto M, Cajaraville MP (2013a) Combined use of native and caged mussels to assess biological effects of pollution through the integrative biomarker approach. Aquat Toxicol 136-137: 32–48.

Mark S, Provencher L, Munro J (2003) Approach for the monitoring and assessment of marine ecosystem health with application to the Mya–Macoma community. Can Tech Rep Fish Aquat Sci 2491: ix + 78 pp.

Masson S, Couillard Y, Campbell PGC, Olsen C, Pinel-Alloul B, Perceval O (2010) Responses of two sentinel species (*Hexagenia limbata*--mayfly; *Pyganodon grandis*--bivalve) along spatial cadmium gradients in lakes and rivers in northwestern Québec. J Environ Monit 12: 143–158.

McCarthy JF, Shugart LR (1990) Biological markers of environmental contamination. In: McCarthy JF, Shugart LR (eds), Biomarkers of environmental contamination. Lewis Publishers, Boca Raton, FL. p 3–14.

Melwani AR, Gregorio D, Jin Y, Stephenson M, Ichikawa G, Siegel E, Crane D, Lauenstein G, Davis JA (2014) Mussel Watch update: long-term trends in selected contaminants from coastal California, 1977-2010. Mar Pollut Bull 81: 291–302.

Miloslavich P, Díaz JM, Klein E, Alvarado JJ, Díaz C, Gobin J, Escobar-Briones E, Cruz-Motta JJ, Weil E, Cortés J, Bastidas AC, Robertson R, Zapata F, Martín A, Castillo J, Kazandjiann A, Ortiz M (2010) Marine biodiversity in the Caribbean: regional estimates and distribution patterns. PloS one, 5(8): e11916.

Montenegro VD, Olivares PA, González MT (2010) Hermaphroditism in Marine Mussel *Perumytilus purpuratus* (Lamarck, 1819), (Mollusca: Mytilidae). Int J Morphol 28: 569–573.

Moore MN, Clarke KR (1982) Use of microstereology and quantitative cytochemistry to determine the effects of crude oil-derived aromatic hydrocarbons on lysosomal structure and function in a marine bivalve mollusc, *Mytilus edulis*. Histochem J 14: 713–718.

Mora Sanchez EM (1990) Catologo de bivalvos marinos del Ecuador. Bol. Cientifico Tecnico, Inst. Nacional de Pesca, Guayaquil, Ecuador 10(1): 1–136.

Morgado ME, Bebianno MJ (2005) A mussel watch in the Ria Formosa lagoon. Ciencias Marinas 31: 231–241

Múgica M, Sokolova IM, Izagirre U, Marigómez I (2015) Season-dependent effects of elevated temperature on stress

biomarkers, energy metabolism and gamete development in mussels. Mar Environ Res 103: 1–10.

Nagelkerken I, Blaber SJM, Bouillon S, Green P, Haywood M, Kirton LG, Meynecke JO, Pawlik J, Penrose HM, Sasekumar A, Somerfield PJ (2008) The habitat function of mangroves for terrestrial and marine fauna: A review Aquat Bot 89: 155–185.

Nascimento IA, Leite MBN, Sansone G, Pereira SA, Smith DH (1998) Stress protein accumulation as an indicator of impact by the petroleum industry in Todos os Santos Bay, Brazil. Aquat Ecosyst Health Manag 1: 101–108.

Naso B, Perrone D, Ferrante MC, Bilancione M, Lucisano A (2005) Persistent organic pollutants in edible marine species from the Gulf of Naples, Southern Italy. Sci Total Environ 343: 83–95.

Nesto N, Romano S, Moschino V, Mauri M, Da Ros L (2007) Bioaccumulation and biomarker responses of trace metals and micro-organic pollutants in mussels and fish from the Lagoon of Venice, Italy. Mar Pollut Bull 55: 469–84.

Nicholson S (2003) Lysosomal membrane stability, phagocytosis and tolerance to emersion in the mussel *Perna viridis* (Bivalvia: Mytilidae) following exposure to acute, sublethal, copper. Chemosphere 52: 1147–1151.

Nieboer E, Richardson DHS (1980) The replacement of the nondescript term Heavy Metal by a biologically and chemically significant classification of metal ions. Environ Pollut 1: 3–26.

Öberg T, Bergström J (1990) Bromine and waste incineration an environmental risk? Organohalogen. Comp 2: 339–342.

Odum EP (1963) Ecology Holt, Rinehart & Winston, New York.

OHI (Ocean Health Index) (2015) Summary of results for countries and territories. Prepared September 25, 2015. Available online at: http://www.oceanhealthindex.org and http://www.ohi-science.org.

Okaniwa N, Miyaji T, Sasaki T, Tanabe K (2010) Shell growth and reproductive cycle of the Mediterranean mussel *Mytilus galloprovincialis* in Tokyo Bay, Japan : relationship with environmental conditions. Plankt Benthos Res 5: 214–220.

Orban E, Di Lena G, Nevigato T, Casini I, Marzetti A, Caproni R (2002) Seasonal changes in meat content, condition index and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two different Italian Food. Chem 77: 57–65.

Orbea A, Cajaraville MP (2006) Peroxisome proliferation and antioxidant enzymes in transplanted mussels of four Basque estuaries with different levels of polycyclic aromatic hydrocarbon and polychlorinated biphenyl pollution. Environ Toxicol Chem 25: 1616–1626.

Ortiz-Zarragoitia M, Bizarro C, Rojo-Bartolomé I, de Cerio OD, Cajaraville MP, Cancio I (2014) Mugilid Fish Are Sentinels of Exposure to Endocrine Disrupting Compounds in Coastal and Estuarine Environments. Marine Drugs 12: 4756-4782.

Ortiz-Zarragoitia M, Cajaraville MP (2010) Intersex and oocyte atresia in a mussel population from the Biosphere's Reserve of Urdaibai (Bay of Biscay). Ecotoxicol Environ Saf 73: 693–701.

Ortiz-Zarragoitia M, Garmendia L, Barbero MC, Serrano T, Marigómez I, Cajaraville MP (2011) Effects of the fuel oil spilled by the Prestige tanker on reproduction parameters of wild mussel populations. J Environ Monit 13: 84–94.

OSPAR Commission (2000a) Quality Status Report 2000 OSPAR Comm London 108 + vii pp.

OSPAR Commission (2000b) Quality Status Report 2000: Region IV–Bay of Biscay and Iberian Coast OSPAR Comm London 134 + xii pp.

OSPAR Commission (2009a) CEMP assessment report : 2008 / 2009 Assessment of trends and concentrations of selected hazardous substances in sediments and biota, London, OSPAR Commission, vol 390/2009 78 pp.

OSPAR Commission (2009b) Status and trend of marine chemical pollution OSPAR Comm London 161 pp.

OSPAR Commission (2013) Levels and trends in marine contaminants and their biological effects – CEMP Assessment report 2012. London, OSPAR Commission, vol 596/2013, 28 pp.

Pampanin DM, Volpato E, Marangon I, Nasci C (2005) Physiological measurements from native and transplanted mussel (*Mytilus galloprovincialis*) in the canals of Venice Survival in air and condition index. Comp Biochem Physiol Part A Mol Integr Physiol 140: 41–52.

Park JJ, Kim H, Kang SW, An CM, Lee SH, Gye MC, Lee JS (2012) Sex ratio and sex reversal in two-year-old class of oyster, *Crassostrea gigas* (Bivalvia: Ostreidae). Dev Reprod. 16: 385–388.

Pellerin J, Amiard JC. (2009) Comparison of bioaccumulation of metals and induction of metallothioneins in two marine bivalves (*Mytilus edulis* and *Mya arenaria*). Comparative Biochemistry and Physiology C 150: 186–195.

Peng J, Wang Y, Wu J, Zhang Y (2007) Evaluation for regional ecosystem health: methodology and research progress. Acta Ecol Sin 27: 4877–4885.

Pérez-Cruz YG, Rangel-Ruiz LJ, Gamboa-Aguilar J (2013) Metales en almejas y sedimentos en la Reserva de la Biósfera "Pantanos de Centla" Tabasco, México. Hidrobiológica 23 (1): 1-8.

Perugini M, Cavaliere M, Giammarino A, Mazzone P, Olivieri V, Amorena M (2004) Levels of polychlorinated biphenyls and organochlorine pesticides in some edible marine organisms from the Central Adriatic Sea. Chemosphere 57: 391–400.

Philippart CJM, van Aken HM, Beukema JJ, Bos OG, Cadée GC, Dekker R (2003) Climate-related changes in recruitment of the bivalve *Macoma balthica*. Limnol Oceanogr 48: 2171–2185.

Phillips DJH, Rainbow PS (1994) Biomonitoring of trace aquatic contaminants. Ed. Chapman and Hall, Boca Raton, Florida, 371 p.

Phillips DJH, Segar DA (1986) Use of bio-indicators in monitoring conservative contaminants: Programme design imperatives. Mar Pollut Bull 17(1): 10-17.

Polanía J (1993) Mangroves of Costa Rica. In: Lacerda LD (editor). Conservation and sustainable utilization of Mangrove Forests in Latin America and Africa Regions. Part 1; Volume 2.

Polanía J, Mainardi V (1993) Mangrove Forests of Nicaragua. In: Lacerda LD (editor). Conservation and sustainable utilization of Mangrove Forests in Latin America and Africa Regions. Part 1; Volume 2.

Ponizovsky AA (2003) Metals, metalloids and radionuclides in the Baltic Sea ecosystem. J Environ Qual 32: 1929.

Pörtner HO (2002) Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. Comp Biochem Physiol - A 132: 739–761.

Poveda G, Waylen PR, Pulwarty RS (2006) Annual and interannual variability of the present climate in northern South America and southern Mesoamerica. Palaeogeogr Palaeoclimatol Palaeoecol 234(1): 3–27.

Powell EN, Klinck JM, Hofmann EE (2011) Generation time and the stability of sex-determining alleles in oyster populations as deduced using a gene-based population dynamics model. J Theor Biol 271: 27–43.

Pozo K, Kukucka P, Vankova L, Pribylova P, Klanova J, Rudolph A, Banguera Y, Monsalves J, Contreras S, Barra R (2015) Polybrominated Diphenyl Ethers (PBDEs) in Concepción Bay, central Chile after the 2010 Tsunami. Mar Pollut Bull 95: 480-483.

Puy-Azurmendi E, Ortiz-Zarragoitia M, Kuster M, Martínez E, Guillamón M, Domínguez C, Serrano T, Barbero MC, López de Alda M, Bayona JM, Barceló D, Cajaraville MP (2010) An integrated study of endocrine disruptors in sediments and reproduction-related parameters in bivalve molluscs from the Biosphere's Reserve of Urdaibai (Bay of Biscay). Mar Environ Res 69 Suppl. S63–66.

Rainbow P (2002) Trace metal concentrations in aquatic invertebrates: why and so what?. Environ Pollut 120: 497–507.

Rainbow PS (2006) Biomonitoring of trace metals in estuarine and marine environments. Australas J Ecotoxicol 12: 107–122.

Ramsar (1999). A Directory of Wetlands of International Importance designated under the Convention on Wetlands of International Importance Especially as Waterfowl Habitat. Ramsar, 1971. Scott Frazier, editor. Compiled by Wetlands International for the Seventh Meeting of the Conference of Contracting Parties to the Ramsar Convention San José, Costa Rica, May 1999.

Rawlins BG, Ferguson AJ, Chilton PJ, Arthurton RS, Rees JG, Baldock JW (1998) Review of agricultural pollution in the Caribbean with particular emphasis on small island developing states. Mar Pollut Bull 36: 658-668.

RBU-Rep (1994) Seguimiento ecotoxicologíco de la ría de la Reserva de la Biosfera de Urdaibai (1993–1994). Final Report Cooperative Agreement University Basque Country & Basque Government Department of Environment, Leioa, Basque Country.

Rebelo MF, Amaral MCR, Pfeiffer WC (2005) Oyster condition index in *Crassostrea rhizophorae* (Guilding 1828) from a heavy-metal polluted coastal lagoon. Braz J Biol 65: 345–51.

Restrepo JD, Kjerfve B (2000a) Magdalena river: Interannual variability (1975–1995) and revised water discharge and sediment load estimates. J Hydrol 235(1–2): 137–149.

Restrepo JD, Kjerfve B (2000b) Water discharge and sediment load from the western slopes of the Colombian Andes with focus on Rio San Juan. J Geol 108(1): 17–34.

Richards W, Bohnsack J (1990) The Caribbean Sea: a large marine ecosystem in crisis In: Sherman K, Alexander L, Gold B (eds), Large marine ecosystems: patterns, processes and yields Washington, DC (USA): American Association for the Advancement of Science, pp 44–53.

Rivera-Monroy VH, Twilley RR, Bone D, Childers DL, Coronado-Molina C, Feller IC, Herrerra-Silviera JA, Jaffe R, Mancera JE, Rejmankova E, Salisbury JE (2004) A conceptual framework to develop long-term ecological research and management objectives in the wider Caribbean Region. BioScience 54(9): 843-856.

Rocher B, Le Goff J, Peluhet L, Briand M, Manduzio H, Gallois J, Devier MH, Geffard O, Gricourt L, Augagneur S, Budzinski H, Pottier D, André V, Lebailly P, Cachot J (2006) Genotoxicant accumulation and cellular defence activation in bivalves chronically exposed to waterborne contaminants from the Seine River. Aquat Toxicol 79: 65–77.

Rodriguez A (1981) Marine and coastal environmental stress in the Wider Caribbean Region. Ambio 10: 283-294.

Rueda M, Urban HJ (1998) Population dynamics and fishery of the fresh-water clam *Polymesoda solida* (Corbiculidae) in Cienaga Poza Verde, Salamanca Island, Colombian Caribbean. Fish Res 39(1): 75-86.

Sabry RC, da Silva PM, Gesteira TCV, Pontinha V de A, Magalhães ARM (2011) Pathological study of oysters *Crassostrea gigas* from culture and *C. rhizophorae* from natural stock of Santa Catarina Island, SC. Brazil Aquaculture 320: 43–50.

Sánchez-Páez H, Alvarez-Leon R, Pinto-Nolla F, Sanchez Alferez AS, Pino-Renjifo JC, Garcia-Hansen I, Acosta-Peñaloza MT (1997). Diagnóstico y Zonificación preliminar de los Manglares del Caribe colombiano. En: Sánchez-Páez H, Alvarez-León R (eds.) Proyecto PD 171/91 Rev. 2 Fase II (Etapa 2) Conservación y Manejo para el Uso Múltiple y Desarrollo de los Manglares en Colombia. Ministerio del Medio Ambiente / OIMT. Santa Fe de Bogotá DC (Colombia), 511 p.

Sands P, Peel J, Fabra A, Mackenzie R (2012) Principles of international environmental law, 3rd ed Cambridge University Press, Cambridge University Press, New York, NY, USA.

Schettino T, Caricato R, Calisi A, Giordano ME, Lionetto MG (2012) Biomarker Approach in Marine Monitoring and Assessment : New Insights and Perspectives. Open Environ Sci 6: 20–27.

Schiedek D, Sundelin B, Readman JW, Macdonald RW (2007) Interactions between climate change and contaminants. Mar Pollut Bull 54: 1845–56.

Schindler, PW, 1991 The regulation of heavy metals in natural aquatic systems In: Vernet, JP (Ed), Heavy Metals in the Environment Elsevier, Amsterdam, pp 95–123.

Segner H, Schmitt-Jansen M, Sabater S (2014) Assessing the impact of multiple stressors on aquatic biota: the receptor's side matters. Environ Sci Technol 48: 7690−7696.

Severeyn HJ, García de Severeyn Y, Ewald JJ (1994) Taxonomic revision of *Polymesoda solida* (Philippi, 1846) (Bivalvia: Corbiculidae), a new name for *Polymesoda arctata*, the estuarine clam of Lake Maracaibo and other estuaries of the tropical Atlantic coasts of America. Ciencia 2: 53-65.

Shi G, Chen Z, Bi C, Li Y, Teng J, Wang L, Xu S (2010) Comprehensive assessment of toxic metals in urban and suburban street deposited sediments (SDSs) in the biggest metropolitan area of China. Environ Pollut 158: 694–703.

Shugart LR, McCarthy JF, Halbrook RS (1992) Biological markers of environmental and ecological contamination: an overview. Risk Anal 12:353–360.

Silva CAR, Rainbow PS, Smith BD (2003) Biomonitoring of trace metal contamination in mangrove-lined Brazilian coastal systems using the oyster *Crassostrea rhizophorae*: comparative study of regions. Hydrobiologia 501: 199–206.

Silva CAR, Smith BD, Rainbow PS (2006) Comparative biomonitors of coastal trace metal contamination in tropical South America (N. Brazil). Mar Envir Res 61: 439–455.

Silva-Benavides A M, Bonilla R (2015) Estructura de la población y distribución de *Anadara tuberculosa* Sowerby (1833) (Mollusca: Bivalvia) en los manglares de Golfito y Playa Blanca de Puerto Jiménez, Golfo Dulce, Costa Rica. Int J Trop Biol 63 (1): 287-298.

Simpson SL, Angel BM, Jolley DF (2004) Metal equilibration in laboratory-contaminated (spiked) sediments used for the development of whole-sediment toxicity tests. Chemosphere 54: 597–609.

Sims RC, Overcash MR (1983) Fate of polynuclear aromatic compounds (PNAs) in soil-plant systems. Residue Rev 88: 1–68.

Singh A (2008) Governance in the Caribbean Sea: Implications for sustainable development United Nations–Nippon Foundation Fellowship Programme, p 122.

Siung-Chang A (1997) A review of marine pollution issues in the Caribbean. Environ Geochem Health 19: 45–55.

Smaal AC, Wagenvoort A, Hemelraad J, Akkerman I (1991) Response to stress of mussels (*Mytilus edulis*) exposed in dutch tidal waters. Comp Biochem Physiol Part C 100: 197–200.

Soclo HH, Garrigues PH, Ewald M (2000) Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: Case studies in Cotonou (Benin) and Aquitaine (France) Areas. Mar Pollut Bull 40: 387–396.

Sokolova IM, Frederich M, Bagwe R, Lannig G, Sukhotin, AA (2012) Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar Environ Res 79: 1–15.

Soto M, Cajaraville MP, Angulo E, Marigómez I (1996) Autometallographic localization of protein-bound copper and zinc in the common winkle, *Littorina littorea*: a light microscopical study Histochem J 28: 689–701.

Soto M, Ireland MP, Marigómez I (2000) Changes in mussel biometry on exposure to metals: implications in estimation of metal bioavailability in "Mussel-Watch"programmes. Sci Total Environ 247: 175–187.

Spongberg AI, Davies P (1998) Organochlorinated pesticide contaminants is Golfo Dulce, Costa Rica. Rev Biol Trop 46 (Suppl 4): 111-124.

Spongberg AL, Witter JD (2008) A review of PCB concentrations in tropical media, 1996-2007. Rev Biol Trop 56 (Suppl 4): 1-9.

Steffen W (2006) The Anthropocene, global change and sleeping giants: where on Earth are we going?. Carbon Balance Manag 1: 3.

Steinberg CE (2012) Environmental stress on animals: adverse or beneficial?. Expert Opin Environ Biol 1: 1–2.

Stentiford GD, Feist SW (2005) A histopathological survey of shore crab (*Carcinus maenas*) and brown shrimp (*Crangon crangon*) from six estuaries in the United Kingdom. J Invertebr Pathol 88: 136–146.

Stern-Pirlot A, Wolff M (2006) Population dynamics and fisheries potential of *Anadara tuberculosa* (Bivalvia: Arcidae) along the Pacific coast of Costa Rica. Rev Biol Trop 54(Suppl 1): 87-99.

Storelli MM, Marcotrigiano GO (2005) Bioindicator organisms: Heavy metal pollution evaluation in the Ionian Sea (Mediterranean Sea - Italy). Environ Monit Assess 102: 159–166.

Strogyloudi E, Angelidis MO, Christides A, Papathanassiou E (2012) Metal concentrations and metallothionein levels in *Mytilus galloprovincialis* from Elefsis bay (Saronikos gulf, Greece). Environ Monit Assess 184. 7189–7205.

Sturludottir E, Gunnlaugsdottir H, Jorundsdottir HO, Magnusdottir E V, Olafsdottir K, Stefansson G (2013) Spatial and temporal trends of contaminants in mussel sampled around the Icelandic coastline. Sci Total Environ 454-455: 500–509.

Suárez MP, Alvarez C, Molist P, San Juan F (2005) Particular aspects of gonadal cycle and seasonal distribution of gametogenic stages of *Mytilus galloprovincialis* cultured in the estuary of Vigo. J Shellfish Res 24. 531–540.

Svärdh L, Johannesson K (2002) Incidence of hemocytes and parasites in coastal populations of blue mussels (*Mytilus edulis*) - Testing correlations with area, season, and distance to industrial plants. J Invertebr Pathol 80: 22–28.

Teclaff LA (1972) International Law and the Protection of the Oceans from Pollution. Fordham Law Rev 40: 529–530.

Tett P, Gowen RJ, Painting SJ, Elliott M, Forster R, Mills DK, Bresnan E, Capuzzo E, Fernandes TF, Foden J, Geider RJ, Gilpin LC, Huxham M, McQuatters-Gollop AL, Malcolm SJ, Saux-Picart S, Platt T, Racault M-F, Sathyendranath S, van

der Molen J, Wilkinson M (2013) Framework for understanding marine ecosystem health. Mar Ecol- Prog Ser 494: 1–27.

Thoma H, Hanschultz G, Knorr E, Hutzinger O (1987) Polybrominated dibenzofurans (PBDF) and dibenzodioxins (PBDD) from the pyrolysis of heat brominated diphenyl ethers, biphenyls and plastic mixtures of these compounds. Chemosphere 16: 277–285.

Tomlinson PB (1986) The botany of mangroves. Cambridge University Press. Cambridge. 413 p.

Torres RR, Tsimplis MN (2012), Seasonal sea level cycle in the Caribbean Sea. J Geophys Res 117: C07011. DOI 10.1029/2012JC008159.

Twilley RR (1998) Mangrove wetlands. In: Messina, MG and Conner WH (eds), Southern Forested Wetlands. Ecology and Management Boca Raton, Florida: Lewis Publishers, pp 445-473.

Twilley RR, Day JW (2012) Mangrove Wetland. In: Day JW, Crump BC, Kemp WM, Yáñez-Arancibia A (eds), Estuarine Ecology. 2nd edition New Jersey: John Wiley & Sons, Inc, Hoboken, pp 165-202.

Twilley RR, Rivera-Monroy VH (2005) Developing performance measures of mangrove wetlands using simulation models of hydrology, nutrient biogeochemistry and community dynamics. J Coast Res 40: 79–93.

UICN / ORMA, 2001 Humedales de Centroamérica: síntesis de veintisiete estudios e iniciativas sobre educación, investigación, manejo y conservación de humedales y zonas costeras / ed. Francisco Pizarro, Claudia Gómez y Rocío Córdoba - 1 ed. – San José, C.R.

UN (United Nations) (1992) Report of the United Nations Conference on Environment and Development: Chapter 17 Protection of the oceans, all kinds of seas, including enclosed and semi-enclosed seas, and coastal areas and the protection, national use and development of their living resources. In: Conference on Environment and Development United Nations, Rio de Janeiro, 3-14 june 1992, pp 1–33.

UNEP (1994) Regional overview of Land-Based Sources of pollution in the Wider Caribbean Region UNEP(OCA)/CAR WG 14/4, 55 pp.

UNEP (1995) The Global Programme of Action for the Protection of the Marine Environment from Land-based Activities. UNEP (OCA)/LBA/IG2/7.

UNEP (2002) Central America and the Caribbean Regional Report, Regionally Based Assessment of Persistent Toxic Substances. UNEP, CH-1219, Geneva-Switzerland 133 pp.

UNEP (2004) Global International Water Assessment (GIWA), Caribbean Sea/Colombia & Venezuela, Central America & Mexico. GIWA Regional Assessment 3b, 3c. University of Kalmar, Kalmar, Sweden.

UNEP (2006) Isaza CFA, Sierra-Correa PC, Bernal-Velasquez M, Londono LM, Troncoso W Caribbean Sea/Colombia & Venezuela, Caribbean Sea/Central America & Mexico. GIWA Regional Assessment 3b, 3c. University of Kalmar, Kalmar, Sweden.

UNEP (2007) Global strategic directions for the regional seas programmes 2008-2012: Enhancing the role of the regional seas conventions and action plans. UNEP (DEPI)/RS 9. 6.

USEPA (2014) National Estuary Program 2008 - 2010 Program Evaluation Report USEPA.

Valdez Domingos FX, Azevedo M, Silva MD, Randi MAF, Freire CA, Silva de Assis HC, Oliveira Ribeiro CA (2007) Multibiomarker assessment of three Brazilian estuaries using oysters as bioindicators. Environ Res 105: 350–363.

Valencia A, Rojo-Bartolomé I, Bizarro C, Cancio I, Ortiz-Zarragoitia M (2017) Alteration in molecular markers of oocyte development and intersex condition in mullets impacted by wastewater treatment plant effluents. Gen Comp Endocrinol 245: 10-18.

Van Lavieren H, Metcalfe CD, Drouillard K, Sale PF, Bouchot GG, Reid R, Vermeulen L (2011) Strengthening Coastal Pollution Management in the Wider Caribbean Region. Proceedings of the 64th Gulf and Caribbean. Fisheries Institute 5: 1–11.

Vargas JA, Acuña-González J, Gómez E, Molina J (2015) Metals in coastal mollusks of Costa Rica. Rev Biol Trop 63: 1007–1019.

Vélez A (1982) Hermafroditismo en la ostra de mangle *Crassostrea rhizophorae*. Bol. Inst. Oceanogr. Univ. Oriente. 21: 129-132.

Viarengo A, Canesi L, Pertica M, Mancinelli G, Accomado R, Smaal A, Orunesu M, Accomando R, Smaal A, Orunesu M (1995) Stress on stress response: a simple monitoring tool in the assessment of a general stress syndrome in mussels. Mar Environ Res 39: 245–248.

Viarengo A, Lafaurie M, Gabrielides GP, Fabbri R, Marro A, Romeo M (2000) Critical evaluation of an intercalibration exercise undertaken in the framework of the MED POL biomonitoring program. Mar Environ Res 49: 1–18.

Viarengo A, Lowe D, Bolognesi C, Fabbri E, Koehler A (2007) The use of biomarkers in biomonitoring: a 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. Comp Biochem Physiol C Toxicol Pharmacol 146: 281–300.

Villalba A, Mourelle SG, Carballal MJ, López C (1997) Symbionts and diseases of farmed mussels *Mytilus galloprovincialis* throughout the culture process in the Rias of Galicia (NW Spain). Dis Aquat Organ 31: 127–139.

Villarroel E, Buitrago E, Lodeiros C (2004) Identification of environmental factors affecting growth and survival of the tropical oyster *Crassostrea rhizophorae* in suspended culture in the Golfo de Cariaco, Venezuela. Rev Cient Ciencias Vet 14: 28–35.

Vivas-Aguas L, Tosic M, Sánchez J, Narváez S, Cadavid B, Bautista P, Betancourt J, Parra J, Echeverri L, Espinosa L (2010) Diagnóstico y evaluación de la calidad ambiental marina en el Caribe y Pacifico colombiano. REDCAM Inf Téc 2010. INVEMAR. Santa Marta, 208 pp.

Vivas–Aguas LJ, Sánchez J, Cadavid B, Betancourt J, Quintero M, Moreno Y, Santana C, Cuadrado I, Ibarra K, Ríos M, Obando P, Sánchez D (2014) Diagnóstico y evaluación de la calidad de las aguas marinas y costeras del Caribe y Pacífico

colombianos. Serie de publicaciones del Invemar No. 4. Red de vigilancia para la conservación y protección de las aguas marinas y costeras de Colombia (REDCAM). Informe Técnico 2013, INVEMAR, Santa Marta, 314 p.

Wallner-Kersanach M, Theede H, Eversberg U, Lobo U (2000) Accumulation and Elimination of Trace Metals in a Transplantation Experiment with Crassostrea rhizophorae. Arch Environ Contam Toxicol 38: 40–45.

Walters BB, Rönnbäck P, Kovacs JM, Crona B, Hussain SA, Badola R, Pimavera JH, Babier E, Dahdouh-Guebas F (2008) Ethnobiology, socio-economics and management of mangrove forests: a review. Aquatic Botany 89(2): 220-236.

Wang C, Lee S-K (2007) Atlantic warm pool, Caribbean low-level jet, and their potential impact on Atlantic hurricanes. Geophys Res Lett 34: L02703.

Ward RD, Friess DA, Day RH, MacKenzie RA (2016) Impacts of climate change on mangrove ecosystems: a region by region overview. Ecosyst Health Sustain 2(4): e01211.

Watanabe I, Satsukawa R (1987) Formation of brominated dibenzofurans from the photolysis of flame retardant decabromodiphenyl ether in hexane solution by UV and sunlight. Bull Environ Contam Toxicol 39: 953–959.

Webster L, Tronczynski J, Korytar P, Booij K, Law R (2010) Determination of parent and alkylated polycyclic aromatic hydrocarbons (PAHs) in biota and sediment. ICES Tech Mar Environ Sci No 45, 26 pp.

Wedderburn J, McFadzen I, Sanger RC, Beesley A, Heath C, Hornsby M, Lowe D (2000) The field application of cellular and physiological biomarkers, in the mussel *Mytilus edulis*, in conjunction with early life stage bioassays and adult histopathology. Mar Pollut Bull 40: 257–267.

Wilhelmsson D, Thompson RC, Holmström K, Lindén O, Eriksson-Hägg H (2013) Charpter 6 Marine Pollution. In: Managing ocean environments in a changing climate. Elsevier Inc, Boston, pp 127–169.

Wolfe DA (1992) Selection of bioindicators of pollution for marine monitoring programmes. Chem Ecol 6: 149–167.

Yoo J-M, Carton JA (1990) Annual and interannual variation of the freshwater budget in the tropical Atlantic Ocean and the Caribbean Sea. J Phys Oceanogr 20(6): 831–845.

Zanette J, Jenny MJ, Goldstone JV, Parente T, Woodin BR, Bainy ACD, Stegeman JJ (2013) Identification and expression of multiple CYP1-like and CYP3-like genes in the bivalve mollusk *Mytilus edulis*. Aquat Toxicol 128-129: 101-112.

Zhou Q, Zhang J, Fu J, Shi J, Jiang G (2008) Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Anal Chim Acta 606: 135–150.

Ziarrusta H, Olivares M, Delgado A, Posada-Ureta O, Zuloaga O, Etxebarria N (2015) Multiscreening determination of organic pollutants in molluscs using matrix solid phase dispersion. J Chromat 1391A:18–30.

Zorita I, Apraiz I, Ortiz-Zarragoitia M, Orbea A, Cancio I, Soto M, Marigómez I, Cajaraville MP (2007) Assessment of biological effects of environmental pollution along the NW Mediterranean Sea using mussels as sentinel organisms. Environ Pollut 148: 236–50.

Zuykov M, Pelletier E, Harper DA (2013) Bivalve mollusks in metal pollution studies: from bioaccumulation to biomonitoring. Chemosphere 93. 201–208.

II. State of the art, hypothesis and objectives

1.1. State of the art

Mangroves are highly complex transitional coastal ecosystems that include 0.7% of world's tropical and subtropical forests and provide a unique ecosystem that represents complex food webs with a strong relationship with neighbouring habitats. In the lasts decades, mangrove-lined coastal ecosystems worldwide have been severely impacted by a combination of natural and anthropogenic stressors including chemical pollution.

In the Wider Caribbean Region (WCR), pollution monitoring programmes carried out in mangrove-lined coastal systems have been hitherto strictly based on chemical analysis. However, contaminants normally are present by cocktails of pollutants in multiple stress scenarios and therefore we need to carry our biological effects assessment together with the chemical analysis. For this purpose, pollution monitoring programmes based on the use of biomarkers as early warning signals of the biological deterioration, is highly recommended by international and intergovernmental conventions and regulations. Unfortunately, the monitoring capacities and facilities can be in many WCR countries far away from those required to conduct sophisticated and most-advanced biomarker-based monitoring using molluscs as sentinel species. However, the application of biomarkers is not necessarily inhibited by monetary and logistic constraints because effortless biomarkers can provide basic knowledge on animal health and water quality and are technically achievable everywhere.

Accordingly, and aware of the technical and logistic limitations (e.g., limited accessibility and difficulties for secure sample transportation and quality *in situ* processing), a toolbox of non-sophisticated and reliable biological effects endpoints was selected to assess the potential of the mangrove bivalve species, as sentinels for pollution monitoring in the WCR and neighbouring areas. This toolbox includes responses easy to measure at population and individual level and histological histopathological analyses; these latter allows scoring responses at systemic and tissue levels on the basis of cheap, solid and straightforward technology.

The Stress-on-Stress (SoS) response was recommended by ICES for monitoring programmes as an indicator of mussel health status. Further on, the successful application of SoS response as biomarker for environmental monitoring has been extended to other bivalve species. SoS response reveals the capacity of bivalves to survive on air and it is interpreted as a measure of resilience. It is not as sensitive as some ICES core biomarkers of general stress (e.g. lysosomal

membrane stability) but is more sensitive than others, and its methodology is simple, rapid and low-cost. Flesh Condition Index (FCI) reflects the physiological status of bivalves and it has been reported that it is reduced on pollutant exposure. Gamete development and reproductive impairment (e.g. alterations in sex-ratio, oocyte atresia, gonad histopathology) were included in the toolbox because the gametogenesis is crucial to understand bivalve health and because reproduction disturbances are well-known biological effects exerted by chemical pollutants. Digestive gland histopathology was also a core element of the toolbox, as a wide range of contaminants, including metals, pesticides and PAHs, is known to provoke histopathological alterations in this tissue.

Currently, pollution monitoring programmes carried out in USA, Canada, Europe and internationally use eastern oysters (*Crassostrea virginica*) and mussels (*Mytilus galloprovincialis*) as biomonitor and sentinel species. These species are not present in the WCR and therefore there is a need for alternative species suitable to be used in this region. In agreement with previous reports, the mangrove cupped oyster, *Crassostrea rhizophorae,* is a potential candidate biomonitor and sentinel for the WCR and other Latin America and tropical areas. Likewise, the slender marsh clam, *Polymesoda arctata,* in the Caribbean and the mangrove cockles, *Anadara tuberculosa and Larkinia grandis,* in the Pacific coast are potential alternative species for mangrove-lined coastal systems devoid or with scarce mangrove cupped oysters.

1.2. Hypothesis

Biomonitoring programmes for the assessment of chemical pollution and ecosystem health disturbance in mangrove-lined coastal systems in tropical and subtropical ecosystems, such as those of the Wider Caribbean Region and Latin America, can be achieved by applying analytical chemistry to determine pollutant tissue burdens together with low-cost and effortless biomarkers, recorded in autochthonous biomonitor and sentinel bivalve species.

1.3. Objectives

On order to test this hypothesis, the following general objectives are to be achieved:

1. To confirm the suitability and to promote the use of the mangrove cupped oyster, *Crassostrea rhizophorae*, as biomonitor species for pollution biomonitoring in mangrove-lined Caribbean coastal systems.
2. To develop a suitable, low-cost and effortless multi-biomarker approach for pollution monitoring in mangrove-lined Caribbean coastal systems using the mangrove cupped oyster, *C. rhizophorae* as sentinel species.
3. To determine the suitability of alternative species such as the slender marsh clam (*Polymesoda arctata*) and the mangrove cockles (*Anadara tuberculosa* and *Larkinia grandis*) as biomonitor and sentinel species for biomonitoring of pollution and associated ecosystem health disturbance in mangrove-lined Caribbean and Pacific coastal systems in Nicaragua.
4. To identify and solve logistic and technical problems associated to the implementation of biomarker-based pollution biomonitoring programmes in mangrove-lined coastal systems.

III. Results and Discussion

Chapter 1. Chemical pollution assessment in mangrove-lined Caribbean coastal systems using the oyster *Crassostrea rhizophorae* as biomonitor species

Scientific Contributions:

Aguirre-Rubí J, Garmendia L, Etxebarria N, Ortiz-Zarragoitia M, Luna-Acosta A, Soto M, Zaldibar B, Izagirre U, Espinoza F, Ahrens MJ, Marigómez I. 2013. Combined biomarker and chemical analysis as a tool to assess coastal ecosystem health in Caribbean mangroves: a pollution biological effects monitoring pilot program. *9th Iberian & 6th Iberoamerican Congress on Environmental Contamination & Toxicology CICTA, Valencia, July 1-4 2013*. Oral presentation.

Aguirre-Rubí J, Luna-Acosta A, Garmendia L, Etxebarria N, Ortiz-Zarragoitia M, Zaldibar B, Soto M, Izagirre U, Espinoza F, Villamil L, Ahrens MJ, Marigómez I. Combining tissue-level biomarkers and chemical analysis to assess ecosystem health in Caribbean mangroves: relevance for pollution monitoring. *25th Annual Meeting of the Society of Environmental Toxicology and Chemistry SETAC Europe, Barcelona, May 3-7 2015*. Oral presentation.

Aguirre-Rubí J, Luna-Acosta A, Etxebarria N, Soto M, Espinoza F, Ahrens MJ, Marigómez I. 2017. Chemical pollution assessment in mangrove-lined Caribbean coastal systems using the oyster *Crassostrea rhizophorae* as biomonitor species. ENVON SCI POLLUT RES, doi:10.1007/s11356-017-9159-2.

Keywords: *Crassotrea rhizophorae*, mangrove cupped oyster, bioaccumulation, ecosystem health, monitoring

Abstract: This paper aims to contribute to pollution biomonitoring in mangrove-lined Caribbean coastal systems using mangrove cupped oysters, *Crassostrea rhizophorae*, as a biomonitor species. Biomonitoring was carried out in 8 localities (3 in Nicaragua and 5 in Colombia) subjected to different types and levels of pollution. Oysters were collected during the rainy and dry seasons of 2012-2013 and the tissue concentrations of metals, PAHs, and POPs were determined. Low tissue concentrations of metals (except Hg) and PAHs, moderate-to-high tissue concentrations of Hg, HCHs, DDTs, detectable levels of chlorpyrifos, PCBs (mainly CB28, CB118, CB138 and CB 153) and BDE85 and negligible levels of musks were recorded in Nicaraguan oysters. Conversely, a distinct profile of POPs was identified in Colombia, where the tissue concentrations of HCHs and DDTs were practically negligible, chlorpyrifos and PBDEs were below detection limits, and the tissue levels of PCBs were low (CB28 and CB52). In contrast, noticeable tissue concentrations of synthetic musk fragrances were recorded in all the Colombian localities in the dry season, and the tissue concentrations of Ag, As, Cd, Pb and PAHs in several localities and in particular seasons ranged from moderate to extremely high. Regarding POPs, the values recorded for HCHs, DDTs and PCBs in Nicaraguan mangrove cupped oysters greatly exceeded the reference values in tissues of *C. rhizophorae* from the Wider Caribbean Region, whereas only the levels of PCBs were occasionally surpassed in Colombia. The differing contaminant profiles for oysters from Nicaragua and Colombia were distinguishable by distinct radar plots and principal component analysis, as well as evidenced in integrated pollution indices. Likewise, seasonality affected oyster tissue contaminant profiles and seemed to depend on seasonal variability in physicochemical properties of mangrove ecosystems as well as on diversification of human activities and practices between the rainy and the dry season, but seemingly not so markedly on oysters' reproductive condition. Considering the diversity, both geographical and environmental, of the studied scenarios, the present results confirm the suitability of *C. rhizophorae* as a biomonitor species at Caribbean regional scale for pollution monitoring programmes in mangrove-lined coastal systems, where seasonal variability is a major factor controlling pollutant mobility and bioavailability.

Resumen: El objetivo de este trabajo es contribuir al biomonitoreo de la contaminación en los sistemas costeros de manglares del Caribe, usando las ostras de manglar *Crassostrea rhizophorae* como una especie biomonitora. Se llevó a cabo el biomonitoreo en ocho localidades (tres en Nicaragua y cinco en Colombia) sujetas a diferentes tipos y niveles de contaminación. Se recoloectaron ostras durante las épocas de lluvia y seca del 2012 – 2013, y se determinaron las concentraciones en tejido de metales, PAHs y POPs. En los tejidos de las ostras de Nicaragua se registraron bajas concentraciones de metales (excepto Hg) y PAHs, concentraciones entre intermedias y altas de Hg, HCHs y DDTs, niveles detectables de clorpirifos, PCBs (sobre todo CB28, CB118, CB138 y CB 153) y BDE85, y niveles insignificantes de almizcles sintéticos. Por el contrario, en Colombia se identificó un perfil distinto de POPs en el que las concentraciones de HCHs y DDTs fueron insignificantes, Clorpirifos y PBDE estuvieron por debajo del límite de detección, y los niveles tisulares de PCBs (CB28 y CB52) fueron bajos. En cambio, se detectaron concentraciones significativas en tejido de almizcles sintéticos en todas las localidades de Colombia en la estación seca. Se determinaron concentraciones tisulares de Ag, As, Cd, Pb y PAHs en varias localidades y en épocas específicas en un rango entre moderadas y extremadamente altas. En lo que respecta a POPs, los valores de HCHs, DDTs y PCBs presentes en los tejidos de la ostra *C. rhizophorae* en Nicaragua excedieron por mucho los valores de referencia en ostra de la Región del Gran Caribe (WCR), mientras que en Colombia solo los niveles de PCBs sobrepasaron ocasionalmente los de referencia. Se distinguieron diferentes perfiles de contaminantes en las ostras de Nicaragua y Colombia mediante distintos diagramas de radar y mediante análisis de componentes principales, así como en los índices integrados de contaminación. Igualmente, los perfiles de contaminantes de los tejidos de las ostras se vieron afectados por la estacionalidad, lo que y parece depender de la variabilidad estacional de las propiedades fisicoquímicas de los ecosistemas de manglares y de la diversificación de la actividad humana entre la época lluvia y la época seca, pero no aparentemente de la condición reproductiva de las ostras. Considerando la diversidad tanto geográfica como ambiental de los escenarios estudiados, los resultados actuales confirman la idoneidad de *C. rhizophorae* como una especie biomonitora a escala regional en el Caribe para llevar a cabo programas de biomonitoreo de la contaminación en sistemas costeros de manglares, donde la variabilidad estacional es un factor importante que controla la movilidad y biodisponibilidad de los contaminantes.

1. Introduction

Mangroves are highly complex transitional coastal ecosystems that include 0.7% of world's tropical and subtropical forests and provide a unique ecosystem that represent complex food webs with a strong relationship with neighbouring habitats (Bosire et al., 2008; Nagelkerken et al., 2008; Giri et al., 2011). In the last four decades, mangrove ecosystems worldwide have been severely impacted by a combination of natural and anthropogenic stressors (Lewis et al., 2011; Bayen, 2012). Particularly, mangrove-lined coastal systems, an important natural resource for tourism, fisheries and storm protection in the Wider Caribbean Region (WCR), are enormously vulnerable to land-based human activities (UNEP, 1994).

Mining, industrial processes (smelters, oil refineries, chemical industry, shipyards), untreated sewage sludge and diffuse sources such as metal piping, traffic and combustion by-products from coal-burning power stations are the main anthopogenic sources of metal pollution in the WCR, with Cd, Hg, and Pb being the metallic pollutants of major concern (UNEP, 2006). Burning of waste, burning of vegetation for clearing land, and crude oil production and transportation result in elevated levels of polycyclic aromatic hydrocarbons (PAHs) at regional scale in the WCR (UNEP, 2002; Fernandez et al., 2007). The improper use and/or disposal of agrochemicals and industrial chemicals, combustion processes and release of by-products contributes to environmentally relevant levels of persistent organic pollutants (POPs), such as DDTs and other pesticides, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) (UNEP, 2006). Indeed, persistent pesticides constitute a major environmental concern in the WCR, where the use of agricultural pesticides has greatlhy increased during the last 30 years and DDT residues have become ubiquitous (Rawlins et al., 1998; Fernandez et al., 2007). It is worth noting that only ~10% of a pesticide are considered to be effective upon application, withthe remaining 90% and its derivatives expected to ultimately enter waterways and ultimately coastal ecosystems via runoff, misapplication and atmospheric transport (UNEP, 1994). PCBs have been used extensively since the 1930s in the WCR (e.g. in electrical transfomers). Correspondingly, they have been detected in water, sediment, seafood and biota samples (Fernandez et al., 2007), although data and monitoring capacity are limited at regional scale for these POPs. Certainly, PCBs and PBDEs are contaminants distinctive of urban and industrialised areas; however, they have been found at increasing levels in developing countries, as a result of poor waste management practices and improper burning (UNEP, 2006). In view of their environmental relevance at

regional scale, POPs ranked second in the priority rankings of Caribbean contaminant categories (GESAMP, 2001). However, with the exception of some pesticides, the monitoring of most of them relies on occasional analyses for research purposes or for impact assessment e.g. after accidental spills (UNEP, 2004; GEF-REPCar, 2011).

Pollution monitoring programmes have been carried out in the WCR coastal systems since the 1970's, under the auspices of intergovernmental institutions (Siung-Chang, 1997; UNEP, 2006). Thus, the UNEP Caribbean Environment Programme (CEP) was established in 1981 in order to promote regional cooperation for protection and sustainable development of the Caribbean Sea. Moreover, several tools (e.g. the protocol for Pollution from Land-based Sources and Activities) were agreed in the Cartagena Convention in 1983 in order to classify water bodies, establish legally binding standards, identify major sources of pollutants and prevent pollution (Siung-Chang, 1997). However, with notable exceptions (e.g. REDCAM in Colombia, Vivas-Aguas et al., 2014), monitoring activities at national scale are to our knowledge irregular and based on sporadic studies carried out by academics and research groups. A crucial issue in both national and regional monitoring programmes is that elsewhere employed technology and assessment methods (e.g. Asia-Pacific Mussel Watch, USA Mussel-Watch and EU Marine Strategy Framework Directive; Goldberg, 1975; Monirith et al., 2003; Kimbrough et al., 2008; Zampoukas et al., 2014) are not easily available or they are unaffordable and unsustainable; which often may be worsened by simple logistic hurdles (e.g. accessibility to sampling sites and sample transporation logistics). Other challenges include the paucity of baseline environmental data (e.g. concentrations of pollutants), the relatively poor baseline knowledge about contamination in mangrove-lined Caribbean coastal systems, and the limited human capacity and regional networking prospects (Rawlins et al., 1998; UNEP, 2004; 2006). Moreover, ongoing monitoring programmes are aimed at recording the concentration of pesticides and other POPs, PAHs and metals in seawater and sediments but not, only exceptionally, in biota (Sericano et al., 1995; UNEP, 2002; 2004; 2006; Rojas de Astudillo et al., 2005; Fernandez et al., 2007; Carvalho et al., 2009a; Vivas-Aguas et al., 2010; 2014; Castañeda-Chávez, 2011; Alfonso et al., 2013; Kanhai et al., 2014; 2015). Indeed, monitoring programmes based on the Mussel-Watch approach using bivalves or other target organisms as biomonitors (Goldberg, 1975; Sericano et al., 1995; Monirith et al., 2003; Kimbrough et al., 2008; 2009) are not systemically carried out, to our knowledge. If this approach is to be implemented in the WCR, research efforts must be addressed to solve logistic and technological questions and to select suitable biomonitor species.

Mangrove cupped oysters, *Crassostrea rhizophorae*, have been quite extensively investigated as potential biomonitor for pollution assessment in mangrove-lined coastal ecosystems (Nascimento et al., 1998; Wallner-Kersanach et al., 2000; Rebelo et al., 2005; Silva et al., 2003; 2006; Valdez-Domingos et al., 2007) because (a) they are filter-feeding sessile organisms inhabiting both clean and polluted sites attached either to mangrove roots or to coastal rocks; (b) they bioaccumulate high concentrations of pollutants in their tissues (Rainbow, 2006; Van Lavieren et al., 2011; Torres et al., 2012; Kanhai et al., 2014); (c) they respond to environmental insult (Silva et al., 2003; Rebelo et al., 2005; Torres et al., 2012); (d) they are widely distributed in tropical regions, e.g. from Caribbean to Southern Brazil; and (e) they are easy to collect including a large range of size classes (Beeby, 2001; Fox, 2001; Basu et al., 2007; Valdez Domingos et al., 2007; Masson et al., 2010). Indeed, C. rhizophorae has already been employed as biomonitor species in pollution monitoring studies aimed at deciphering spatial and temporal trends in chemical pollutants in tropical coastal zones in Brazil (Silva et al., 2003; Rebelo et al., 2005; Torres et al., 2012).

The present investigation aims at expanding the use of the mangrove cupped oyster, *C. rhizophorae*, as biomonitor species for pollution biomonitoring in mangrove-lined Caribbean coastal systems. For this purpose, a pilot field study was carried out in 8 localities (3 in Nicaragua and 5 in Colombia) subjected to different types and levels of pollution, in two sampling campaigns during 2012-2013. Samples were collected in the rainy and dry seasons. The tissue concentration of metals, PAHs, and POPs in oysters was recorded as a measure of the nature and levels of bioavailable chemical pollutants and their seasonal variability in different representative scenarios of mangrove-lined Caribbean coastal systems including subtidal oyster reefs in coastal lagoons, intertidal prop roots of magrove trees and intertidal rocky shores.

2. Material and Methods

2.1. Sampling sites and sample collection

In Nicaragua, subtidal (<1 m depth) oyster reefs were studied in two localities (Fig. 1): Bluefields (sampling sites: Punta Lora and Half Way Cay) and Pearl Lagoon (sampling site: Pigeon Cay). Punta Lora was considered as a prospective reference site (far away from urban settlements) whilst Half Way Cay and Pigeon Cay were selected as potentially polluted areas

influenced by aquatic transport and urban discharges (GEF-REPCar, 2011; Ebanks-Mongalo et al., 2013). In Colombia, intertidal prop roots of mangrove trees were studied in Cartagena Bay and Barbacoas Bay (Fig. 2) and intertidal rocky shores in Santa Marta Bay (Fig. 3).

In Cartagena Bay, a mangle islet 200 m north of Isla Maparadita (0.5 Km offshore the Terminal of Cartagena Port) and Isla Brujas were selected as seemingly polluted sites, as shown in previous studies (Vivas-Aguas et al., 2010; 2014). Isla Brujas is an islet adjacent to the industrial zone of Mamonal (oil refineries, petrochemicals, and asphalt, cement and smelting plants) that also receives the direct impact of the Dique Channel. This channel was open 5 centuries ago to connect the Magdalena River with the Cartagena Bay for navigation and constitutes a major source of sediments and chemical pollution in the Cartagena Bay (Vivas-Aguas et al., 2014). In addition, Isla Barú in Barbacoas Bay was selected as a potential reference site; however, it also may be influenced by the Magdalena River to which it communicates by smaller channels via the Dique Channel since the 1950's (Gómez-Giraldo et al., 2009).

In Santa Marta Bay, the Marina Santa Marta was selected as a sampling site subject to strong anthropogenic influence (Garcia et al., 2012), whereas the nearby Taganga Harbour was a priori considered as a reference site. Sampling was carried out over one year (2012-2013) in the rainy season (October 2012) and in the dry season (March 2013). Seawater surface temperature was in the range of 30-32ºC during both seasons.

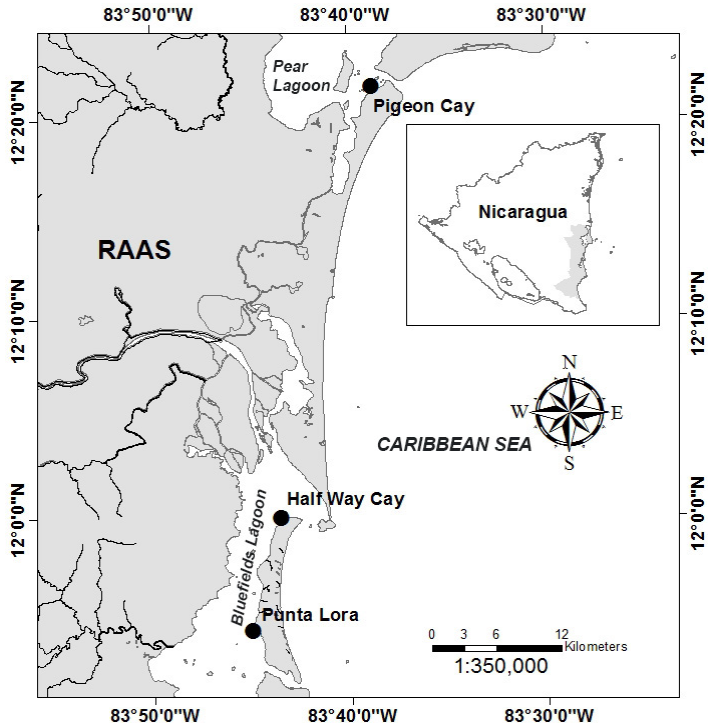


Fig. 1. Caribbean coastal maps from Nicaragua, showing the localities where oysters *Crassostrea rhizophorae* were collected in 2012-2013. Nicaraguan coast: Pigeon Cay (12°21'42.50"N-83°38'32.11"W); Half Way Cay (11°59'58.73"N-83°43'28.24"W) and Punta Lora (11°54'21.02"N-83°44'58.68"W).

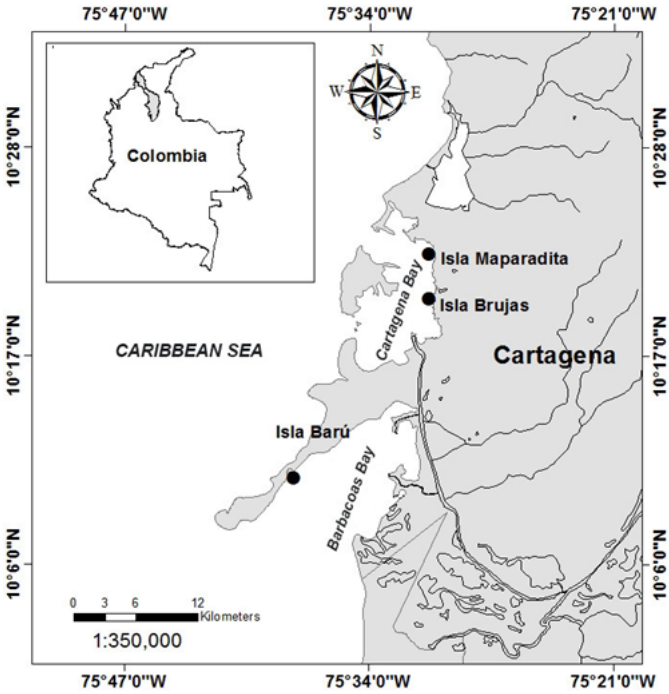


Fig. 2. Caribbean coastal maps from Cartagena-Colombia, showing the localities where oysters *Crassostrea rhizophorae* were collected in 2012-2013. Isla Barú (10°10'56.02"N-75°38'21.74"W); Isla Brujas (10°19'59.07"N-75°30'47.24"W) and Isla Maparadita (10°22'21.09"N-75°30'48.65"W) in Cartagena Bays.

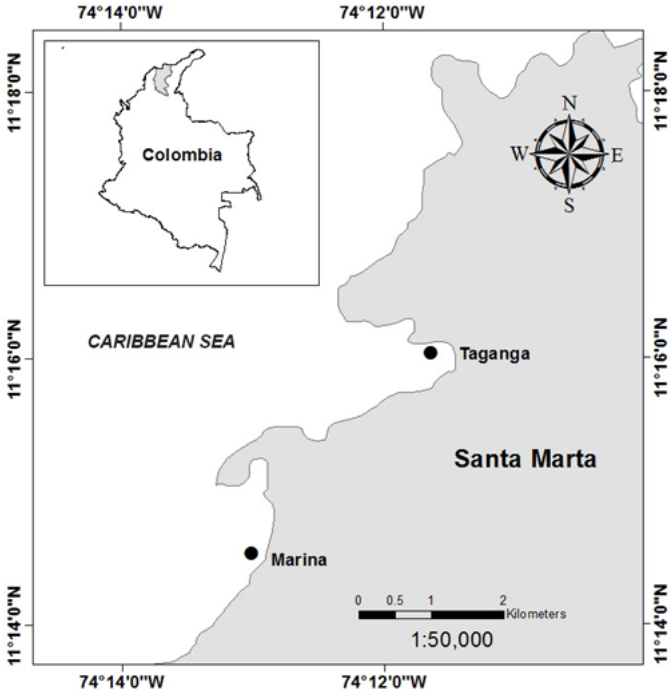


Fig. 3. Caribbean coastal maps from Santa Marta-Colombia, showing the localities where oysters *Crassostrea rhizophorae* were collected in 2012-2013. Marina Santa Marta (11°14'31.59"N-74°13'05.24"W) and Taganga (11°16'07.68"N-74°11'37.37"W) in Santa Marta Bay.

Up to 85 mangrove cupped oysters (*Crassostrea rhizophorae*) were collected per sampling site, of which 60 were used for biological effects assessment (Chapter 2) and the remaining 25 for the chemical analyses presented herein. Upon collection, oysters were placed in 15 L plastic boxes (2 individuals / L) in seawater at ambient temperature and transported to the laboratory (for 3-6 h) before processing. Due to logistic problems, sampling could not be conducted in Pigeon Cay during the dry season.

2.2. Tissue concentrations of contaminants

Pools of mangrove cupped oysters (25 individuals) were homogenised and freeze-dried before being stored (at -20ºC) and analysed. For the analysis of metals (Ag, Al, As, Cd, Cr, Cu, Hg, Ni, Pb, Ti, V and Zn), 100 mg of freeze-dried samples were digested in a microwave oven (Multi-wave 3000, Anton Paar, Austria) and the extracts were first filtered and then measured in an Inductively Coupled Plasma - Mass Spectrometer (ICP-MS; NexION 300 Perkin Elmer, USA) (Bartolomé et al., 2010; Navarro et al., 2010). For the analysis of the organic contaminants, 0.3 g of freeze-dried samples and 0.3 g of Florisil dispersant were manually blended in a glass mortar and were transferred to a 10-mL glass syringe containing 0.6 g of deactivated silica and 4.0 g of activated silica. The target analytes were eluted with 25 mL of dichloromethane, and the eluate was evaporated to dryness using N_2 blowdown and reconstituted to a final volume of 140 L of n-hexane. All these extracts were analysed by Gas Chromatography - Mass Spectrometry (GC–MS) using an Agilent 7890A gas chromatograph coupled to an Agilent 7000 triple quadrupole mass spectrometer and an Agilent 7693 autosampler (Agilent Technologies). The MassHunter WorkStation Acquisition Software (Version B.05.02/Build 5.2.365.0, Agilent Technologies, 2008) was used for data acquisition and automatic integration and quantification of the results. This method was designed to measure up to 40 non-polar or slightly non-polar organic pollutants such as PAHs, PCBs, PBDEs, organochlorine pesticides (OCPs), organophosphorus pesticides and musk fragrances (Ziarrusta et al., 2015).

2.3. Chemical pollution indices

Pollutant concentrations in mangrove cupped oyster tissues were compared with available environmental quality criteria for individual pollutants (OSPAR Commission, 2005; 2009; 2013; Kimbrough et al., 2008; Green et al., 2012): Background Reference Concentrations (BRCs); Background Assessment Criteria (BACs), Highest Background Concentrations (HBCs) and

Highest Low Concentrations (HLC). BRCs are intended to provide baseline or reference concentrations, and to describe environmental conditions under which there is no anthropogenic influence on the concentrations of the target substances in the environment (Davies, 2004; OSPAR Commission, 2005). BACs describe the threshold value for the background level, using data from reference sites (OSPAR Commission, 2005; 2009; 2013). HBCs correspond to the upper limit to Class I in the Norwegian marine pollution monitoring approach, which recognises five classes from Class I, insignificantly polluted, to Class V, extremely polluted (Green et al. 2012). HLCs correspond to the upper limit of the Low Concentration Range in the NOAA's mussel watch pollution monitoring; this recognises three concentration ranges (low, medium and high) for the tissue concentration of pollutants in oysters (Kimbrough et al., 2008).

The Chemical Pollution Index (CPI; Bellas et al., 2011; 2014; Beiras et al., 2012) was calculated for each site and season. For this purpose, Concentration Factors (CF) were calculated for each pollutant by dividing the tissue pollutant concentrations (C_{tiss}) by the corresponding environmental quality criterion (C_{eqc}): $CF=C_{tiss}/C_{eqc}$. Then, the following formula was applied: $CPI=\Sigma_i[log(CF_i)]$. Likewise, the Pollution Load Index (PLI; Tomlinson et al., 1980) was computed for each site and season by obtaining the n-root from the n-CFs that were obtained for all the pollutants, according to the following equation: $PLI = \sqrt[n]{CF_1 \, X \, CF_2 \ldots \ldots CF_n}$.

2.4. Statistical analyses

Statistical analyses were carried out with the aid of SPSS version 22 statistical package (IBM SPSS, Armonk, NY, USA). The normality of data distribution (Shapiro-Wilk's test) and homogeneity of variance (Levene's test) were determined before proceeding with subsequent analyses. Due to the high variability between samples, even after normal distribution transformation of data, non-parametric analyses were carried out. Significant differences between localities and seasons in pollutant tissue concentrations in oysters were analyzed by the Z score test (p<0.05). Pearson's correlation between tissue concentrations of pollutants was carried out for Nicaraguan (N=5) and Colombian (N=10) oysters, separately; also between PLI and CPI for the complete set of studied localities. Principal component analysis (PCA) was performed (N. variables = 43) on the basis of leverage correlation after normalization and standardisation of the data, using "*The Unscramble" v. 7.1* software (Camo, Norway).

3. Results

3.1. Nicaragua

Overall, metal tissue concentrations were not high (Table 1) and after applying the Z score test differences between seasons could only be established with a significance level of $p<0.1$. Likewise, metal tissue concentrations were slightly higher in Half Way Cay than in Punta Lora, and statistical significance was established with $p<0.1$, especially in the rainy season (Table 1). PAH tissue concentration was apparently higher in the dry season than in the rainy season (Table 2). In the rainy season, the highest values were recorded in Pigeon Cay and the lowest in Punta Lora, with Half Way Cay values in between (Table 2). In the dry season, tissue PAH concentration increased up to 8 fold in Punta Lora but remained seemingly unchanged in Half Way Cay (Table 2). The most relevant PAHs found were acenaphthylene, acenaphthene, pyrene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and dibenzo[ah]anthracene, especially in Pigeon Cay in the rainy season but also, to a lesser extent, in Half Way Cay and Punta Lora in the dry season (Table 2).

The sum of the 16 USEPA PAHs (except napthalene) was much lower in Punta Lora during the rainy season than in any other Nicaraguan sample, which was accompanied by low values of $\sum_{HMW}$PAHs and $\sum_{LMW}$PAHs and a high $\sum_{LMW}$PAH/$\sum_{HMW}$PAHs ratio (Table 2). Unlike in the case of PAHs, POP tissue concentrations were higher in the rainy season than in the dry season (Table 3); nevertheless, significant differences after applying the Z score test could only be established at the level of $p<0.1$ in most of the cases. HCHs and DDTs and their derivatives were recorded in oyster tissues in all the samples analyzed, irrespective of the locality and the season (Table 3). Interestingly, the highest tissue concentrations of pesticides (α-HCH, β-HCH and DDT derivatives such as 4,4-DDE) were recorded in Punta Lora in the rainy season (Table 3). Musk fragrances were always below detection limits. The tissue concentrations for CB28, CB118 and CB153 were high and similar between localities and seasons, whilst for other individual PCBs (CB52, CB101, CB138 and CB180) tissue concentrations were higher in the dry season than in the rainy season, and especially high in Pigeon Cay (Table 3). Only one of the eight analysed PBDEs (BDE85) was found in oyster tissues at measurable levels; this showed markedly high values in the rainy season in Pigeon Cay and Punta Lora (Table 3). According to the Pearson's correlation analysis carried out (Tabla 4), there existed association (a) amongst $_{LMW}$PAHs (other than acenaphthylene and pyrene) and some $_{HMW}$PAHs, such as benzo[a]anthracene, chrysene, benzo[b]fluoranthene+benzo[k]fluo-ranthene, as well as As, chlorpyrifos and PCBs; (b) amongst

benzo[a]pyrene, benzo[ghi]pyrene and indeno[1,2,3-cd]pyrene; and (c) between $_{HMW}$PAHs and HCHs (+) and $_{HMW}$PAHs and DDE (-).

The pollutant profile for each locality and season is illustrated in radar plots in Fig. 4. For this purpose, the following variables were used to construct the radar plots using a spreadsheet: (a) sum of tissue concentrations of metals upon removal of outliers such as Al, Cu, Ti and Zn; (b) sum of 16 US EPA priority PAHs (USEPA, 2001) except naphthalene; (c) sum of musks (HHCB +AHTM); (d) sum of 7 ICES PCBs (OSPAR Commission, 2010); and (e) sum of OCPs (HCHs + DDTs). It can clearly be seen that (a) pollutant tissue concentrations were higher in the rainy season than in the dry season in all localities; (b) PCBs and OCPs were the most representative pollutants; and (c) OCPs seem to constitute a potential problem were elevated in Punta Lora during the rainy season.

Table 1. Metal tissue concentration in *Crassostrea rhizophorae* (µg/g) from shallow subtidal oyster reefs of Nicaraguan mangrove lagoons. The superscript letters (a, b and c) indicate roughly significant differences between groups (p<0.1). LOD, limit of detection.

	LOD	RAINY SEASON			DRY SEASON	
		Pigeon Cay	Half Way Cay	Punta Lora	Half Way Cay	Punta Lora
Ag	0.0015	1.88^{a}	2.08^{a}	2.98^{b}	0.92^{a}	0.89^{a}
Al	15.3	469.67^{a}	665.83^{b}	225.42^{c}	408.77^{a}	488.05^{a}
As	0.01	6.81^{a}	4.23^{b}	2.96^{b}	4.39^{b}	4.59^{b}
Cd	0	1.39^{a}	2.50^{a}	1.78^{a}	1.78^{a}	3.59^{b}
Cr	0.22	0.79^{a}	1.04^{b}	0.36^{a}	0.54^{a}	0.39^{a}
Cu	0.11	557.13^{a}	541.19^{a}	220.02^{b}	487.66^{a}	357.22^{a}
Hg	0.004	0.16^{a}	0.18^{a}	0.12^{a}	0.08^{b}	0.13^{a}
Ni	0.54	1.00^{a}	1.21^{a}	0.54^{b}	0.75^{a}	1.38^{a}
Pb	0.015	0.25^{a}	0.33^{a}	0.21^{a}	0.26^{a}	0.39^{b}
Ti	0.94	18.32^{a}	24.17^{b}	11.15^{a}	11.27^{a}	12.68^{a}
V	0	1.43^{a}	2.07^{b}	0.73^{a}	1.07^{a}	1.44^{a}
Zn	2.36	1525.61^{a}	2544.21^{b}	936.76^{a}	1945.92^{a}	1490.22^{a}
∑Metals		**2584.44**^{a}	**3789.04**^{b}	**1403.03**^{c}	**2863.41**^{a}	**2360.97**^{a}

Positive CPI values, always <1, were recorded in Pigeon Cay in the rainy season and in Half Way Cay and Punta Lora in the dry season (Fig. 5a). Overall, the main pollutants contributing to these CPI values were As, Cd, benzo[a]pyrene, HCHs, DDTs and PCBs (SM 1). PLI values were

always <50, although PLI recorded in Pigeon Cay at the rainy season was 43.8 (Fig. 5b). Benzo[a]pyrene in the dry season and, generally, HCHs and DDTs were found to be the major contributors to PLI (SM 2).

Table 2. PAH tissue concentration in *Crassostrea rhizophorae* (ng/g) from shallow subtidal oyster reefs of Nicaraguan mangrove lagoons. The superscript letters (a, b and c) indicate significant differences between groups (p<0.05; p<0.1, when shown between brackets). The following compounds were under detection limits in all the samples (superscript numbers refer to the sums and indices given in the table where they are integrated): Flu (LOD=0.07)$^{(1)}$; Flr (LOD=0.34)$^{(1)}$. LOD, limit of detection; udl, under detection limits; n/a, not applicable.

	LOD	RAINY SEASON			DRY SEASON	
		Pigeon Cay	Half Way Cay	Punta Lora	Half Way Cay	Punta Lora
Acy (1)	1	30^{a}	30^{a}	udl^{b}	26^{a}	31^{a}
Ace (1)	1	52	29	29	38	38
Phe (1)	0.49	udl^{a}	udl^{a}	udl^{a}	23^{a}	83^{b}
Ant	1	22^{a}	5^{b}	4^{b}	10^{b}	9^{b}
Pyr (1)	1	29	udl	udl	udl	25
Benz[a]A (2)	1	24	6	5	13	12
Chr (2)	1	24^{a}	5^{b}	7^{b}	12^{b}	11^{b}
B[b]F +B[k]F (2)	1	23	udl	3	10	10
B[a]P (2)	1	64^{a}	50^{a}	udl^{b}	45^{a}	55^{a}
B[ghi]P	1	19^{a}	20^{a}	udl^{b}	17^{a}	22^{a}
Ind (2)	2	26^{a}	26^{a}	udl^{b}	23^{a}	28^{a}
D[ah]A (2)	2	29^{a}	34^{a}	udl^{b}	30^{a}	40^{a}
∑PAHs (16; except Naph)		**342**$^{(a)}$	**204**$^{(a)}$	**48**$^{(b)}$	**248**$^{(a)}$	**365**$^{(a)}$
$\sum_{HMW}$PAHs$^{\Sigma(2)}$ (carcinogenic)		**190**$^{(a)}$	**121**$^{(a)}$	**15**$^{(b)}$	**133**$^{(a)}$	**156**$^{(a)}$
$\sum_{LMW}$PAHs$^{\Sigma(1)}$ (%∑PAHs)		**111**$^{(a)}$ **(32%)**	**59**$^{(a)}$ **(29%)**	**29**$^{(a)}$ **(60%)**	**87**$^{(a)}$ **(35%)**	**177**$^{(b)}$ **(48%)**
Ind / B[ghi]P		**1.37**	**1.30**	**n/a**	**1.35**	**1.27**
$\sum_{LMW}$PAHs / $\sum_{HMW}$PAHs		**0.58**$^{(a)}$	**0.49**$^{(a)}$	**1.93**$^{(b)}$	**0.65**$^{(a)}$	**1.13**$^{(a)}$
Ind / (Ind + B[ghi]P)		**0.58**	**0.57**	**n/a**	**0.58**	**0.56**

Table 3. POP tissue concentration in *Crassostrea rhizophorae* (ng/g) from shallow subtidal oyster reefs of Nicaraguan mangrove lagoons. The superscript letters (a, b and c) indicate significant differences between groups ($p<0.05$; $p<0.1$, when shown between brackets). The following compounds were under detection limits in all the samples (superscript numbers refer to the sums and indices given in the table where they are integrated): 2.4-DDD (LOD=5)[3]; 4.4-DDT (LOD=50)[3]; 2.4-DDE (LOD=5)[3]; HHCB (LOD=8.8)[4]; AHTN (LOD=10)[4]; CB180 (LOD=1)[5]; BDE28 (LOD=7.2)[6]; BDE47 (LOD=1.3)[6]; BDE66 (LOD=0.94)[6]; BDE99 (LOD=6.6)[6]; BDE100 (LOD=7.5)[6]; BDE153 (LOD=50)[6]; BDE154 (LOD=50)[6]. LOD, limit of detection; udl, under detection limits; n/a, not applicable.

	LOD	RAINY SEASON			DRY SEASON	
		Pigeon Cay	Half Way Cay	Punta Lora	Half Way Cay	Punta Lora
α-HCH	50	60$^{(a)}$	udl$^{(a)}$	102$^{(b)}$	udl$^{(a)}$	udl$^{(a)}$
β-HCH	25	89$^{(a)}$	90$^{(a)}$	102$^{(a)}$	87$^{(a)}$	116$^{(b)}$
γ-HCH	2	25$^{(a)}$	14$^{(a)}$	9$^{(a)}$	17$^{(a)}$	30$^{(b)}$
δ-HCH	25	88^{a}	85^{a}	74^{b}	85^{a}	90^{a}
∑HCHs		**262**	**189**	**287**	**189**	**236**
4.4-DDE[3]	2	36^{a}	206^{a}	992^{b}	35^{a}	35^{a}
2.4-DDT[3]	25	113$^{(a)}$	101$^{(b)}$	90$^{(c)}$	105$^{(b)}$	99$^{(b)}$
∑DDTs$^{\Sigma(3)}$		**149**^{a}	**307**^{a}	**1082**^{b}	**140**^{a}	**134**^{a}
4.4-DDT/4.4-DDE		**0**	**0**	**0**	**0**	**0**
2.4-DDD/4.4-DDE		**0**	**0**	**0**	**0**	**0**
(2.4-DDD+4.4-DDE)/ ∑DDTs		**0.24**$^{(a)}$	**0.67**$^{(a)}$	**0.92**$^{(b)}$	**0.25**$^{(a)}$	**0.26**$^{(a)}$
∑OCPs		**411**^{a}	**496**^{a}	**1369**^{b}	**329**^{a}	**370**^{a}
Chlorpyrifos	2	11$^{(a)}$	udl$^{(b)}$	udl$^{(b)}$	5$^{(b)}$	udl$^{(b)}$
∑Musks$^{\Sigma(4)}$		**0**	**0**	**0**	**0**	**0**
CB28[5]	1	111$^{(a)}$	100$^{(a)}$	99$^{(a)}$	116$^{(b)}$	101$^{(a)}$
CB52[5]	1	18^{a}	udl^{b}	udl^{b}	udl^{b}	udl^{b}
CB101[5]	1	19^{a}	udl^{b}	udl^{b}	udl^{b}	udl^{b}
CB118[5]	1	124	121	111	121	118
CB138[5]	1	22^{a}	udl^{b}	udl^{b}	udl^{b}	udl^{b}
CB153[5]	1	47$^{(a)}$	29$^{(b)}$	28$^{(b)}$	36$^{(b)}$	35$^{(b)}$
∑PCBs$^{\Sigma(5)}$ **(PCB_7)**		**341**$^{(a)}$	**250**$^{(b)}$	**238**$^{(b)}$	**273**$^{(b)}$	**254**$^{(b)}$
BDE85[6]	1	325^{a}	udl^{b}	89^{b}	udl^{b}	udl^{b}
∑PBDEs$^{\Sigma(6)}$		**325**^{a}	udl^{b}	**89**^{b}	udl^{b}	udl^{b}
∑POPs		**1088**^{a}	**746**^{a}	**1696**^{b}	**607**^{a}	**624**^{a}

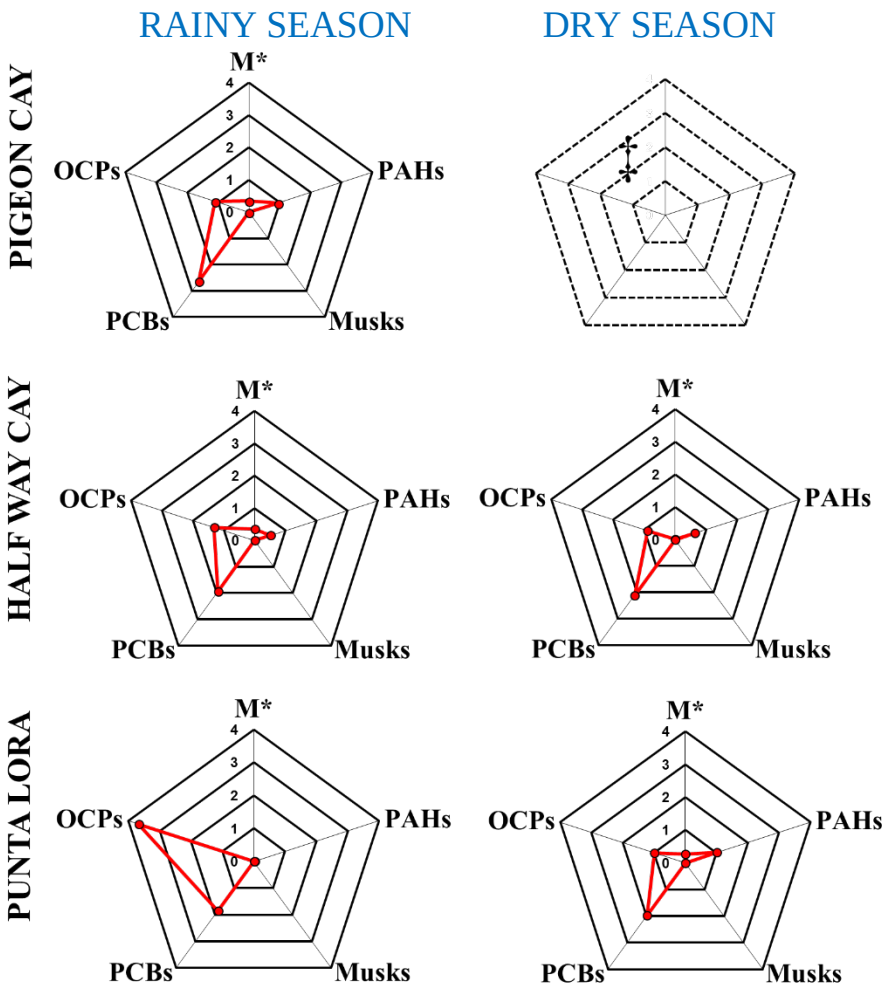


Fig. 4. Radar plots obtained using the total concentration of metals (other than Al, Cu, Ti and Zn: M*; µg/g dry-wt), the sum of the 16 USEPA priority PAHs (ng/g dry-wt), the sum of musks (ng/g dry-wt), the sum of the 7 ICES PCBs (ng/g dry-wt) and the sum of OCPs (ng/g dry-wt), as variables to depict the tissue pollutant profile (red lines) for each locality and each season in oysters collected from

3.2. Colombia

With the exception of oysters from Taganga Bay, metal tissue concentrations were higher in the rainy season than in the dry season and not very dissimilar amongst localities, with the exception of the Zn tissue concentration (Table 4; Z score test, $p<0.05$). In Taganga, the tissue concentrations of Al, As, Cr, Ni, Ti and V were significantly higher in the dry season than in the rainy season (Table 5). PAH tissue concentrations were higher in the dry season than in the rainy season except in Isla Brujas where exactly the opposite was observed (Table 6). In the dry season, the most abundant PAH was phenanthrene in all the localities, although fluorene and pyrene were also relevant in Isla Brujas (Table 6). In the rainy season, in contrast, fluorene and anthracene were more concentrated in all the localities, and the levels of carcinogenic PAHs, especially chrysene, but also pyrene, benzo[a]anthracene, benzo[ghi]pyrene and indeno[1,2,3-cd]pyrene, were notably elevated at Isla Brujas (Table 6). Likewise, POP tissue concentrations were noticeably higher in the dry season than in the rainy season, especially in Isla Brujas and Taganga (Table 7). The levels of HCHs, DDTs, chlorpyrifos and PBDEs in oyster tissues were always below the detection limit (Table 7). The tissue concentrations of PCBs and, more markedly, of musk fragrances were higher

in the dry season than in the rainy season in all the localities studied (Table 7). Besides, musk fragrances were below detection limits in Marina Santa Marta and Taganga Bay during the rainy season. According to the Pearson's correlation analysis carried out (Table 8), there was association (a) amongst different metals and of these with CB138; (b) amongst $\sum_{HMW}$PAHs; and (c) between musk fragrances and of these with CB52. Radar plots, constructed as above detailed, were used to illustrate the pollutant profile in Colombian oyster tissues (Fig. 6). It can be observed that pollutant levels were higher in the dry season than in the rainy season. Isla Brujas was the locality most influenced by PAHs. Musk fragrances gained relevance in the dry season in all localities, especially in Taganga. PCBs and OCPs lacked any relevance in the pollutant profile of Colombian oyster tissues.

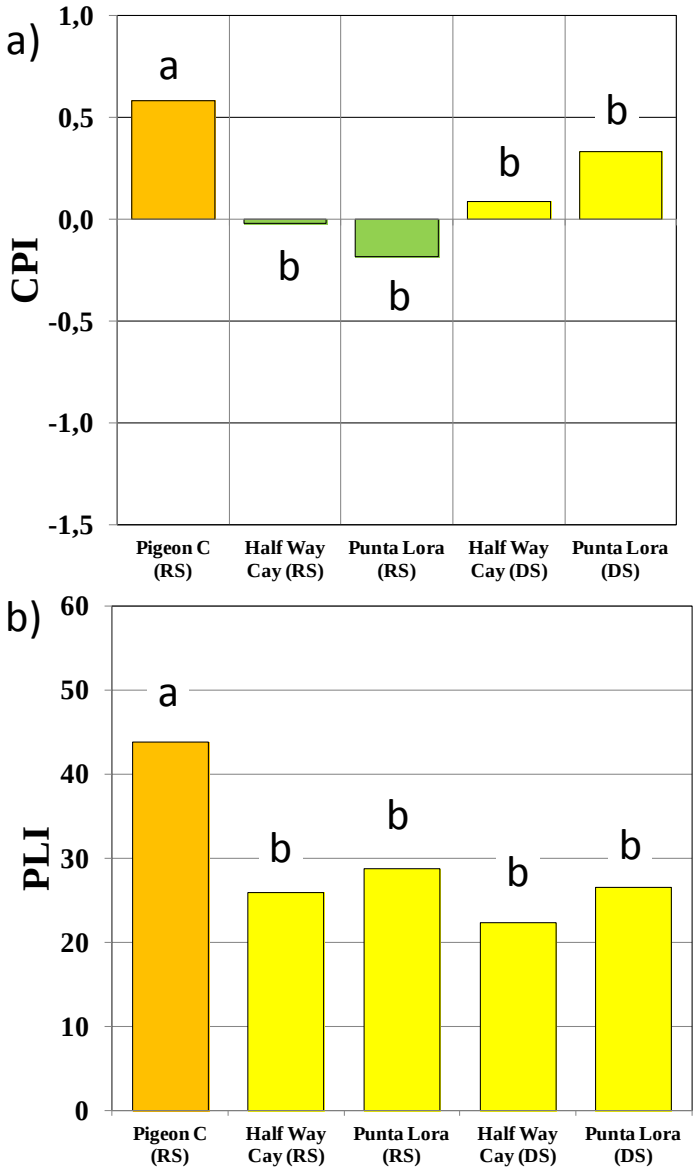


Fig. 5. (a) Chemical Pollution Index (CPI) in oysters from Nicaragua. CPI is categorized into three groups: 'low pollution' when CPI ≤ 0, 'moderate pollution' when 0 < CPI ≤ 1 and 'high pollution' when CPI > 1 (according to Bellas et al., 2011; 2014). (b) Pollution Load Index (PLI) derived from the tissue concentrations of pollutants in oysters from Nicaragua (Tomlinson et al., 1980). A PLI of 100 correspond to very contaminated sites and a PLI > 50 requires a potential risk and moderate contamination (Angulo, 1996). Letters (a and b) indicate significant differences between groups ($p<0.05$) after the Z-score test. RS, rainy season; DS, dry season.

Table 4. Matrix of Pearson's correlation coefficients between tissue concentrations of pollutants in mangrove cupped oysters, *C. rhizophorae*, from Nicaraguan mangrove lagoons collected at both rainy and dry seasons. Significant correlations (N=5; $p<0.05$) are indicated by asterisks (positive ones in yellow and negative ones in orange).

	Ti	Pb	As	Cu	Acy	Ace	Ant	Pyr	B[a]A	Chr	B[b]F +B[k]F	B[a]P	B[ghi] P	Ind	D[ah]A	$\sum_{HMW}$ PAHs	$\sum_{LMW}$ PAHs	γ-HCH	δ-HCH	4,4-DDE	2,4-DDT	Chlorp	CB 28	CB 52	CB 101	CB 138
V	0.89*																									
Hg	0.89*																									
Cd	0	0.92*																								
Cr	0.96*	0.12	0.41																							
Ni	0.5	0.93*	0.38																							
Ace	0	-0.08	0.93*	0.5	0.49																					
Ant	0.12	-0.15	0.96*	0.56	0.47	0.99*																				
B[a]A	0.05	-0.09	0.95*	0.55	0.51	1.00*	0.99*	0.77																		
Chr	-0.01	-0.22	0.91*	0.44	0.36	0.99*	0.99*	0.76	0.99*																	
B[b]F +B[k]F	-0.1	-0.14	0.89*	0.4	0.39	0.99*	0.97*	0.8	0.98*	0.99*																
B[a]P	0.47	0.56	0.82	0.82	0.98*	0.66	0.65	0.62	0.68	0.55	0.57															
B[ghi]P	0.45	0.74	0.63	0.75	1.00*	0.44	0.41	0.49	0.46	0.31	0.35	0.96*														
Ind	0.46	0.69	0.66	0.78	1.00*	0.48	0.46	0.49	0.51	0.36	0.39	0.97*	1.00*													
D[ah]A	0.38	0.8	0.53	0.67	0.97*	0.35	0.31	0.44	0.37	0.21	0.26	0.92*	0.99*	0.98*												
$\sum_{HMW}$PAHs	0.34	0.47	0.88*	0.78	0.93*	0.78	0.76	0.71	0.79	0.68	0.7	0.98*	0.91*	0.93*	0.86											
$\sum_{LMW}$PAHs	-0.14	0.72	0.5	0.17	0.68	0.53	0.42	0.79	0.51	0.41	0.51	0.69	0.72	0.7	0.75	0.74										
α-HCH	-0.26	-0.76	-0.13	-0.53	-0.8	0.03	0.08	0	0	0.18	0.12	-0.66	-0.83	-0.81	-0.89*	-0.57	-0.51									
γ-HCH	-0.03	0.63	0.68	0.29	0.71	0.69	0.6	0.89*	0.66	0.58	0.66	0.77	0.74	0.73	0.73	0.82	0.97*									
δ-HCH	0.3	0.71	0.71	0.66	0.96*	0.59	0.55	0.66	0.6	0.47	0.52	0.96*	0.97*	0.97*	0.96*	0.95*	0.84	0.87*								
4,4-DDE	-0.29	-0.57	-0.7	-0.78	-0.97*	-0.59	-0.55	-0.49	-0.61	-0.47	-0.51	-0.96*	-0.96*	-0.97*	-0.94*	-0.95*	-0.71	-0.74	-0.96*							
2,4-DDT	0.37	0.04	0.93*	0.88*	0.75	0.85	0.88*	0.53	0.88*	0.81	0.79	0.85	0.68	0.73	0.59	0.89*	0.38	0.53	0.71	-0.8						
Chlorpyrifos	0.09	-0.4	0.86	0.59	0.33	0.91*	0.94*	0.51	0.93*	0.94*	0.90*	0.5	0.26	0.31	0.14	0.61	0.16	0.34	0.35	-0.45	0.87*					
CB28	-0.21	-0.31	0.53	0.55	0.36	0.64	0.63	0.12	0.67	0.63	0.63	0.42	0.3	0.35	0.26	0.52	0.15	0.21	0.34	-0.56	0.73	0.77				
CB52	0.28	-0.3	0.89*	0.49	0.28	0.88*	0.93*	0.68	0.88*	0.92*	0.87*	0.48	0.21	0.26	0.09	0.57	0.18	0.4	0.33	-0.3	0.76	0.90*	0.41			
CB101	0.28	-0.3	0.89*	0.49	0.28	0.88*	0.93*	0.68	0.88*	0.92*	0.87*	0.48	0.21	0.26	0.09	0.57	0.18	0.4	0.33	-0.3	0.76	0.90*	0.41			
CB118	0.54	0.25	0.84	0.97*	0.88*	0.66	0.7	0.41	0.71	0.59	0.58	0.92*	0.83	0.87*	0.76	0.91*	0.39	0.51	0.8	-0.89*	0.95*	0.67	0.62	0.56	0.56	
CB138	0.28	-0.3	0.89*	0.49	0.28	0.88*	0.93*	0.68	0.88*	0.92*	0.87*	0.48	0.21	0.26	0.09	0.57	0.18	0.4	0.33	-0.3	0.76	0.90*	0.41	1.00*	1.00*	
CB153	0.05	-0.09	0.95*	0.55	0.51	1.00*	0.99*	0.77	1.00*	0.99*	0.98*	0.68	0.46	0.51	0.37	0.79	0.51	0.66	0.6	-0.61	0.88*	0.93*	0.67	0.88*	0.88*	0.88*
BDE85	0.16	-0.48	0.73	0.27	0.01	0.77	0.83	0.59	0.77	0.85	0.79	0.22	-0.06	-0.01	-0.18	0.33	0.01	0.22	0.07	-0.03	0.56	0.82	0.29	0.96*	0.96	0.96*

Positive CPI values (<1) were only recorded in Isla Brujas in the rainy season (Fig. 7a), with As, Cd, benzo[a]anthracene, chrysene and benzo[ghi]pyrene as the main pollutants contributing to this CPI value (SM 1). PLI values were always <50, although PLI recorded in Isla Brujas at the rainy season was 44.0, and PLI values recorded in the dry season in Isla Brujas and Isla Maparadita were, respectively, 19.9 and 11.1 (Fig. 7b). Cadmium, benzo[a]anthracene, chrysene and benzo[ghi]pyrene were found to be the major contributors to PLI in Isla Brujas in the rainy season whilst in the dry season, the main contributors in both Isla Brujas and Isla Maparadita were HHCB, phenathrene and Cd (SM 2).

4. DISCUSSION

4.1. Metals

In Nicaragua, metal tissue concentrations in mangrove cupped oysters were low, according to proposed criteria for oysters and mussels (Silva et al., 2003, Kimbrough et al., 2008, Alfonso et al., 2013, Solaun et al., 2013), with some exceptions. Mercury was found at moderate levels in the dry season and in Punta Lora during the rainy season and at high levels in Half Way Cay and Pigeon Cay during the rainy season. This can be attributed to the presence of artisanal gold mining upstream the Escondido River (Dumailo, 2003; MARENA, 2010), which is a major source of Hg pollution (Cordy et al., 2011). Cadmium was found at intermediate levels in Punta Lora during the dry season, and Cu at intermediate levels in all the localities. Urban runoff and sewage are typical sources for these metals, especially when there is no sanitation system, as is the case (Silva et al., 2001). In addition, antifouling paints, widely used because navigation is the main transportation means in the area, are a potential source for Cu (Wallner-Kersanach et al., 2000). Overall, metal tissue concentrations were higher in Half Way Cay than in Punta Lora during the rainy season, which is consistent because Half Way Cay is located at the sediment deposition zone of the lagoon, whilst Punta Lora is along the outflow of the secondary channel of the lagoon. In Colombia, the tissue concentration of As, Cd, Cr, Cu and Zn in oysters was relatively high when compared with concentration reported in other Caribbean localities (Campos, 1990; Silva et al., 2001; Cogua et al., 2012). In addition, tissue concentration of Hg in Marina Santa Marta during the dry season, and the tissue concentration of Pb in Isla Barú and Isla Brujas in the rainy season and in Marina Santa Marta and Taganga at both seasons were moderate (Kimbrough et al., 2008). Extremely high Cd tissue concentration were recorded in Isla Barú and Isla Brujas during the rainy season and in

Isla Maparadita at the dry season, whilst moderate-to-high levels were found in Isla Brujas during the dry season and in Isla Maparadita during the rainy season, according to NOAA criteria for *C. virginica* (Kimbrough et al., 2008). The values greatly exceeded those reported for *C. corteziensis* from coastal lagoons in Mexico (Wider Caribbean Region) as indicative of polluted areas (5.34 µg Cd/ g dry-wt; Frias Espiricueta et al., 2009). The presence of high levels of Cd in Isla Brujas and Isla Maparadita can be easily explained by the industrial activities carried out in these localities as well as by the influence of sediment loads received from the Dique Channel. Conversely, the concentration of Pb, Cr and Cd in seawater was determined to be below detection limits (Vivas-Aguas et al., 2014), which suggests that these metals may be associated with sediments and particulate matter and therefore they could not be detected by seawater analysis. These findings highlight the need to use biomonitors for metal pollution assessment rather than seawater analysis, as established since the pionering proposal of Goldberg, (1975). Likewise, the high levels of Cd recorded in the Isla Barú oysters' tissues at the rainy season suggest that sediments and water of the Magdalena River are an important source of metal pollution, since metals were not detectable in seawater (Vivas-Aguas et al., 2014) but are detected in oyster tissues as a result of bioaccumulation.

In Marina Santa Marta oysters, the tissue concentration of As, Cu and Zn can be considered moderate (Kimbrough et al., 2008), as well as Ni tissue concentrations in Taganga oysters in the dry season. The main source of metal pollution in this area are likely to be port activitie, since local metal industries and mining are absent (Vivas-Aguas et al., 2014). Thus, antifouling paints and vessel repair activities in the marina constitute the most likely sources of Cu and Zn in Marina Santa Marta (Wallner-Kersanach et al., 2000). The medium tissue concentration of As in Marina Santa Marta oysters and, more specifically, the high concentration of this metal recorded in Taganga oysters during the dry season are difficult to explain. According to geochemical data, As is a major potential pollutant in Colombia, as it is widespread throughout the country in the form of arsenopyrite associated with widely disseminated gold ores (Alonso et al., 2013). However, specific measurements carried out in sediments of the Ciénaga Grande de Santa Marta swamp revealed very low As concentrations (Perdomo et al., 1998). Nevertheless, Taganga cannot be considered a pristine reference site because together with musk fragrances (see below) and the metal aforementioned, also the tissue levels of Cr were moderately high (e.g., 6.39 µg Cr/g dry-wt in *C. rhyzophorae* fromTaganga *vs.* 1-1.8 µg Cr /g dry-wt in *C. rhizophorae* from the nearby central Venezuelan coast; legal limit for shellfish consumption is 5 µg Cr/g dry-wt). The most

probable source of As, musk fragrances and Cr are municipal wastewater discharges from Santa Marta that are delivered into Taganga Bay via a submarine outfall. On the other hand, Cd and Pb tissue concentration in oysters were low, in agreement with the low concentration of these metals detected in seawater during the study period (Vivas-Aguas et al., 2014).

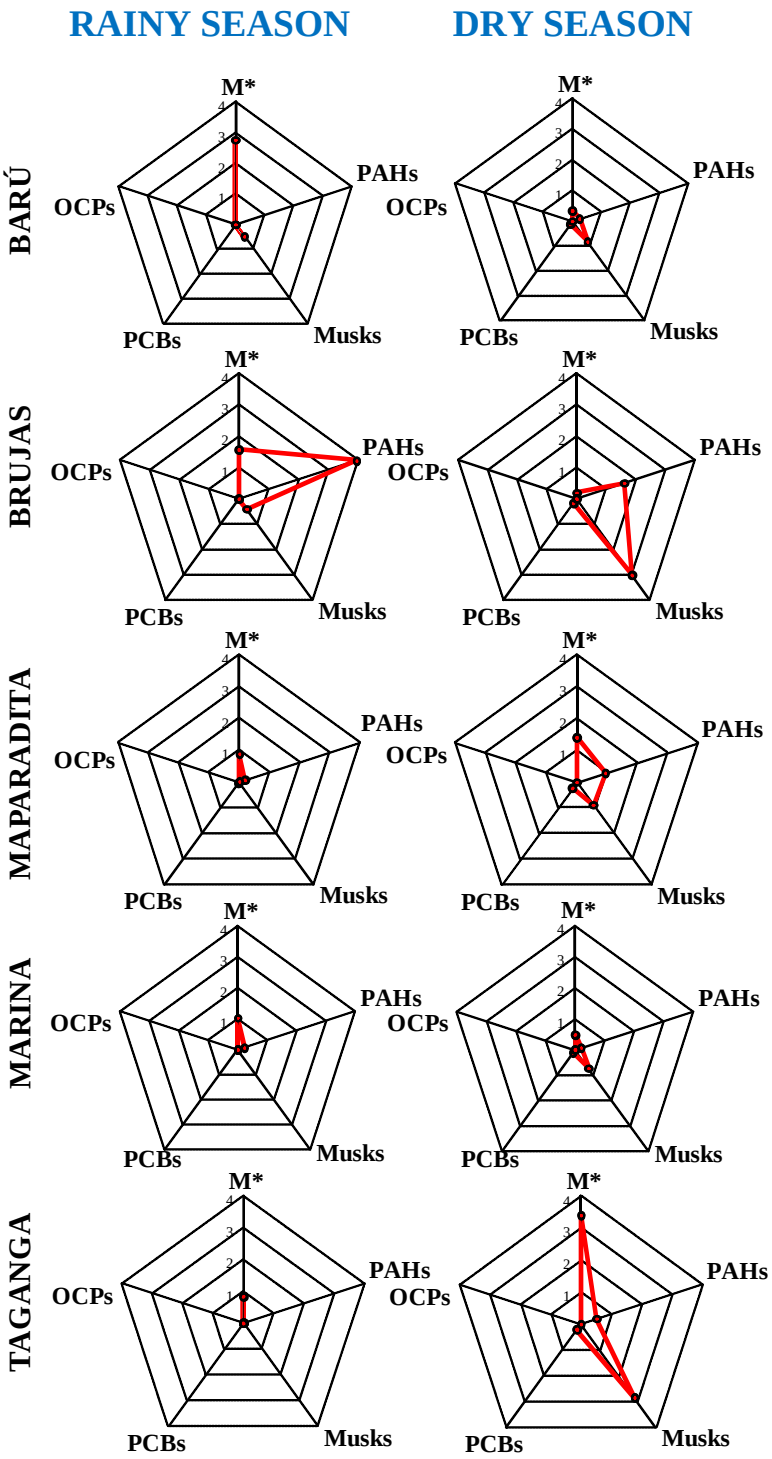


Fig. 6. Radar plots obtained using the total concentration of metals (other than Al, Cu, Ti and Zn: M*; µg/g dry-wt), the sum of the 16 USEPA priority PAHs (ng/g dry-wt), the sum of musks (ng/g dry-wt), the sum of the 7 ICES PCBs (ng/g dry-wt) and the sum of OCPs (ng/g dry-wt), as variables to depict the tissue pollutant profile (red lines) for each locality and each season in oysters collected from Colombia.

Table 5. Metal tissue concentration in *Crassostrea rhizophorae* (µg/g) from intertidal roots/docks of Colombian mangrove swamps. The superscript letters (a, b and c) indicate significant differences between groups ($p<0.05$; $p<0.1$, when shown between brackets). LOD, limit of detection.

	LOD	RAINY SEASON					DRY SEASON				
		Isla Barú	Isla Brujas	Isla Maparadita	Marina	Taganga	Isla Barú	Isla Brujas	Isla Maparadita	Marina	Taganga
Ag	0.0015	$1.08^{(a)}$	$1.52^{(b)}$	$0.89^{(a)}$	$0.21^{(a)}$	$1.24^{(a)}$	$0.48^{(a)}$	$0.41^{(a)}$	$1.47^{(c)}$	$0.06^{(d)}$	$0.28^{(a)}$
Al	15.3	351.01^{a}	317.84^{a}	260.03^{a}	609.10^{a}	522.14^{a}	190.17^{a}	90.45^{a}	58.35^{a}	302.04^{a}	949.40^{b}
As	0.01	8.17^{a}	4.97^{a}	4.67^{a}	13.06^{a}	7.82^{a}	6.87^{a}	6.48^{a}	7.04^{a}	10.60^{a}	33.03^{b}
Cd	0	28.03^{a}	16.60^{b}	6.95^{b}	0.92^{b}	1.08^{b}	2.54^{b}	3.43^{b}	15.88^{a}	0.76^{b}	1.14^{b}
Cr	0.22	0.79^{a}	1.40^{a}	4.71^{a}	3.21^{a}	4.44^{a}	2.11^{a}	0.28^{a}	0.23^{a}	1.22^{a}	6.39^{b}
Cu	0.11	62.42^{a}	184.73^{a}	82.11^{a}	206.10^{a}	105.18^{a}	66.45^{a}	38.72^{a}	54.89^{a}	465.80^{b}	113.04^{a}
Hg	0.004	$0.09^{(a)}$	$0.06^{(a)}$	$0.06^{(a)}$	$0.11^{(a)}$	$0.09^{(a)}$	$0.04^{(a)}$	$0.03^{(b)}$	$0.07^{(a)}$	$0.13^{(c)}$	$0.11^{(a)}$
Ni	0.54	1.07^{a}	1.09^{a}	0.66^{a}	1.27^{a}	1.70^{a}	0.41^{a}	0.49^{a}	0.43^{a}	0.64^{a}	2.34^{b}
Pb	0.015	$0.60^{a(a)}$	$0.54^{a(a)}$	$0.41^{a(a)}$	$0.69^{a(a)}$	$0.56^{a(a)}$	$0.37^{a(a)}$	$0.22^{a(b)}$	$0.15^{b(a)}$	$0.64^{a(a)}$	$0.79^{a(c)}$
Ti	0.94	10.97^{a}	8.72^{a}	5.99^{a}	32.72^{a}	30.74^{a}	5.74^{a}	3.47^{a}	2.33^{a}	21.07^{a}	72.64^{b}
V	0	1.25^{a}	1.23^{a}	1.25^{a}	1.82^{a}	2.29^{a}	0.76^{a}	0.63^{a}	0.38^{a}	1.14^{a}	4.16^{b}
Zn	2.36	495.46^{a}	1951.81^{a}	1401.70^{a}	2302.36^{a}	2093.90^{a}	1271.46^{a}	718.69^{a}	488.58^{a}	3541.70^{b}	1900.18^{a}
∑Metals		**960.93** ^{a}	**2490.53** ^{a}	**1769.43** ^{a}	**3171.57** ^{a}	**2771.19** ^{a}	**1547.40** ^{a}	**863.30** ^{a}	**629.80** ^{a}	**4345.81**^{b}	**3083.50** ^{a}

Table 6. PAH tissue concentration in *Crassostrea rhizophorae* (ng/g) from intertidal roots/docks of Colombian mangrove swamps. The superscript letters (a, b and c) indicate significant differences between groups ($p<0.05$; $p<0.1$, when shown between brackets). The following compounds were under detection limits in all the samples (superscript numbers refer to the sums and indices given in the table where they are integrated): [b]F+B[k]F (LOD=1)[(2)]; B[a]P (LOD=1)[(2)]; D[ah]A (LOD=2)[(2)]. LOD, limit of detection; udl, under detection limits; n/a, not applicable.

	LOD	RAINY SEASON					DRY SEASON				
		Isla Barú	Isla Brujas	Isla Maparadita	Marina	Taganga	Isla Barú	Isla Brujas	Isla Maparadita	Marina	Taganga
Acy [(1)]	1	udl	udl	udl	udl	udl	udl	udl	udl	udl	udl
Ace [(1)]	1	udl	udl	udl	udl	udl	udl	udl	udl	udl	udl
Flu [(1)]	0.07	0.8^{a}	16.1^{a}	udl^{a}	37.9^{b}	udl^{a}	udl	udl	udl	udl	udl
Phe [(1)]	0.49	$udl^{a(a)}$	$25.4^{a(a)}$	$udl^{a(a)}$	$udl^{a(a)}$	$udl^{a(a)}$	$117.4^{a(a)}$	$290.2^{a(b)}$	333.4^{b}	$106.2^{a(a)}$	$206.5^{a(a)}$
Ant	1	$40.2^{a(a)}$	$3.5^{a(b)}$	$1.5^{a(b)}$	49.6^{b}	$42.1^{a(a)}$	$udl^{a(b)}$	$udl^{a(b)}$	$udl^{a(b)}$	$udl^{a(b)}$	$udl^{a(b)}$
Flr [(1)]	0.34	udl^{a}	6.4^{a}	2.4^{a}	udl^{a}	udl^{a}	2.0^{a}	120.8^{b}	8.5^{a}	udl^{a}	udl^{a}
Pyr [(1)]	1	udl^{a}	59.1^{a}	8.5^{a}	0.9^{a}	udl^{a}	udl^{a}	144.8^{b}	udl^{a}	udl^{a}	udl^{a}
Benz[a]A [(2)]	1	udl^{a}	101.8^{b}	42.6^{a}	2.7^{a}	udl^{a}	udl^{a}	udl^{a}	udl^{a}	udl^{a}	udl^{a}
Chr [(2)]	1	udl^{a}	837.1^{b}	45.2^{a}	22.0^{a}	udl^{a}	udl^{a}	udl^{a}	udl^{a}	udl^{a}	udl^{a}
B[ghi]P	1	udl	202.8	udl	udl	udl	udl	udl	udl	udl	udl
Ind [(2)]	2	udl	47.3	udl	udl	udl	udl	udl	udl	udl	udl
∑PAHs (16; except Naph)		**41.0^{a}**	**1299.5^{b}**	**100.3^{a}**	**113.1^{a}**	**42.1^{a}**	**119.4^{a}**	**555.8^{a}**	**341.9^{a}**	**106.2^{a}**	**206.5^{a}**
$\sum_{HMW}$PAHs $^{\Sigma(2)}$ (carcinogenic)		**n/a**	**986.3**	**87.8**	**24.7**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**
$\sum_{LMW}$PAHs $^{\Sigma(1)}$ (%∑PAHs)		**0.8 (2%)**	**81.6 (6%)**	**10.9 (11%)**	**38.8 (34%)**	**n/a**	**n/a**	**265.6 (48%)**	**8.5 (2%)**	**n/a**	**n/a**
Ind / B[ghi]P		**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**
$\sum_{LMW}$PAHs / $\sum_{HMW}$PAHs		**n/a**	**0.1**	**0.1**	**1.6**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**
Ind / (Ind + B[ghi]P)		**n/a**	**0.2**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**

Table 7. POP tissue concentration in *Crassostrea rhizophorae* (ng/g) from intertidal roots/docks of Colombian mangrove swamps. The superscript letters (a, b and c) indicate significant differences between groups ($p<0.05$; $p<0.1$, when shown between brackets). The following compounds were under detection limits in all the samples (superscript numbers refer to the sums and indices given in the table where they are integrated): α-HCH (LOD=50)[3]; β-HCH (LOD=25)[3]; γ-HCH (LOD=2)[3]; δ-HCH (LOD=25)[3]; 4.4-DDE (LOD=2)[4]; 2.4-DDD (LOD=5)[4]; 2.4-DDT (LOD=25)[4]; 4.4-DDT (LOD=50)[4]; Chlorpyrifos (LOD=2); CB101 (LOD=1)[6]; CB118 (LOD=1)[6]; CB153 (LOD=1)[6]; BDE28 (LOD=7.2)[7]; BDE47 (LOD=1.3)[7]; BDE66 (LOD=0.94)[7]; BDE85 (LOD=1)[7]; BDE99 (LOD=6.6)[7]; BDE100 (LOD=7.5)[7]; BDE153 (LOD=50)[7]; BDE154 (LOD=50)[7]. LOD, limit of detection; udl, under detection limits; n/a, not applicable.

	LOD	RAINY SEASON					DRY SEASON				
		Isla Barú	Isla Brujas	Isla Maparadita	Marina	Taganga	Isla Barú	Isla Brujas	Isla Maparadita	Marina	Taganga
∑HCHs$^{\Sigma(3)}$		**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
∑DDTs $^{\Sigma(4)}$		**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
∑OCPs		**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
HHCB $^{(5)}$	8.8	11.1^{a}	9.6^{a}	0.4^{a}	udl^{a}	udl^{a}	19.6^{a}	71.0^{b}	20.9^{a}	17.5^{a}	67.1^{c}
AHTN $^{(5)}$	10	0.2$^{(a)}$	udl$^{(a)}$	udl$^{(a)}$	udl$^{(a)}$	udl$^{(a)}$	29.1$^{(a)}$	48.7$^{(b)}$	34.2$^{(a)}$	24.6$^{(a)}$	37.8$^{(a)}$
∑Musks $^{\Sigma(5)}$		**11.3^{a}**	**9.6^{a}**	**0.4^{a}**	**0.0^{a}**	**0.0^{a}**	**48.6^{a}**	**119.6^{b}**	**55.0^{a}**	**42.1^{a}**	**104.8^{a}**
CB28 $^{(6)}$	1	udl	3.7	5.7	0.6	udl	udl	udl	udl	udl	udl
CB52 $^{(6)}$	1	udl	udl	udl	udl	udl	14.4	21.0	27.6	13.5	24.1
CB138 $^{(6)}$	1	udl	udl	udl	udl	udl	udl	udl	udl	1.5	3.8
CB180$^{(6)}$	1	udl	udl	udl	udl	0.8	udl	udl	1.7	udl	udl
∑PCBs$^{\Sigma(6)}$ (PCB_7)		**0.0$^{(a)}$**	**3.7$^{(a)}$**	**5.7$^{(a)}$**	**0.6$^{(a)}$**	**0.8$^{(a)}$**	**14.4$^{(a)}$**	**21.0$^{(a)}$**	**29.3$^{(b)}$**	**15.0$^{(a)}$**	**27.9$^{(a)}$**
∑PBDEs$^{\Sigma(7)}$		**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
∑POPs		**11.3$^{a(a)}$**	**13.3$^{a(a)}$**	**6.1$^{a(a)}$**	**0.6$^{a(a)}$**	**0.8$^{a(a)}$**	**63.0$^{a(a)}$**	**140.6$^{a(b)}$**	**84.4$^{a(a)}$**	**84.0$^{a(a)}$**	**158.3^{b}**

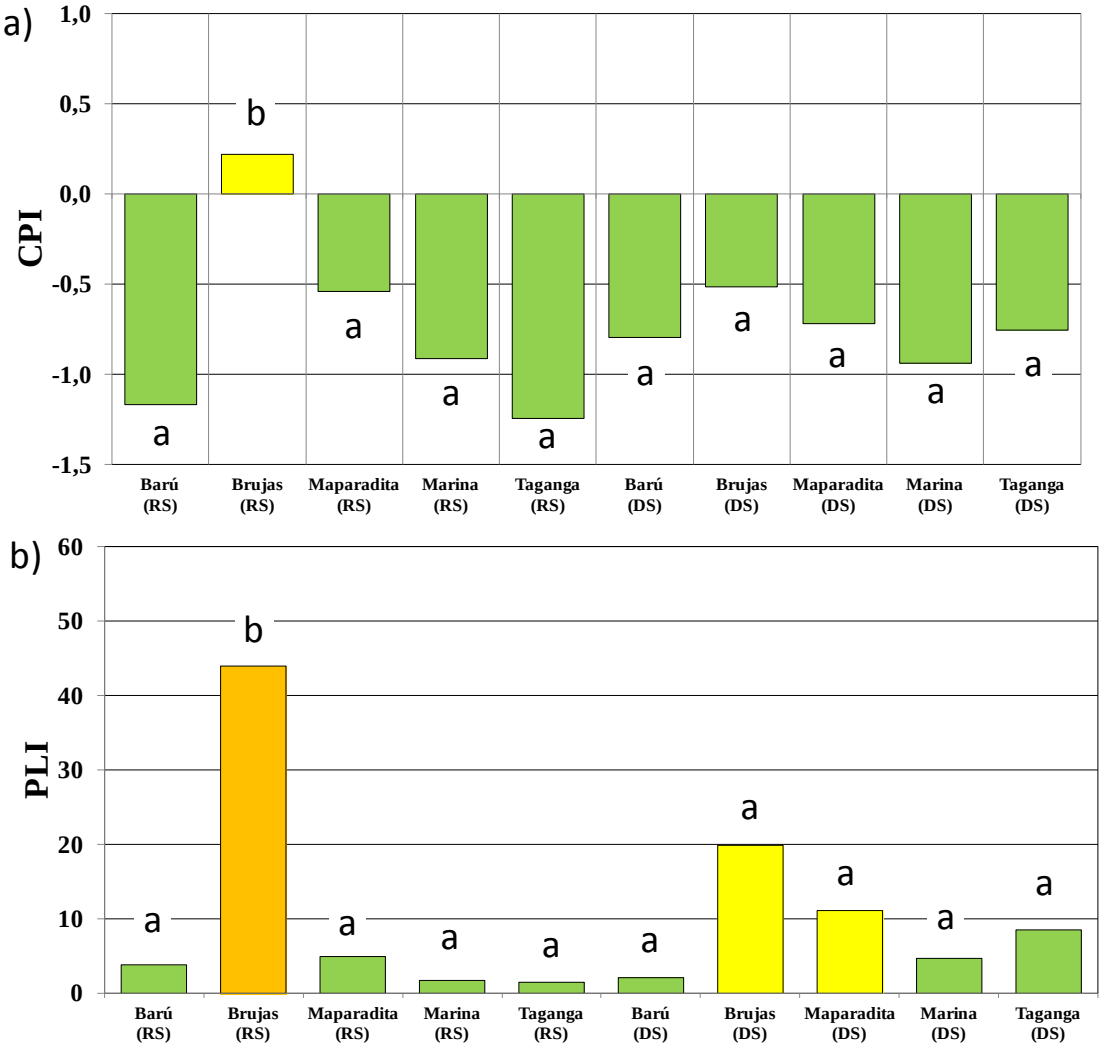


Fig. 7. (a) Chemical Pollution Index (CPI) in oysters from Nicaragua (a) and Colombia (b). CPI is categorized into three groups: 'low pollution' when CPI ≤ 0, 'moderate pollution' when 0 < CPI ≤ 1 and 'high pollution' when CPI > 1 (according to Bellas et al., 2011; 2014). (b) Pollution Load Index (PLI) derived from the tissue concentrations of pollutants in oysters from Colombia (Tomlinson et al., 1980). A PLI of 100 correspond to very contaminated sites and a PLI > 50 requires a potential risk and moderate contamination (Angulo, 1996). Letters (a and b) indicate significant differences between groups ($p<0.05$) after the Z-score test. RS, rainy season; DS, dry season.

Seasonality. Both in Nicaragua and Colombia, metal tissue concentrations were higher at the rainy season than at the dry season, except in Taganga where the tissue concentrations of Al, As, Cr, Ni, Ti and V were higher in the dry season than in the rainy season. In contrast, the concentrations of Cd, Cr and Pb in seawater from Cartagena Bay and Barbacoas Bay were lower in the rainy season in 2012 than in the dry season in 2013 (Vivas-Aguas et al., 2014). It is conceivable that greater loads of suspended matter during the rainy season contribute to enhanced metal uptake and accumulation (Rebelo et al., 2003). Thus, Rebelo et al., (2003) found that the tissue concentration of Cd and Zn were the highest during the rainy season in *C. rhizophorae* from Sepetiba Bay (Brazil) and Alfonso et al., (2013) reported that the tissue concentrations of Al, Cd

and Ni were highest during the rainy season in *C. rhizophorae* from Buche and Mochima estuaries in Venezuela. Likewise, the highest tissue concentration of Pb and Zn was recorded in the rainy season of *C. gigas* and *C. cortezienzes* (Jara-Marini et al., 2008; Osuna-Martínez et al., 2011). However, contradictory patterns have been reported regarding seasonality of metal tissue concentrations in mangrove oysters. The Pb tissue concentration was higher in the dry season than in the rainy season in mangrove cupped oysters from Trinidad and Tobago (Kanhai et al., 2014) and in mangrove oysters *C. cortezienzes* from the Gulf of California (Páez-Osuna and Osuna-Martínez, 2015); and Amado-Filho et al., (2008) did not find any significant difference in metal tissue concentrations between rainy and dry seasons in mangrove cupped oysters from Todos os Santos Bay (Brazil). In these cases, seasonal trends would depend on the local variability in hydrodynamics of sediment particles, independently of the season (Rebelo et al., 2003). For instance, Páez-Osuna and Osuna-Martínez, (2015) concluded that for the majority of the metals the tissue concentration in mangrove oysters from the Gulf of California coastal lagoons was greater during the dry season than during the rainy season due to regionally relevant upwelling; whilst for Hg, high levels in the rainy season were associated with transport of materials from the watershed to the lagoon.

4.2. Polycyclic Aromatic Hydrocarbons (PAHs)

Tissue concentrations of PAHs reported in bivalves from Nicaragua within the framework of the International Mussel Watch are in the range of <100 ng/g for ∑PAHs (Sericano et al., 1995). In the present study, slightly higher concentrations were found for total PAHs except for Punta Lora in the rainy season. Nevertheless, the tissue levels of total PAHs recorded can be regarded as low (e.g., in *C. virginica*, values of ∑PAHs in the range of 47-828 ng/g are considered low; Kimbrough et al., 2008). In the case of Colombia, the values of ∑PAHs recorded in all the localities in the rainy season and in Isla Barú and Marina Santa Marta in the dry season were around or below the range of <100 ng/g (Sericano et al., 1995). As a striking exception, oysters from Isla Brujas had much higher PAH concentrations (presenting medium levels according to Kimbrough et al., 2008). Isla Brujas is located in the vicinity of an oil refinery and terminal, as well as the Mamonal complex and, most relevantly, it receives direct sediment input from the Dique Channel. In agreement, the same location has been reported to present the highest concentrations of dissolved and dispersed hydrocarbons in seawater during the study period, especially in the rainy season (Vivas-Aguas et al., 2014). ∑PAHs were >100 ng/g in all the localities in the dry season;

however, the values recorded can be considered low according to the criteria of Kimbrough et al., (2008). Similarly, total PAHs in *C. rhizophorae* tissues were in the range of 109-362 ng/g dry-wt in Trinidad and Tobago (Kanhai et al., 2015) and in the range of 66.8-240.7 ng/g dry-wt in Guadeloupe in the Lesser Antilles (Ramdine et al., 2012). However, a potential risk cannot be disregarded (Neff et al., 2005) neither in Nicaragua nor in Colombia; as potentially carcinogenic PAHs (PAH_{HMW}; IARC, 1987) were recorded in Nicaragua (except in Punta Lora in the rainy season) and in Isla Maparadita and Marina Santa Marta and, most outstandingly, in Isla Brujas in the rainy season. On the other hand, PAH tissue concentrations were considerably greater in the dry season than in the rainy season, especially in Punta Lora for phenanthrene and pyrene, and in all the Colombian localities for phenanthrene.

Regarding the likely origin of the PAHs, the diagnostic ratios Phe/Ant and Flr/Pyr (Neff et al., 2005) could not be calculated as some of the compounds were below detection limits. Instead, the ratio of $_{LMW}$PAHs to $_{HMW}$PAHs was used as chemical indicator of the petrogenic vs pyrolytic origin the PAHs (Baumard et al., 1998; Soclo et al., 2000). The ratio was >1 in Punta Lora and in Marina Santa Marta in the rainy season, indicating a predominant petrogenic origin whilst it was <0.65 in Half Way Cay and Pigeon Cay at both seasons and, most markedly, in Isla Brujas and Isla Maparadita in the rainy season. These results revealed a pyrolytic origin of the PAHs in these localities. In Punta Lora in the dry season, both petrogenic and pyrolytic sources of PAHs seem to be superimposed, as the value of $_{LMW}$PAHs/$_{HMW}$PAHs was close to 1. PAH isomer pair ratios are indicative of whether PAHs are derived from biomass, coal, and petroleum combustion (Yunker et al., 2002; Oros and Ross 2005). Thus, Ind/(Ind+B[ghi]P) isomer pair ratio (Yunker et al., 2002; Oros and Ross 2005) showed that in mangrove cupped oysters from Punta Lora in the dry season and in Pigeon Cay and Half Way Cay the PAHs were derived primarily from biomass and coal combustion (Ind/(Ind+B[ghi]P)=0.56-0.58). Meanwhile, this ratio indicated that petroleum combustion was the main source of PAHs in Isla Brujas in the rainy season (Ind/(Ind+B[ghi]P)=0.20-0.50; Oros and Ross, 2005). The likely source for these high PAH concentrations is the nearby oil refinery and asphalt plant (Mamonal industrial zone). The same approach was used to conclude that petroleum combustion was a major source of PAHs in San Francisco Estuary sediments and mussels and that only minor amounts of the PAHs in bivalves were derived from biomass (e.g., grasses, wood, and wood soot) and coal combustion (Pereira et al., 1999; Oros and Ross, 2004; 2005).

Table 7. Matrix of Pearson's correlation coefficients between tissue concentrations of pollutants in mangrove cupped oysters, *C. rhizophorae*, from intertidal roots/docks of Colombian mangrove swamps collected at both rainy and dry seasons. Significant correlations (N=10; $p<0.05$) are indicated by asterisks (positive ones in yellow and negative ones in orange).

	Ag	Al	Ti	V	Hg	Pb	Cd	As	Cr	Ni	Cu	Zn	Flu	Phe	Ant	Flr	Pyr	B[a]A	Chr	B[ghi]P	Ind	$\sum_{HMW}$ PAHs	$\sum_{LMW}$ PAHs	HHCB	AHTN	CB 28	CB 52	CB 138
Al	-0.32																											
Ti	-0.41	0.96*																										
V	-0.27	0.97*	0.97*																									
Hg	-0.34	0.64	0.65*	0.54																								
Pb	-0.37	0.87*	0.77*	0.77*	0.78*																							
Cd	0.67*	-0.32	-0.43	-0.34	-0.16	-0.18																						
As	-0.47	0.85*	0.94*	0.88*	0.54	0.63*	-0.33																					
Cr	-0.22	0.80*	0.76*	0.84*	0.33	0.58	-0.49	0.62																				
Ni	-0.07	0.95V	0.91*	0.96*	0.53	0.77*	-0.18	0.77*	0.75*																			
Cu	-0.43	0.15	0.19	0.05	0.68*	0.49	-0.31	0.1	-0.06	0																		
Zn	-0.46	0.42	0.43	0.34	0.67*	0.64*	-0.58	0.25	0.32	0.29	0.89*																	
Flu	-0.14	0.28	0.13	0.07	0.24	0.35	-0.09	0.02	0.04	0.16	0.23	0.29																
Phe	-0.09	-0.29	-0.04	-0.16	-0.28	-0.57	-0.09	0.21	-0.32	-0.25	-0.23	-0.37	-0.38															
Ant	0.08	0.35	0.18	0.19	0.38	0.42	0.13	-0.05	0.13	0.37	-0.04	0.06	0.52	-0.61														
Flr	-0.17	-0.41	-0.3	-0.32	-0.57	-0.53	-0.13	-0.19	-0.4	-0.33	-0.29	-0.36	-0.16	0.53	-0.27													
Pyr	-0.03	-0.38	-0.33	-0.31	-0.61	-0.44	-0.03	-0.26	-0.4	-0.28	-0.22	-0.28	-0.03	0.38	-0.3	0.93*												
Benz[a]A	0.5	-0.11	-0.25	-0.11	-0.27	0.02	0.3	-0.31	-0.02	-0.03	0.07	0.09	0.25	-0.35	-0.23	-0.12	0.23											
Chr	0.49	-0.06	-0.18	-0.09	-0.21	0.07	0.33	-0.23	-0.16	0.04	0.13	0.13	0.32	-0.25	-0.17	-0.08	0.27	0.94*										
B[ghi]P	0.49	-0.06	-0.17	-0.08	-0.21	0.07	0.33	-0.22	-0.18	0.04	0.13	0.12	0.3	-0.23	-0.17	-0.07	0.28	0.92*	1.00*									
Ind	0.49	-0.06	-0.17	-0.08	-0.21	0.07	0.33	-0.22	-0.18	0.04	0.13	0.12	0.3	-0.23	-0.17	-0.07	0.28	0.92*	1.00*	1.00*								
$\sum_{HMW}$ PAHs	0.49	-0.07	-0.19	-0.09	-0.22	0.07	0.33	-0.24	-0.14	0.03	0.12	0.12	0.31	-0.26	-0.17	-0.08	0.27	0.95*	1.00*	1.00*	1.00*							
$\sum_{LMW}$ PAHs	-0.11	-0.35	-0.3	-0.31	-0.56	-0.43	-0.09	-0.23	-0.4	-0.28	-0.22	-0.28	0.06	0.4	-0.21	0.96*	0.98*	0.12	0.17	0.17	0.17	0.16						
HHCB	-0.39	0.13	0.32	0.27	-0.2	-0.12	-0.22	0.53	0	0.16	-0.22	-0.23	-0.35	0.74*	-0.5	0.66*	0.56	-0.28	-0.19	-0.16	-0.16	-0.2	0.56					
AHTN	-0.39	-0.18	0.08	-0.05	-0.25	-0.4	-0.31	0.32	-0.21	-0.2	-0.12	-0.21	-0.44	0.92*	-0.65*	0.58	0.41	-0.44	-0.34	-0.32	-0.32	-0.36	0.42	0.86*				
CB28	0.32	-0.13	-0.28	-0.11	-0.27	-0.06	0.14	-0.33	0.24	-0.14	-0.05	0.02	0.12	-0.42	-0.22	-0.15	0.06	0.78	0.52	0.47	0.47	0.55	-0.01	-0.38	-0.5			
CB52	-0.24	-0.13	0.13	0	-0.12	-0.39	-0.2	0.39	-0.17	-0.13	-0.12	-0.23	-0.43	0.96*	-0.64*	0.36	0.19	-0.43	-0.34	-0.31	-0.31	-0.35	0.2	0.75*	0.95*	-0.49		
CB138	-0.48	0.71*	0.84*	0.79*	0.54	0.58	-0.34	0.93*	0.55	0.64*	0.28	0.37	-0.21	0.26	-0.31	-0.18	-0.21	-0.21	-0.16	-0.15	-0.15	-0.17	-0.23	0.57	0.41	-0.24	0.46	
CB180	0.57	-0.29	-0.18	-0.22	-0.04	-0.51	0.18	-0.17	-0.21	-0.14	-0.26	-0.32	-0.22	0.45	-0.01	-0.11	-0.22	-0.22	-0.17	-0.15	-0.15	-0.17	-0.2	-0.14	0.15	-0.24	0.37	-0.21

Seasonality. In Nicaragua, $_{LMW}$PAHs were dominant (60% of the total PAHs) in mangrove cupped oysters from Punta Lora at the rainy season whilst $_{LMW}$PAH% values were below 35% in Half Way Cay and Pigeon Cay, and values in Punta Lora at the dry season reached 49%. Hellou et al., (1993) and Gaspare et al., (2009) associated the dominance of $_{LMW}$PAHs to waterborne exposure, contrasting with dominance of $_{HMW}$PAHs for exposure through sediment and suspended matter. According to these criteria, Punta Lora oysters were exposed predominantly to waterborne PAH fractions in the rainy season and those from Half Way Cay and Pigeon Cay in Nicaragua in both seasons. Interestingly, Punta Lora in the dry season seemingly constituted an intermediate case, with oysters exposed to a mixture of water-borne and sediment or suspended matter associated compounds ($_{LMW}$PAHs = 48% of PAH_{total}). In Colombia, $_{LMW}$PAH% values were below 35% in all the localities in the rainy season (in Taganga it could not be calculated) and in Isla Maparadita in the dry season, indicating that oysters were exposed predominantly to waterborne PAH fractions, according to the criteria proposed by Hellou et al., (1993) and Gaspare et al., (2009). Conversely, in Isla Brujas in the dry season ($_{LMW}$PAHs = 48% of PAH_{total}) oysters were seemingly exposed to a mixture of waterborne and sediment or suspended matter associated PAH compounds.

4.3. Persistent Organic Pollutants (POPs)

Organochlorine pesticides (OCPs). HCHs and DDTs are common pollutants in coastal areas and estuaries resulting mainly from agricultural practices and insect control (de Brito et al., 2002), which seems to be the case of Nicaraguan Caribbean mangroves. Bluefields Lagoon receives water load from the Escondido River basin, where corn, banana, sugarcane, oil palm and coconut intensive agriculture has promoted the elevated application of pesticides for the last 50 years (GEF-REPCar, 2011; Ebanks-Mongalo et al., 2013). In agreement, dieldrin, DDTs and terbufos were recently found in water, sediment and mangrove cupped oysters from Bluefields at concentrations over environmental quality criteria (Ebanks-Mongalo et al., 2013). Likewise, high concentrations of HCHs and DDTs have been reported in water and sediments in other Nicaraguan Caribbean localities (GEF-REPCar, 2011). The use of DDTs is banned but public health institutions have permission to use DDT for sanitation purposes and DDT derivatives are released to the environment, especially in the rainy season, to fight against mosquitoes (Bodin et al., 2011). Thus, ∑DDT in *C. virginica* from Coastal Lagoons in the Gulf of Mexico was highest in rainy season, when runoff transports DDT previously applied to nearby crop fields and urban areas (Castañeda-

Chávez, 2011). The levels of total DDTs recorded herein are higher, especially in Punta Lora in the rainy season, than the ∑DDTs reported in bivalves from Nicaragua within the framework of the International Mussel Watch (<10 ng/g dry-wt; Sericano et al., 1995). Likewise, they are much higher than in crab eggs (*C. granulate*) from impacted mangroves in Guanabara Bay at Brazil (98 ng/g dry-wt; de Souza et al., 2009). However, the values of ΣDDTs recorded are lower than those recorded in other areas; e.g. ΣDDTs of 156.2 ng/g dry-wt were reported in mangrove cupped oysters from the vicinity of Paranaguá City (Liebezeit et al., 2011) and ∑DDT as high as 864 ng/g dry-wt was reported in freshwater snails from Hanoy City water canals (Nhan et al., 2001). The predominance of DDE amongst DDT and its derivatives indicates that exposure is chronic or at least long lasting (Castañeda-Chávez, 2011). In agreement, DDE was a major contributor to the DDT in oysters from Paranaguá City, as these were chronically affected by sewage discharge. Alike, DDE was the main POP compound found in tissues of oysters, *C. virginica* and *C. rhizophorae*, from Términos lagoon at the Gulf of Mexico (Carvalho et al., 2009a; 2009b) and in mussels, *Perna perna*, from Brazilian bays (Galvao et al., 2014). Therefore, the high levels of 4.4-DDE found in oysters from Half Way Cay and, most outstandingly, in those from Punta Lora, suggest that the source of DDTs is most likely long-lasting and uninterrupted. Likewise, significant levels of HCHs were recorded in Nicaraguan mangrove cupped oysters, which were of lower magnitude than the DDT levels, especially in Punta Lora in the rainy season. As pointed out by Nhan et al., (2001), who also found lower tissue concentrations of HCHs than of DDTs in freshwater snails from water canals in the region of Hanoi, there is no reason to believe that the amounts of HCHs used as pesticide have been lower than those of DDT. However, the solubility in water of HCHs is much higher and their half-lives much shorter than in the case of DDT and, therefore, the average environmental levels are lower in the long term. Nevertheless, the values of ∑HCHs were similar to those reported in *C. virginica* and *C. rhizophorae* from Mexico (3-32 ng/g dry-wt; Carvalho et al., 2009a) and in bivalves from the Wider Caribbean region (12 ng γ-HCH/g dry-wt; Van Lavieren et al., 2011) but higher than those found in tissues of oysters from Senegal (1 ng /g dry-wt; Bodin et al., 2011) and in freshwater snails, *Angulyagra* sp. from water canals in the region of Hanoi (0.51 ng /g dry-wt; Nhan et al., 2001).

On the other hand, OCPs were below detection levels in the tissues of oysters collected from the Colombian localities in both seasons. In the early 2000's, DDT and its metabolites, heptachlor, aldrin and HCHs were widespread pollutants in surface waters in Santa Marta Bay as the result of runoff from upstream plantations of coffee and banana, but in the recent decade these chemicals

have been below detection limits in tributary rivers as result of regulatory restrictions for the use of these OCPs (Vivas-Aguas et al., 2014). Similarly, a marked reduction in the OCP levels in seawater and sediments have been recorded in Cartagena Bay and Barbacoas Bay during the last decade (Vivas-Aguas et al., 2010). Thus, in the studied Colombian localities, OCP concentrations in surface waters were below detection limits in the 2012-2013 rainy and dry seasons (Vivas-Aguas et al., 2014), in agreement with our present records of OCP tissue levels in mangrove cupped oysters.

Chlorpyrifos. Chlorpyrifos is a broad spectrum organophosphate insecticide widely used on food crops; however few data are available about its concentration in marine biota. The range of chlorpyrifos in the tissues of marine bivalves along the US coast, including oysters (*C. rhyzophorae* and other species) and mussels, ranges from "non-detected" to 53 ng/g dry-wt, with a regional average of 0.78 ng/g dry-wt (Wade et al., 1998). In the present study, this organophosphate pesticide was only detected in Nicaraguan mangrove cupped oysters (in Half Way Cay in the dry season and Pigeon Cay in the rainy season) in the range of 5-11 ng/g dry-wt and not in the Colombian localities. In agreement, chlorpyrifos was recorded in sediments and mangrove cupped oysters from Bluefields in the 2011 rainy season (Ebanks-Mongalo et al., 2013) but not in surface waters of Cartagena Bay in the 2012-2013 period (Vivas-Aguas etal., 2014). Thus, in Nicaraguan Caribbean mangroves, high levels of chlorpyrifos seem to be associated with currently ongoing agricultural practices. In Colombia, however, this organophosphate pesticide was detected in previous years at toxic levels in surface waters influenced by the Dique Channel input (intensive agriculture in the Magdalena River basin) and by agrochemical industry sited at the Harbour area (Vivas-Aguas et al., 2010); with very high levels in the 2011 rainy season but below detection limits in the 2012-2013 period (Vivas-Aguas et al., 2014). It seems that the environmental levels of this pesticide can be highly erratic with transient peaks after recent application or discharges followed by low levels after a while. Indeed, chlorpyrifos has low solubility in water (thus it is usuallyassociated to suspended organic matter) and a relative short half-life (approx. 7 days), and is volatile in water and metabolised by aquatic animals (Serrano et al., 1997). Consequently, the absence of a seasonal or geographical pattern can be associated to changes in the application and timing, so that detectable tissue concentrations might correspond to samples collected just after recent applications of the pesticide, as suggested by Wade et al., (1998). Nevertheless, this should not be overlooked because short pulses of chlorpyrifos can exert long-term toxicity to marine invertebrates (CCME, 2008).

Musk fragrances. Synthetic musks are ubiquitous contaminants in the marine environment. Galaxolide (HHCB) and Tonalide (AHTN) are the major musk fragrances in sewage discharged into estuaries. The tissue concentration of musks recorded in Isla Barú, Isla Brujas and Isla Maparadita in the rainy season are within the range of those previously reported for marine molluscs (e.g., <12 ng/g dry-wt for HHCB and <17 ng/g dry-wt for AHTN in North Sea mussels; Rüdel et al., 2006). However, much higher concentrations were recorded in the dry season (interestingly, the top touristic season) in all the localities, in the range of 15-70 ng/g dry-wt for HHCB and 25-50 ng/g dry-wt for AHTN. Nevertheless, these values are still much below those recorded by for HHCB in mussels (1700 ng/g lipid = ~140 ng/g dry-wt; assuming 1% fat and 80% hydration) and clams (3000 ng/g lipid = ~240 ng/g dry-wt; assuming 1% fat and 80% hydration) from densely populated and industrialised zones of Canada (Gatermann et al., 1999). The high HHCB and AHTN concentrations in Taganga Bay and Isla Brujas during the dry season are most probably due to wastewater discharges from nearby sewage outfalls.

Polychlorinated biphenyls (PCBs). The levels of total PCBs recorded in Nicaraguan *C. rhizophorae* were lower than those recorded in crab eggs from impacted mangroves in Guanabara Bay in Brazil (570 ng/g dry-wt; de Souza et al., 2009) but higher than ∑PCBs reported in bivalves from Nicaragua within the framework of the International Mussel Watch (<10 ng/g dry-wt; Sericano et al., 1995) and for freshwater snails from the region of Hanoi (<60 ng/g dry-wt; Nhan et al., 2001). PCBs are essentially of industrial origin and therefore the tissue concentrations found in Nicaraguan mangrove cupped oysters are much lower than those found in harbors and industrial areas of industrialised countries (e.g., 2500 ng/g in mussels from Marseille harbor in France; Villeneuve et al., 1999). Nevertheless, remarkable tissue concentrations of CB28, CB118 and CB153 were recorded in all the Nicaraguan localities and seasons whilst CB52, CB101, CB138 and CB180 were found at lower concentrations that were highest at the dry season and were especially high in Pigeon Cay. All these PCB congeners have been recognised as non- or poorly metabolised in molluscs and therefore they are bioaccumulated (Kannan et al., 1995; Nhan et al., 2001). Similarly, CB28 and CB153 were the two individual PCBs recorded at highest tissue concentrations in brown mussels (*Perna perna*) from Southeastern Brazil bays (Galvao et al., 2014). Data on seasonality are not conclusive. In the present study, no seasonal trend was observed for CB28, CB118 and CB153 tissue concentrations, but CB52, CB101, CB138 and CB180 peaked up at the dry season. In contrast, *C. gasar* collected from the Saloum Delta (Senegal) had

systematically higher PCB levels (by a factor of 2–3) during the rainy season compared to individuals sampled at the dry season (Bodin et al., 2011).

Polybrominated diphenyl ethers (PBDEs). PBDEs are bioaccumulative halogenated compounds used as flame retardants in automobile, textile and, mainly, electronics industries (Kimbrough et al., 2009) and are considered to be emerging environmental contaminants. BDE85 showed markedly high values in the rainy season in Pigeon Cay and Punta Lora, with values of total PBDEs in the range of 89-325 ng/g dry-wt. These values are much higher than previously reported in bivalves (Moon et al., 2007; Oros et al., 2007) and cannot be associated with a particular source at local scale.

Seasonality. The tissue concentrations of HCHs, DDTs, chlorpyrifos, PCBs and PBDEs in Nicaraguan mangrove cupped oysters were higher in the rainy season than in the dry season, especially for ∑OCPs in Punta Lora. In contrast, the levels of POPs in Colombian mangrove cupped oysters were higher during the dry season than during the rainy season, especially in Marina Santa Marta and Taganga. It seems, therefore, that the seasonal trend in the tissue concentration of POPs is not the same in Nicaraguan and Colombian oysters. In *C. gasar* from Senegal, high tissue concentrations of POPs in the rainy season were attributed to recent inputs from the surroundings though rainfall and river runoff (Bodin et al., 2011). Likewise, POP tissue concentrations in *C. virginica* (lipid base) from Coastal Lagoons in the Gulf of Mexico were also highest in rainy season (Castañeda-Chávez, 2011). It is conceivable that high POP tissue concentrations in the rainy season are the consequence of enhanced runoff that transports POPs from crop fields and urban areas to receiving waterways. This can be especially relevant in tropical countries where rainfall is intense during the rainy season and intensive agricultural practices lead to a high input of pesticides over watersheds (Daam and Van Den Brink, 2010), like in the Nicaraguan mangroves, and less relevant in more industrial areas such as the Colombian mangrove system studied herein.

4.4. Pollution biomonitoring and assessment

Principal Component Analysis of the tissue pollutant concentrations showed a 71% variance accounted for by 3 principal components (PC-1: 43%; PC-2: 17%; and PC-3: 11%; Fig. 8-10). Communality was high (>74%) for all the items. PC-1 was interpreted in terms of rural *vs.* urban sources of pollution, with high agricultural activities, aquatic transport and rural wastewater disposal in the positive part of its axis (with low-to-moderate scores but high correlation loadings for Ace, Acy, B[a]P, B[b]F+B[k]F, D[ah]A, Ind, γ-HCH, δ-HCH, β-HCH, 4.4-DDE, 2,4-DDT, CB28, CB118 and CB153) and urban pollutants in the negative part (with moderate scores and correlation loadings for Flr, AHTN and HHCB). PC-2 was interpreted in terms of metal pollution, essentially because scores for the tissue concentrations of Al, As, Cr, Ni, Pb, Ti, V and Zn were higher than the average (unlike for Cd and Ag), with high scores and high correlation loadings for Ni, Ti and V, and moderate scores and correlation loadings for Al, As, Cr, Pb, V and Zn. PC-3 was interpreted as seasonality. It was not strongly correlated with any pollutant tissue concentration and only low-to-moderate scores and correlation loadings were found for Hg, B[a]A, B[ghi]P, Chr, Ind, Chlorpyrifos, HHCB, AHTN, CB52 and CD138. However, the tissue concentrations of these pollutants exhibited the most marked seasonal variability, especially in Colombia, where the samples (localities) were clearly discriminated between the positive and the negative part of the PC-3 axis. Thus, at least 3 consistent clusters were outlined upon combining pairs of PCs (Fig. 8-10). In the PC-2 vs PC-1 biplot (Fig. 8): (a) samples corresponding to Nicaraguan localities at both seasons were clustered lying very markedly towards the positive part of PC-1, characterized by moderate loading (values) of the oyster tissue concentrations of PAHs, OCPs and PCBs (CB28, CB118 and CB153), (b) samples from Marina Santa Marta and Taganga at both seasons were clustered at the centre of the top left quadrant around moderate-to-high concentrations of metals such as As, Cr and Pb, and (c) samples from Isla Barú during the dry season were clustered nearby moderate levels of musk fragrances. In addition, samples from Isla Brujas and Isla Maparadita at the dry season were clearly discriminated from any other sample, being located towards the extreme bottom left quadrant (high levels of Flr and moderate-to-high of Cd, and Phe). In the PC-3 vs PC-1 biplot (Fig. 9), the Nicaraguan cluster remained as in the previous biplot but samples from Colombia appeared in two clusters: (a) rainy season samples in the top left quadrant, together with the highest loading values for Cd and Flu in Brujas and to a lesser extent in Isla Maparadita and Marina Santa Marta; and (b) dry season samples in the top left quadrant, together with the high loading values for AHTN and CB52, and moderate values for As,

Phe and HHCB. Finally, the PC-3 vs PC-2 biplot (Fig. 10) discriminated all the samples both by location and season, although the loadings were in most of the cases low-to-moderate and only the 28% of the variance was accounted for. Samples from Marina Santa Marta and Taganga were clustered depending on the season, with moderate-to-high loadings for metals with less (e.g., Al, As, Cr, Ni, Pb, Ti, V and Zn) and more (e.g., As and Hg) marked seasonal trends. Samples from Barú, Brujas and Maparadita during the rainy season appeared in a cluster associated to Chr, B[a]A and B[ghi]P and to a lesser extent to Cd, Ant, Flu and Ind). Finally, samples from Isla Barú, Isla Brujas and Isla Maparadita during the dry season and Pigeon Cay during the rainy season were distributed in the bottom left quadrant, without a clear pollutant profile as, except for AHTN and CB52, correlation loadings are low-to-moderate.

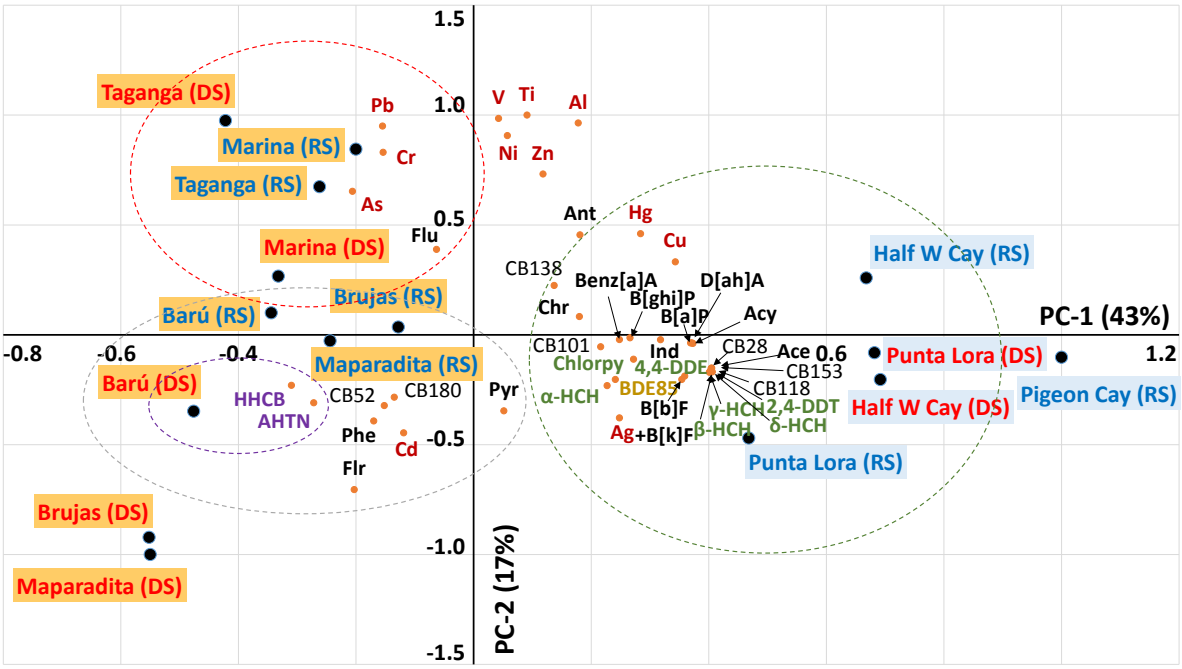


Fig. 8. Principal Component Analysis of the tissue pollutant distributions for oysters, *C. rhizophorae*, sampled in the Nicaraguan and Colombian Caribbean in the rainy and the dry season. Samples and variables are shown in biplots of combinations of two principal components identified (PC-2 vs PC-1). Four clusters can be distinguished in biplot among the samples and are related to the tissue pollutant distributions in oysters. RS, rainy season; DS, dry season.

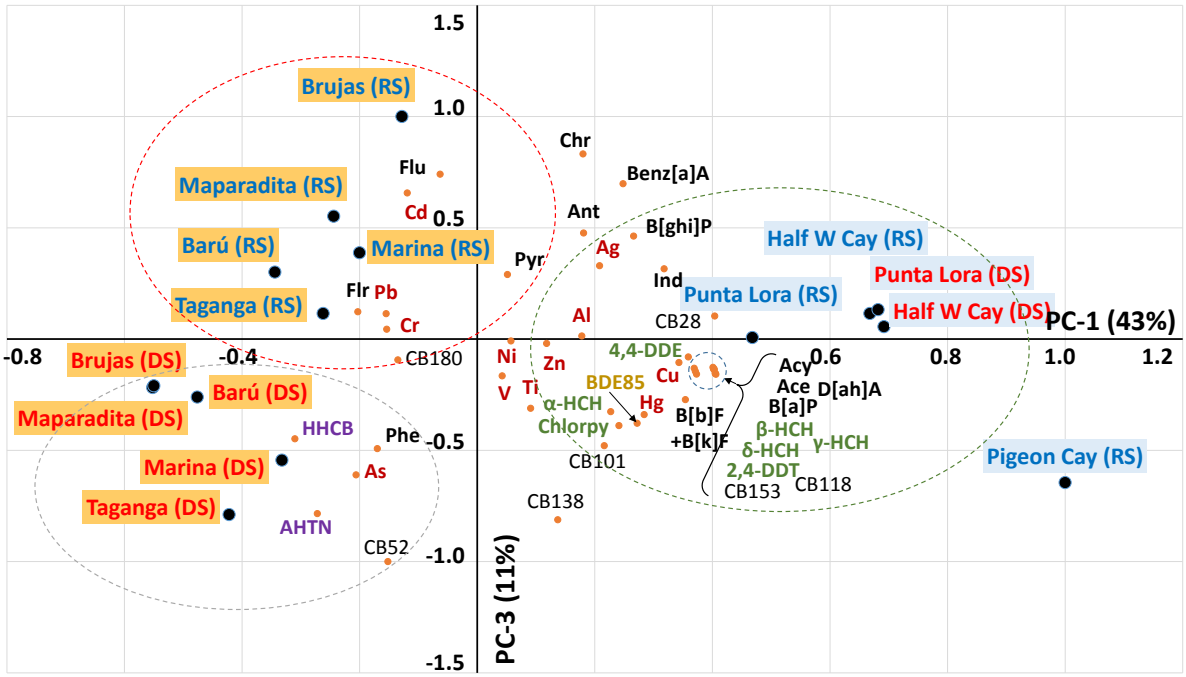


Fig. 9. Principal Component Analysis of the tissue pollutant distributions for oysters, *C. rhizophorae*, sampled in the Nicaraguan and Colombian Caribbean in the rainy and the dry season. Samples and variables are shown in biplots of combinations of two principal components identified PC-3 vs PC-1. Three clusters can be distinguished in biplot among the samples and are related to the tissue pollutant distributions in oysters. RS, rainy season; DS, dry season.

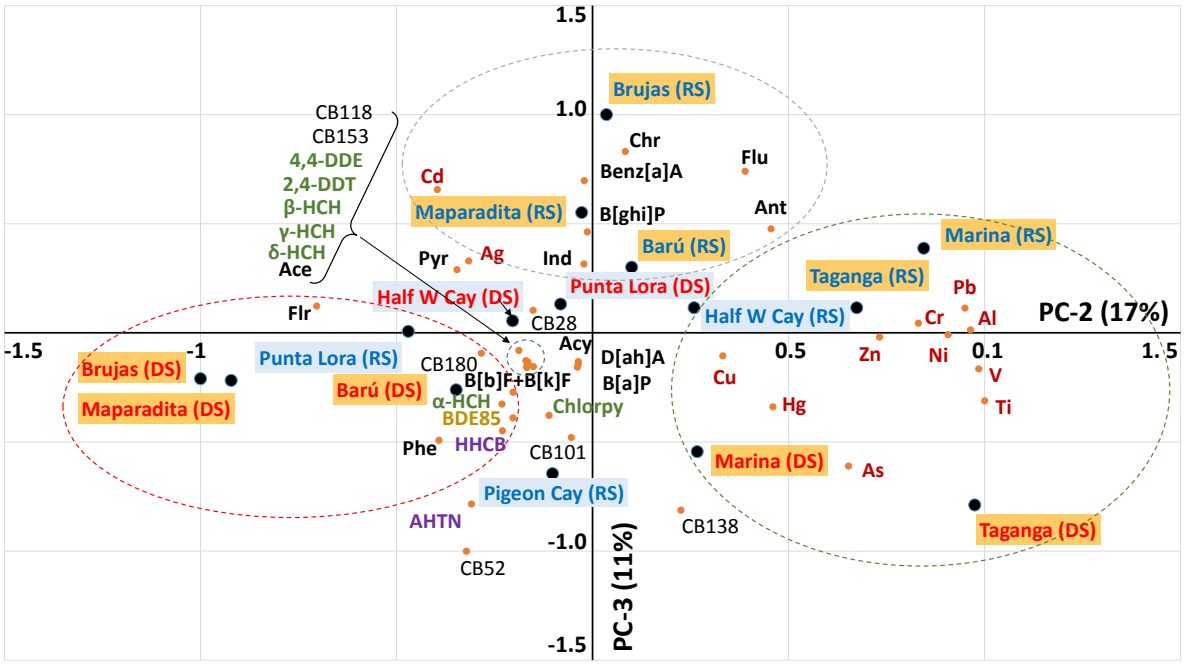


Fig. 10. Principal Component Analysis of the tissue pollutant distributions for oysters, *C. rhizophorae*, sampled in the Nicaraguan and Colombian Caribbean in the rainy and the dry season. Samples and variables are shown in biplots of combinations of two principal components identified PC-3 vs PC-2. Three clusters can be distinguished in biplot among the samples and are related to the tissue pollutant distributions in oysters. RS, rainy season; DS, dry season.

In summary, based on the determination of pollutant tissue levels in *C. rhizophorae*, the present study revealed that the pollution profile was different between the two studied geographical areas, Nicaragua and Colombia; as shown by both radar plot pollution profiles (Fig. 4 and 6) and PCA (Fig. 8-10). Likewise, the levels of pollutants were different, which was clearly reflected in the CPI and PLI values (Figs. 5 and 7), which were significantly correlated to each other following a linear regression model (Fig. 11). Therefore, CPI and PLI seem to be fully comparable integrative estimates of pollutants' levels. In addition, seasonality was a crucial factor affecting contamination profiles and concentrations and seemed to indicate seasonal variability in physicochemical properties of mangrove ecosystems as well as on diversification of human activities and practices (e.g., pest control, sanitation, tourism) between the rainy and the dry season, rather than the oysters' reproductive cycle (Chapter 2).

Overall, low tissue concentrations of metals (except Hg) and PAHs, moderate-to-high tissue concentrations of Hg, HCHs, DDTs, detectable levels of chlorpyrifos, PCBs (mainly CB28, CB118, CB138 and CB 153) and BDE85 (in Pigeon Cay) and negligible levels of musks were recorded in Nicaraguan oysters. Conversely, a distinct profile of POPs was identified in Colombia, where the tissue concentrations of HCHs and DDTs were practically negligible, chlorpyrifos and PBDEs were below detection limits, and the tissue levels of PCBs were low (CB28 in the rainy season in Isla Brujas, Isla Maparadita and Marina Santa Marta; and CB52 in all the localities at the dry season). In contrast, noticeable tissue concentrations of musk fragrances were recorded in all the localities in the dry season, and the levels of Ag, As, Cd, Pb, and PAHs in several localities and in particular seasons ranged from moderate to extremely high. In tissues of *C. rhizophorae* from the Wider Caribbean Region (including Mexico, Jamaica and Trinidad) mean values of 12 ng γ-HCH/g dry-wt, 4 ng PCBs/g dry-wt and 1 ng 4,4-DDE/g dry-wt have been reported (Van Lavieren et al., 2011). The values recorded in Nicaraguan mangrove cupped oysters largely exceeded these references, whereas only the levels of PCBs were occasionally surpassed in Colombia. These profiles and levels of pollutants are useful to identify potential pollution problems and pollutant sources in the studied regions but they do not provide reliable indication of the deleterious effects that these pollutants may exert to biota and ecosystems, as recommended for pollution monitoring programs (OSPAR Commission, 2013). For this reason, biological effects assessment was carried out in a parallel investigation aimed at relating oyster health condition to pollutant tissue levels (Chapter 2).

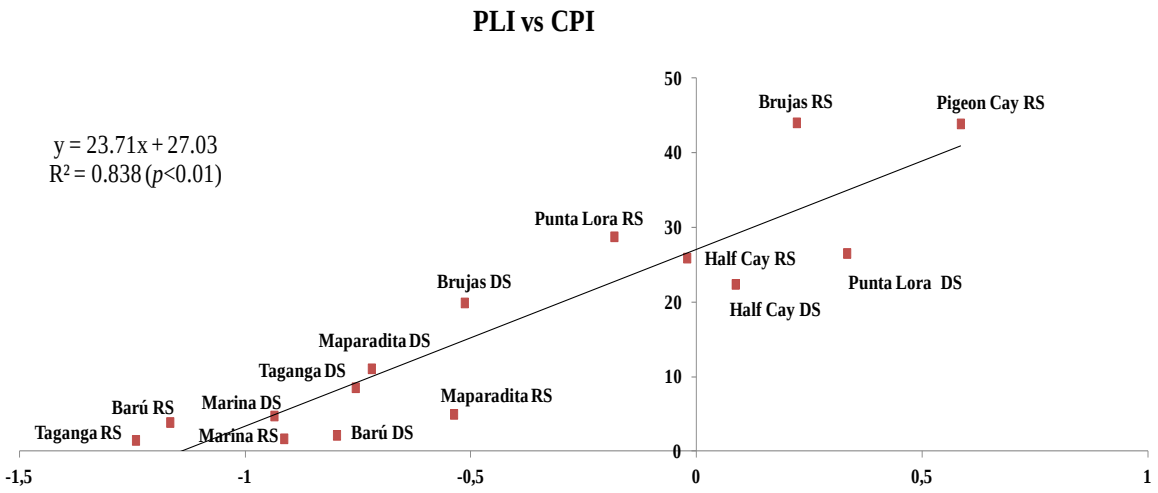


Fig. 11. Linear regression of PLI against CPI. RS, rainy season; DS, dry season.

In conclusion, mangrove cupped oysters, *C. rhizophorae*, are suitable biomonitors for assessing pollution levels in Caribbean mangroves, including diverse habitats such as lagoons and swamps and over a wide geographical coastal region from Nicaragua to Colombia. Nevertheless, seasonality is crucial to properly design and conduct pollution monitoring programmes. National and regional monitoring programmes in the WCR such as IMA in Trinidad and Tobago (Siung-Chang, 1997), GIWA in the WCR (UNEP, 2006) and REDCAM in Colombia (Vivas-Aguas et al., 2014) would be greatly improved by including measurement of tissue concentrations of chemical pollutants in mangrove cupped oysters in addition to measurements of the levels of pollutants in water and sediments, as regularly done in other regions of the world such as Pacific Asia, Europe, Canada and the USA (Monirith et al., 2003; Kimbrough et al., 2008; Zampoukas et al., 2014).

5. References

Alfonso JA, Handt H, Mora A, Vásquez Y, Azocar J, Marcano E (2013) Temporal distribution of heavy metal concentrations in oysters *Crassostrea rhizophorae* from the central Venezuelan coast. Mar Pollut Bull 73: 394–398.

Alonso DL, Latorre S, Castillo E, Brandão PFB (2013) Environmental occurrence of arsenic in Colombia: A review. Environ Pollut 186: 272–281.

Amado-Filho GM, Salgado LT, Rebelo MF, Rezende CE, Karez CS, Pfeiffer WC (2008) Heavy metals in benthic organisms from Todos os Santos Bay, Brazil. Brazilian J Biol 68: 95–100.

Angulo E (1996) The Tomlinson Pollution Load Index applied to heavy metal,'Mussel-Watch'data: a useful index to assess coastal pollution. Sci Total Environ 187(1): 19-56.

Bartolomé L, Etxebarria N, Martínez-Arkarazo I, Raposo JC, Usobiaga A, Zuloaga O (2010) Distribution of organic microcontaminants, butyltins, and metals in mussels from the Estuary of Bilbao. Arch Environ Contam Toxicol 59: 244–254.

Basu N, Scheuhammer AM, Bursian SJ, Elliott J, Rouvinen-Watt K, Chan HM (2007) Mink as a sentinel species in environmental health. Environ Res 103: 130–44.

Baumard P, Budzinski H, Michon Q, Garrigues P, Burgeot T, Bellocq J (1998) Origin and Bioavailability of PAHs in the Mediterranean Sea from Mussel and Sediment Records. Estuar Coast Shelf Sci 47: 77–90.

Bayen S (2012) Occurrence, bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems: A review. Environ Int 48: 84–101.

Beeby A (2001) What do sentinels stand for? Environ Pollut 112: 285–298.

Beiras R, Durán I, Parra S, Urrutia MB, Besada V, Bellas J, Viñas L, Sánchez-Marín P, González-Quijano A, Franco MA, Nieto O, González JJ (2012) Linking chemical contamination to biological effects in coastal pollution monitoring. Ecotoxicology 21: 9–17.

Bellas J, Albentosa M, Vidal-Liñán L, Besada V, Franco MA, Fumega J, González-Quijano A, Viñas L, Beiras R (2014) Combined use of chemical, biochemical and physiological variables in mussels for the assessment of marine pollution along the N-NW Spanish coast. Mar Environ Res 96: 105–117.

Bellas J, Nieto O, Beiras R (2011) Integrative assessment of coastal pollution: development and evaluation of sediment quality criteria from chemical contamination and ecotoxicological data. Cont Shelf Res 31: 448–456.

Bodin N, N'Gom Ka R, Le Loc'h F, Raffray J, Budzinski H, Peluhet L, de Morais LT (2011) Are exploited mangrove molluscs exposed to persistent organic pollutant contamination in Senegal, West Africa? Chemosphere 84: 318–327.

Bosire JO, Dahdouh-Guebas F, Walton M, Crona BI, Lewis RR, Field C, Kairo JG, Koedam N (2008) Functionality of restored mangroves: A review. Aquat Bot 89: 251–259.

Campos NH (1990) La contaminación por metales pesados en la Ciénaga Grande de Santa Marta, Caribe Colombiano. Caldasia 16: 231–243.

Carvalho FP, Villeneuve JP, Cattini C, Rendón J, de Oliveira JM (2009a) Pesticide and PCB residues in the aquatic ecosystems of Laguna de Terminos, a protected area of the coast of Campeche, Mexico. Chemosphere 74: 988–995.

Carvalho FP, Villeneuve JP, Cattini C, Rendón J, de Oliveira JM (2009b) Ecological risk assessment of PCBs and other organic contaminant residues in Laguna de Terminos, Mexico. Ecotoxicology 18: 403–416.

Castañeda-Chávez MR (2011) DDT in *Crassostrea virginica* (Gmelin, 1791) of coastal lagoons in the Gulf of Mexico. J Agric Sci 3: 183–193.

CCME (2008) Canadian water quality guidelines for the protection of aquatic life: Chlorpyrifos. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg (Canada), 9 pp.

Cogua P, Campos-Campos NH, Duque G (2012) Concentración de mercurio total y metilmercurio en sedimento y seston de la Bahía de Cartagena, Caribe Colombiano. Bol Investig Mar y Costeras 41: 267–285.

Cordy P, Veiga MM, Salih I, Al-Saadi S, Console S, Garcia O, Mesa LA, Velásquez-López PC, Roeser M (2011) Mercury contamination from artisanal gold mining in Antioquia, Colombia: The world's highest per capita mercury pollution. Sci Tot Envir 410–411: 154–160.

Daam MA, Van Den Brink PJ (2010) Implications of differences between temperate and tropical freshwater ecosystems for the ecological risk assessment of pesticides. Ecotoxicology 19: 24–37.

Davies IM (2004) Background/Reference Concentrations (BRCS) for the UK. Fisheries Research Services Contract Report No 05/04, Aberdeen.

de Brito APX, De Andrade Brüning IMR, Moreira I (2002) Chlorinated pesticides in mussels from Guanabara Bay, Rio de Janeiro, Brazil. Mar Pollut Bull 44: 79–81.

de Souza AS, Torres JPM, Meire RO, Neves RC, Couri MS, Serejo CS (2009) Organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) in sediments and crabs (*Chasmagnathus granulata*, Dana, 1851) from mangroves of Guanabara Bay, Rio de Janeiro State, Brazil. Analysis 73: 1–18.

Dumailo S (2003) Evaluación de la problemática ambiental por medio del estudio de los fenómenos de sedimentación y contaminación en la Laguna de Bluefields, RAAS, Nicaragua. MSc Thesis, Univ Nac Aut Nicaragua.

Ebanks-Mongalo BF, Suárez-Sánchez J, Siu-Estrada E, Montoya-Arguello JJ, Mairena-Valdivia DA, Flores-Pacheco A, Van der Wal JC, Valencia-Quintana P (2013) Concentración de plaguicidas en agua, sedimentos y ostiones (*Crassostrea rhizophorae*) de la Laguna de Bluefields, RAAS, Nicaragua. WANI: 49-54.

Fernandez A, Singh A, Jaffé R (2007) A literature review on trace metals and organic compounds of anthropogenic origin in the Wider Caribbean Region. Mar Pollut Bull 54: 1681–1691.

Fox GA (2001) Wildlife as sentinels of human health effects in the Great Lakes--St. Lawrence basin. Environ Health Perspect 109: 853–861.

Frías-Espericueta MG, Osuna-López I, Bañuelos-Vargas I, López-López G, Muy-Rangel MD, Izaguirre-Fierro G, Rubio-Carrasco W, Meza-Guerrero PC, Voltolina D (2009) Cadmium, copper, lead and zinc contents of the mangrove oyster, *Crassostrea corteziensis*, of seven coastal lagoons of NW Mexico. Bull Environ Contam Toxicol 83: 595–599.

Galvao P, Henkelmann B, Longo R, Dorneles PR, Torres JPM, Malm O, Schramm KW (2014) Partition of organochlorine concentrations among suspended solids, sediments and brown mussel *Perna perna*, in tropical bays. Chemosphere 114: 9–15.

García F, Palacio C, Garcia U (2012) Water quality at Santa Marta coastal area (Colombia). Dyna 79: 85–94.

Gaspare L, Machiwa JF, Mdachi SJM, Streck G, Brack W (2009) Polycyclic aromatic hydrocarbon (PAH) contamination of surface sediments and oysters from the

inter-tidal areas of Dar es Salaam, Tanzania. Environ Pollut 157: 24–34.

Gatermann R; Hellou J; Huhnerfuss H; Rimkus G; Zitko V (1999) Polycyclic and nitro musks in the environment: A comparison between Canadian and European aquatic biota. Chemosphere 38: 3431-3441.

GEF-REPCar (2011) Agricultural pesticide residues in Caribbean coastal waters. Colombia, Costa Rica and Nicaragua 2008-2011. GEF-REPCar Rpt UNEP, 63 pp.

GESAMP (2001) Protecting the oceans from land-based activities – Land-based sources and activities affecting the quality and uses of the marine, coastal and associated freshwater environment, Rep. Stud. GESAMP No. 71. UNEP, Nairobi.

Giri C, Ochieng E, Tieszen LL, Zhu Z, Singh A, Loveland T, Masek J, Duke N (2011) Status and distribution of mangrove forests of the world using earth observation satellite data. Glob Ecol Biogeogr 20: 154–159.

Goldberg ED (1975) The Mussel Watch: a first step in global marine monitoring. Mar Pollut Bull 6: 111.

Gómez-Giraldo A, Osorio A, Toro FM, Osorio JD, Alvarez OA, Arrieta A (2009) Circulation pattern in the "Barbacoas" bay and its influence on sediment transport to the "Rosario" islands. Avances Rec Hidraul 20: 21-40.

Green NW, Schøyen M, Øxnevad S, Ruus A, Høgåsen T, Beylich B, Håvardstun J, Rogne ÅKG, Tveiten L (2012) Hazardous Substances in Fjords and Coastal Waters - 2011. Levels, Trends and Effects. Long-term Monitoring of Environmental Quality in Norwegian Coastal Waters. NIVA.

Hellou J, Upshall C, Payne JF, Naidu S, Paranjape MA (1993) Total unsaturated-compounds and polycyclic aromatic-hydrocarbons in mollusks collected from waters around Newfoundland. Arch Environ Contam Toxicol 24: 249–257.

IARC (1987) Monographs on the evaluation of the carcinogenic risk of chemicals to humans. In: Overall Evaluation of Carcinogenicity: an Updating of IAPC Monographs Vols 1–42. International Agency for Research on Cancer, Lyon, France (Suppl. 7).

Jara-Marini ME, Soto-Jiménez MF, Páez-Osuna F (2008) Trace metals accumulation patterns in a mangrove lagoon ecosystem, Mazatlán Harbor, southeast Gulf of California. J Envir Sci Hlth 43A: 995-1005.

Kanhai LDK, Gobin JF, Beckles DM, Lauckner B, Mohammed A (2014) Metals in sediments and mangrove oysters (*Crassostrea rhizophorae*) from the Caroni Swamp, Trinidad. Environ Monit Assess 186: 1961–1976.

Kanhai LDK, Gobin JF, Beckles DM, Lauckner B, Mohammed A (2015) Polycyclic aromatic hydrocarbons (PAHs) in *Crassostrea rhizophorae* and *Cathorops spixii* from the Caroni Swamp, Trinidad, West Indies. Environ Sci Pollut Res 22: 1366–1379.

Kannan N, Reusch TBH, Schulz-Bull DE, Petrick G, Duinker JC (1995) Chlorobyphenyls: model compounds for metabolism in food chain organisms and their potential use as ecotoxicological stress indicators by application of the metabolic slope concept. Environ Sci Technol 29: 1851–1859.

Kimbrough KL, Johnson WE, Lauenstein GG, Christensen JD, Apeti DA (2008) An assessment of two decades of contaminant monitoring in the nation's coastal zone. NOAA Technical Memorandum NOS NCCOS 74, 118 pp.

Kimbrough KL, Johnson WE, Lauenstein GG, Christensen JD, Apeti DA (2009) An assessment of polybrominated diphenyl ethers (PBDEs) in sediments and bivalves of the U.S. coastal zone. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 94, 87 pp.

Lewis M, Pryor R, Wilking L (2011) Fate and effect of anthropogenic chemicals in mangrove ecosystems: a review. Envir Pollut 159: 2328–2346.

Liebezeit G, Brepohl D, Rizzi J, Guebert F, Krome M, MacHado E, Pijanowska U (2011) DDT in biota of Paranaguá Bay, Southern Brazil: Recent input and rapid degradation. Water Air Soil Pollut 220: 181–188.

MARENA (2010). Caracterización de la cuenca No. 61. Río Escondido. Ministerio del Ambiente y los Recursos Naturales de Nicaragua, DGPN & DRHCH, 38 pp.

Masson S, Couillard Y, Campbell PGC, Olsen C, Pinel-Alloul B, Perceval O (2010) Responses of two sentinel species (*Hexagenia limbata*-mayfly; *Pyganodon grandis*-bivalve) along spatial cadmium gradients in lakes and rivers in northwestern Québec. J Environ Monit 12: 143–158.

Monirith I, Ueno D, Takahashi S, Nakata H, Sudaryanto A, Subramanian A, Karuppiah S, Ismail A, Muchtar M, Zheng J, Richardson B, Puidente M, Hue ND, Tana TS, Tkalin AV, Tanabe S (2003) Asia–Pacific mussel watch: monitoring contamination of persistent organochlorine compounds in coastal waters of Asian countries. Mar Pollut Bull 46: 281–300.

Moon H-B, Kannan K, Lee S-J, Choi M (2007) Polybrominated diphenyl ethers (PBDEs) in sediment and bivalves from Korean coastal waters. Chemosphere 66: 243–251.

Nagelkerken I, Blaber SJM, Bouillon S, Green P, Haywood M, Kirton LG, Meynecke JO, Pawlik J, Penrose HM, Sasekumar A, Somerfield PJ (2008) The habitat function of mangroves for terrestrial and marine fauna: A review. Aquat Bot 89: 155–185.

Nascimento IA, Leite MBN, Sansone G, Pereira SA, Smith DH (1998) Stress protein accumulation as an indicator of impact by the petroleum industry in Todos os Santos Bay, Brazil. Aquat Ecosyst Health Manag 1: 101–108.

Navarro P, Bartolomé L, Raposo JC, Zuloaga O, Arana G, Etxebarria N (2010) Preparation of a reference mussel tissue material for polycyclic aromatic hydrocarbons and trace metals determination. Anal Chim Acta 675: 91–96.

Neff JM, Stout SA, Gunster DG (2005). Ecological risk assessment of polycyclic aromatic hydrocarbons in sediments: Identifying sources and ecological hazards. Integr Environ Assess Manag 1: 22–33.

Nhan DD, Carvalho FP, Am NM, Tuan NQ, Yen NTH, Villeneuve J-P, Cattini C (2001) Chlorinated pesticides and PCBs in sediments and molluscs from freshwater canals in the Hanoi region. Environ Pollut112: 311–320.

Oros DR, Ross JRM (2004) Polycyclic aromatic hydrocarbons in San Francisco Estuary sediments. Mar Chem 86: 169–184.

Oros DR, Ross JRM (2005) Polycyclic aromatic hydrocarbons in bivalves from the San Francisco estuary: Spatial distributions, temporal trends, and sources (1993-2001). Mar Environ Res 60: 466–488.

Oros DR, Ross JRM, Spies RB, Mumley T (2007) Polycyclic aromatic hydrocarbon (PAH) contamination in San Francisco Bay: A 10-year retrospective of monitoring in an urbanized estuary Environ Res 105: 101–118.

OSPAR Commission (2005) Agreement on Background Concentrations for Contaminants in Seawater, Biota and Sediment. OSPAR Agreement 2005-6, London, 4 pp.

OSPAR Commission (2009) Background Document on CEMP Assessment Criteria for QSR 2010. In: Monitoring and Assessment Series. OSPAR Commission, London.

OSPAR Commission (2010) Quality Status Report 2010. OSPAR Commission, London.

OSPAR Commission (2013) Levels and trends in marine contaminants and their biological effects – CEMP Assessment report 2012.

Osuna-Martínez CC, Páez-Osuna F, Alonso-Rodríguez R (2011) Cadmium, copper, lead and zinc in cultured oysters under two contrasting climatic conditions in coastal Lagoons from SE Gulf of California, Mexico. Bull Environ Contam Toxicol 87: 272–275.

Páez-Osuna F, Osuna-Martínez CC (2015) Bioavailability of cadmium, copper, mercury, lead, and zinc in subtropical coastal lagoons from the southeast Gulf of California using mangrove oysters (*Crassostrea corteziensis* and *Crassostrea palmula*). Arch Environ Contam Toxicol 68: 305–316.

Perdomo L, Ensminger I, Espinosa LF, Elster C, Wallner-Kersanach M, Schnetter M-L (1998) The mangrove ecosystem of the Ciénaga Grande de Santa Marta (Colombia): observations on regeneration and trace metals in sediment. Mar Pollut Bull 37: 393-403.

Pereira WE, Hostettler FD, Luoma SN, van Geen A, Fuller CC, Anima RJ (1999) Sedimentary record of anthropogenic and biogenic polycyclic aromatic hydrocarbons in San Francisco Bay, California. Mar Chem 64: 99–113.

Rainbow PS (2006) Biomonitoring of trace metals in estuarine and marine environments. Australas J Ecotoxicol 12: 107–122.

Ramdine G, Fichet D, Louis M, Lemoine S (2012) Polycyclic aromatic hydrocarbons (PAHs) in surface sediment and oysters (*Crassostrea rhizophorae*) from mangrove of Guadeloupe: Levels, bioavailability, and effects. Ecotox Environ Safety 79: 80–89.

Rawlins BG, Ferguson AJ, Chilton PJ, Arthurton RS, Rees JG, Baldock JW (1998) Review of agricultural pollution in the Caribbean with particular emphasis on small island developing states. Mar Pollut Bull 36: 658-668.

Rebelo MF, Amaral MCR, Pfeiffer WC (2005) Oyster condition index in *Crassostrea rhizophorae* (Guilding 1828) from a heavy-metal polluted coastal lagoon. Braz J Biol 65: 345–51.

Rebelo MF, Rebouças do Amaral MC, Pfeiffer WC (2003) High Zn and Cd accumulation in the oyster *Crassostrea rhizophorae*, and its relevance as a sentinel species. Mar Pollut Bull 46: 1354–8.

Rimkus GG (1999) Polycyclic musk fragrances in the aquatic environment. Toxicology Letters 111: 37–56

Rojas de Astudillo L, Yen IC, Bekele I (2005) Heavy metals in sediments, mussels and oysters from Trinidad and Venezuela. Rev Biol Trop 53: 41–51.

Rüdel H, Böhmer W, Schröter-Kermani C (2006) Retrospective monitoring of synthetic musk compounds in aquatic biota from German rivers and coastal areas. J Environ Monit 8: 812–823

Sericano JL, Wade TL, Jackson TJ, Brooks JM, Tripp BW, Farrington JW, Mee LD, Readmann JW, Villeneuve JP, Goldberg ED (1995) Trace organic contamination in the Americas: An overview of the US National Status & Trends and the International "Mussel Watch" programmes. Mar Pollut Bull 31: 214–225.

Serrano R, López FJ, Hernández F, Peña JB (1997) Bioconcentration of chlorpyrifos, chlorfenvinphos, and methidathion in *Mytilus galloprovincialis*. Bull Environ Contam Toxicol 59: 968–75.

Silva CAR, Rainbow PS, Smith BD (2003) Biomonitoring of trace metal contamination in mangrove-lined Brazilian coastal systems using the oyster *Crassostrea rhizophorae*: comparative study of regions. Hydrobiologia 501: 199–206.

Silva CAR, Rainbow PS, Smith BD, Santos ZL (2001) Biomonitoring of trace metal contamination in the Potengi estuary, Natal (Brazil), using the oyster *Crassostrea rhizophorae*, a local food source. Water Res 35: 4072–4078.

Silva CAR, Smith BD, Rainbow PS (2006) Comparative biomonitors of coastal trace metal contamination in tropical South America (N. Brazil). Mar Envir Res 61: 439–455.

Siung-Chang A (1997) A review of marine pollution issues in the Caribbean. Environ Geochem Health 19: 45–55.

Soclo HH, Garrigues P, Ewald M (2000) Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: case studies in Cotonou (Benin) and Aquitaine (France) areas. Mar Pollut Bull 40: 387–396.

Solaun O, Rodríguez JG, Borja A, González M, Saiz-Salinas JI (2013) Biomonitoring of metals under the water framework directive: Detecting temporal trends and abrupt changes, in relation to the removal of pollution sources. Mar Pollut Bull 67: 26–35.

Tomlinson DL, Wilson JG, Harris CR, Jeffrey DW (1980) Problems in assessment of heavy metals in estuaries and the formation of Pollution Index. Helgol Meeresunters 33: 566–575.

Torres RJ, Cesar A, Pereira CDS, Choueri RB, Abessa DMS, do Nascimento MRL, Fadini PS, Mozeto AA (2012) Bioaccumulation of polycyclic aromatic hydrocarbons and mercury in oysters (*Crassostrea rhizophorae*) from Two Brazilian estuarine zones. Int J Oceanogr; 8 pp. ID 838320; http://dx.doi.org/10.1155/2012/838320.

UNEP (1994) Regional overview of Land-Based Sources of pollution in the Wider Caribbean Region. UNEP (OCA)/CAR WG 14/4, 55 pp.

UNEP (2002) Central America and the Caribbean Regional Report, Regionally Based Assessment of Persistent Toxic Substances. UNEP, CH-1219, Geneva-Switzerland, 133 pp.

UNEP (2004) Global International Water Assessment (GIWA), Caribbean Sea/Colombia & Venezuela, Central America & Mexico. GIWA Regional Assessment 3b, 3c. University of Kalmar, Sweden.

UNEP (2006) Isaza, C.F.A., Sierra-Correa, P.C., Bernal-Velasquez,M., Londono, L.M. and W. Troncoso. Caribbean Sea/Colombia & Venezuela, Caribbean Sea/Central America & Mexico. GIWA Regional Assessment 3b, 3c. University of Kalmar, Sweden.

USEPA (2001) Guidance for Reporting Toxic Chemicals: Polycyclic Aromatic Compounds Category, EPA 260-B-01-03, Washington, DC, 40 pp.

Valdez-Domingos FX, Azevedo M, Silva MD, Randi MAF, Freire CA, Silva de Assis HC, Oliveira Ribeiro CA (2007) Multibiomarker assessment of three Brazilian estuaries using oysters as bioindicators. Environ Res 105: 350–363.

Van Lavieren H, Metcalfe CD, Drouillard K, Sale PF, Bouchot GG, Reid R, Vermeulen L (2011) Strengthening Coastal Pollution Management in the Wider Caribbean Region. Proceedings of the 64th Gulf and Caribbean Fisheries Institute 5: 1–11.

Villeneuve JP, Carvalho FP, Fowler SW, Cattini C (1999) Levels and trends of PCBs, chlorinated pesticides and petroleum hydrocarbons in mussels from the NW Mediterranean coast: comparison of concentrations in 1973/1974 and 1988/1989. Sci Total Environ 237-238: 57–65.

Vivas-Aguas L, Tosic M, Sánchez J, Narváez S, Cadavid B, Bautista P, Betancourt J, Parra J, Echeverri L, Espinosa L (2010) Diagnóstico y evaluación de la calidad ambiental marina en el Caribe y Pacifico colombiano. REDCAM Inf Téc 2010. INVEMAR. Santa Marta, 208 pp.

Vivas–Aguas LJ, Sánchez J, Cadavid B, Betancourt J, Quintero M, Moreno Y, Santana C, Cuadrado I, Ibarra K, Ríos M, Obando P, Sánchez D (2014) Diagnóstico y evaluación de la calidad de las aguas marinas y costeras del Caribe y Pacífico colombianos. Serie de publicaciones del Invemar No. 4. Red de vigilancia para la conservación y protección de las aguas marinas y costeras de Colombia (REDCAM). Informe Técnico 2013, INVEMAR, Santa Marta, 314 p.

Wade TL, Sericano JL, Gardinali PR, Wolff G, and Chambers L (1998) NOAA's 'Mussel Watch' Project: Current use organic compounds in bivalves. Mar Pollut Bull 37: 20-26.

Wallner-Kersanach M, Theede H, Eversberg U, Lobo U (2000) Accumulation and Elimination of Trace Metals in a Transplantation Experiment with Crassostrea rhizophorae. Arch Environ Contam Toxicol 38: 40–45.

Yunker MB, Macdonald RW, Vingarzan R, Mitchell RH, Goyette D, Sylvestre S (2002) PAHs in the Fraser River basin: A critical appraisal of PAH ratios as indicators of PAH source and composition. Org Geochem 33: 489–515.

Zampoukas N., Palialexis A., Duffek A., Graveland J., Giorgi G., Hagebro C., Hanke G., Korpinen S., Tasker M., Tornero V., Abaza V., Battaglia P., Caparis M., Dekeling R., Frias Vega M., Haarich M., Katsanevakis S., Klein H., et al., (2014) Technical guidance on monitoring for the Marine Strategy Framework Directive. European Commission, Report EUR 26499–Joint Research Centre–Institute for Environment and Sustainability, Luxembourg: Publications Office of the European Union, 166 pp.

Ziarrusta H, Olivares M, Delgado A, Posada-Ureta O, Zuloaga O, Etxebarria N (2015) Multiscreening determination of organic pollutants in molluscs using matrix solid phase dispersion. J Chromat 1391A: 18–30.

SM 1. CPI calculations for tissue concentrations of pollutants in mangrove cupped oysters, *C. rhizophorae*, from Nicaraguan mangrove lagoons and intertidal roots/docks of Colombian mangrove swamps, collected at both rainy (RS) and dry seasons (DS). Background tissue concentration values (BTC) were selected for calculations according to the following references (Ref.): BRC (Davies, 2004; OSPAR Commission, 2005); BAC (OSPAR Commission, 2013); HBC (Rimkus, 1999); LOD: limit of detection.

Ref.	BTC	Chemical Pollutant	Pigeon C (RS)	Half Way Cay (RS)	Punta Lora (RS)	Half Way Cay (DS)	Punta Lora (DS)	Isla Barú (RS)	Isla Brujas (RS)	Isla Maparadita (RS)	Marina (RS)	Taganga (RS)	Isla Barú (DS)	Isla Brujas (DS)	Isla Maparadita (DS)	Marina (DS)	Taganga (DS)
BRC	0.78	**Ag**	0.38	0.43	0.58	0.07	0.06	0.14	0.29	0.06	-0.57	0.20	-0.21	-0.28	0.28	-1.11	-0.44
BRC	21.00	**As**	2.05	1.85	1.69	1.86	1.88	2.13	1.92	1.89	2.34	2.12	2.06	2.03	2.07	2.25	2.74
BAC	0.66	**Cd**	1.36	1.62	1.47	1.47	1.78	2.67	2.44	2.06	1.19	1.26	1.63	1.76	2.42	1.10	1.28
BRC	1.80	**Cr**	1.12	1.24	0.78	0.95	0.81	1.12	1.37	1.89	1.73	1.87	1.55	0.67	0.58	1.31	2.03
BAC	0.06	**Hg**	0.43	0.48	0.30	0.12	0.34	0.18	0.00	0.00	0.26	0.18	-0.18	-0.30	0.07	0.34	0.26
BRC	2.40	**Ni**	1.22	1.30	0.95	1.10	1.36	1.25	1.26	1.04	1.33	1.45	0.83	0.91	0.86	1.03	1.59
BAC	1.20	**Pb**	0.62	0.74	0.54	0.64	0.81	1.00	0.95	0.83	1.06	0.97	0.79	0.56	0.40	1.03	1.12
BAC	11.00	**Phe**	-3.04	-3.04	-3.04	0.32	0.88	-3.04	0.36	-3.04	-3.04	-3.04	1.03	1.42	1.48	0.98	1.27
BAC	12.20	**Flr**	-3.09	-3.09	-3.09	-3.09	-3.09	-3.09	-0.28	-0.71	-3.09	-3.09	-0.79	1.00	-0.16	-3.09	-3.09
BAC	9.00	**Pyr**	0.51	-2.95	-2.95	-2.95	0.44	-2.95	0.82	-0.02	-1.00	-2.95	-2.95	1.21	-2.95	-2.95	-2.95
BAC	2.50	**Benz[a]A**	0.98	0.38	0.30	0.72	0.68	-2.40	1.61	1.23	0.03	-2.40	-2.40	-2.40	-2.40	-2.40	-2.40
BAC	8.10	**Chr**	0.47	-0.21	-0.06	0.17	0.13	-2.91	2.01	0.75	0.43	-2.91	-2.91	-2.91	-2.91	-2.91	-2.91
BAC	1.40	**B[a]P**	1.66	1.55	-2.15	1.51	1.59	-2.15	-2.15	-2.15	-2.15	-2.15	-2.15	-2.15	-2.15	-2.15	-2.15
BAC	2.50	**B[ghi]P**	0.88	0.90	-2.40	0.83	0.94	-2.40	1.91	-2.40	-2.40	-2.40	-2.40	-2.40	-2.40	-2.40	-2.40
BAC	2.40	**Ind**	1.03	1.03	-2.38	0.98	1.07	-2.38	1.29	-2.38	-2.38	-2.38	-2.38	-2.38	-2.38	-2.38	-2.38
BAC	0.60	**α-HCH**	2.00	-1.78	2.23	-1.78	-1.78	-1.78	-1.78	-1.78	-1.78	-1.78	-1.78	-1.78	-1.78	-1.78	-1.78
BAC	0.90	**γ-HCH**	1.44	1.19	1.00	1.28	1.52	-1.95	-1.95	-1.95	-1.95	-1.95	-1.95	-1.95	-1.95	-1.95	-1.95
BCR	3.90	**DDTs**	1.58	1.90	2.44	1.56	1.54	-2.59	-2.59	-2.59	-2.59	-2.59	-2.59	-2.59	-2.59	-2.59	-2.59
HBC	0.50	**HHCB**	-1.70	-1.70	-1.70	-1.70	-1.70	1.35	1.28	-0.10	-1.70	-1.70	1.59	2.15	1.62	1.54	2.13
BAC	10.20	**PCBs**	1.52	1.39	1.37	1.43	1.40	-3.01	-0.44	-0.25	-1.23	-1.11	0.15	0.31	0.46	0.17	0.44
LOD	50.00	**BDE85**	0.81	-3.70	0.25	-3.70	-3.70	-3.70	-3.70	-3.70	-3.70	-3.70	-3.70	-3.70	-3.70	-3.70	-3.70

SM 2. PLI calculations for tissue concentrations of pollutants in mangrove cupped oysters, *C. rhizophorae*, from Nicaraguan mangrove lagoons and intertidal roots/docks of Colombian mangrove swamps, collected at both rainy (RS) and dry seasons (DS). Background tissue concentration values (BTC) were selected for calculations according to the following references (Ref.): BRC (Davies, 2004; OSPAR Commission, 2005); BAC (OSPAR Commission, 2013); HBC (Rimkus, 1999); LOD: limit of detection.

Ref.	BTC	Chemical Pollutant	Pigeon C (RS)	Half Way Cay (RS)	Punta Lora (RS)	Half Way Cay (DS)	Punta Lora (DS)	Isla Barú (RS)	Isla Brujas (RS)	Isla Maparadita (RS)	Marina (RS)	Taganga (RS)	Isla Barú (DS)	Isla Brujas (DS)	Isla Maparadita (DS)	Marina (DS)	Taganga (DS)
BRC	0.78	**Ag**						1.38		1.14		1.59			1.88		
BAC	0.66	**Cd**						42.47	25.15	10.53	1.39	1.64	3.85	5.20	24.06		
BRC	1.80	**Cr**						0.44		2.62	1.78	2.47	1.17				3.55
HLC	211.00	**Cu**														2.21	
BAC	0.06	**Hg**						1.50			1.83	1.50	0.67			2.17	1.83
BRC	2.40	**Ni**										0.71					
BAC	11.00	**Phe**											10.67	26.38	30.31	9.65	18.77
BAC	12.20	**Flr**												9.90			
BAC	9.00	**Pyr**												16.09			
BAC	2.50	**Benz[a]A**							40.72	17.04	1.08						
BAC	8.10	**Chr**							103.35	5.58	2.72						
BAC	1.40	**B[a]P**	45.71	35.71		32.14	39.29										
BAC	2.50	**B[ghi]P**							81.12								
BAC	2.40	**Ind**		10.83		9.58	11.67		19.71								
BAC	0.60	**α-HCH**	100.00		170.00												
BAC	0.90	**γ-HCH**	27.78	15.56	10.00	18.89	33.33										
BCR	3.90	**DDTs**	38.21	78.72	277.44	35.90	34.36										
HBC	0.50	**HHCB**						22.20	19.20				39.20	142.00	41.80	35.00	134.20
BAC	10.20	**PCBs**	33.43	24.51	23.33	26.76	24.90			0.56			1.41		2.87	1.47	2.74
LOD	50.00	**BDE85**			1.78												

Chapter 2. Assessment of ecosystem health disturbance in mangrove-lined Caribbean coastal systems using the oyster *Crassostrea rhizophorae* as sentinel species

Scientific Contributions:

Aguirre-Rubí J, Garmendia L, Etxebarria N, Ortiz-Zarragoitia M, Luna-Acosta A, Soto M, Zaldibar B, Izagirre U, Espinoza F, Ahrens MJ, Marigómez I. 2013. Combined biomarker and chemical analysis as a tool to assess coastal ecosystem health in Caribbean mangroves: a pollution biological effects monitoring pilot program. *9th Iberian & 6th Iberoamerican Congress on Environmental Contamination & Toxicology CICTA, Valencia, July 1-4 2013*. Oral presentation.

Aguirre-Rubí J, Luna-Acosta A, Ortiz-Zarragoitia M, Zaldibar B, Izagirre U, Villamil L, Marigómez I. submitted. Assessment of ecosystem health disturbance in mangrove-lined Caribbean coastal systems using the oyster *Crassostrea rhizophorae* as sentinel species. SCI TOT ENVIR.

Keywords: mangrove cupped oyster, biomarkers, condition, reproduction, histopathology, ecosystem health, monitoring

Abstract: This investigation was aimed at contributing to develop a suitable multi-biomarker approach for pollution monitoring in Caribbean mangroves and coastal zones using as sentinel species the mangrove cupped oyster, *Crassostrea rhizophorae*. A pilot field study was carried out in 8 localities (3 in Nicaragua and 5 in Colombia) subjected to different levels and type of pollution. Samples were collected in the rainy and dry seasons in 2012-2013. The biological effects at different levels of biological complexity (stress on stress response, reproduction, condition index, tissue-level biomarkers and histopathological condition) were determined as indicators of health disturbance, integrated as IBR/n index, and compared with tissue burden of chemical pollutants in order to achieve an integrative biomonitoring approach. Though modulated by natural variables and confounding factors, different indicators of health disturbance alone and in combination were related to the presence of different profiles and levels of chemical pollutants present at low-to-moderate levels. Different mixtures of a variety of persistent (e.g., As, Cd, PAHs) and emerging chemical pollutants (e.g. musk fragrances) in combination with different levels of organic and particulate matter resulting from upwelling and sewage discharges, and environmental factors (salinity, temperature) elicited a different degree of disturbance in ecosystem health condition in mangrove-lined Caribbean coastal ecosystems, as reflected in sentinel *C. rhizophorae*. As a result, IBR/n was correlated with pollution indices such as PLI and CPI, even though the levels of biological anomalies used as indicators of health disturbance and the levels of pollutants were in general terms low-to-moderate in a real field situation influenced and modulated by biological variables and environmental factors that varied seasonally. Our study supports the use of simple methodological approaches to diagnose anomalies in the health status of mangrove cupped oysters from different localities and to identify potential causing agents and reflect disturbances in ecosystem health. Consequently, the easy methodological approach used herein is useful for the assessment of health disturbance in a variety Caribbean coastal ecosystems using mangrove cupped oysters as sentinel species.

Resumen: Esta investigación tuvo como objetivo contribuir a desarrollar un enfoque de multi-biomarcadores apropiado para el monitoreo de la contaminación en manglares y zonas costeras del Caribe, usando como especie centinela la ostra de manglar *Crassostrea rhizophorae*. Se llevó a cabo un estudio piloto de campo en ocho localidades (tres en Nicaragua y cinco en Colombia) sujetas a diferentes niveles y tipos de contaminación. Se recolectaron muestras en las épocas de lluvia y seca del 2012 y 2013. Se determinaron efectos biológicos a diferentes niveles de complejidad biológica (respuesta de estrés-sobre-estrés, reproducción, índice de condición, biomarcadores tisulares y condición histopatológica) como indicadores de perturbaciones en la salud, y se integraron en el índice IBR/n. Seguidamente dichos efectos biológicos se compararon con la carga tisular de contaminantes químicos con la finalidad de lograr un enfoque integral de biomonitoreo. Aunque modulados por variables naturales y factores de confusión, se relacionaron, por separado y en conjunto, diferentes indicadores de alteraciones de salud con los diferentes perfiles y niveles de contaminantes químicos, que estaban presentes a niveles entre bajos y medios. Diferentes mezclas de diversos contaminantes químicos persistentes (ejemplo: As, Cd, PAHs) y emergentes (ejemplo: almizcle sintéticos) en combinación con diferentes niveles de materia orgánica y particulada (proveniente de efluentes de aguas residuales, afloramientos) y diferentes factores ambientales (salinidad, temperatura), provocaron diferente grado de alteración en la condición de la salud de los ecosistemas costeros de manglares del Caribe, según se reflejó en la especie centinela *C. rhizophorae*. Como resultado, aunque los niveles de anomalía biológica usados como indicadores de la alteración de la salud y los niveles de contaminantes fueron, en términos generales, de bajos a medios en una situación real de campo y, además, estaban influenciados y modulados por variables biológicas y factores ambientales que variaban con la época, el IBR/n se correlacionó con los índices de contaminación PLI y CPI. Nuestra investigación confirma que pueden usarse enfoques metodológicos sencillos para diagnosticar anomalías en el estado de salud de la ostra de manglar, lo que sirve para identificar potenciales agentes causantes y reflejar perturbaciones en la salud del ecosistema. En consecuencia, este enfoque metodológico sencillo es útil para evaluar perturbaciones de la salud de diversos ecosistemas costeros del Caribe, usando la ostra de manglar como especie centinela.

1. Introduction

Mangrove ecosystems are threatened by a combination of natural disasters, tourism, aquaculture, deforestation and chemical pollution (Ellison, 2004; Defew et al., 2005; Fernandez et al., 2007; Polidoro et al., 2010; Lewis et al., 2011; Bayen 2012). In mangroves of the Wider Caribbean Region (WCR, UNEP Regional Seas Programme), pollution monitoring programmes have been carried out since the 1970's to determine the concentrations of pesticides and other persistent organic pollutants (POPs), PAHs and metals in seawater, sediments, seafood and biomonitor species (Sericano et al., 1995; Rojas de Astudillo et al., 2005; Fernandez et al., 2007; Carvalho et al., 2009; Castañeda-Chávez 2011; Alfonso et al., 2013; Kanhai et al., 2014; 2015). Particularly, mangrove cupped oysters (*Crassostrea rhizophorae*) have been proposed as biomonitors for pollution monitoring in mangrove ecosystems (Nascimento et al., 1998; Wallner-Kersanach et al., 2000; Rebelo et al., 2005; Silva et al., 2003; 2006; da Silva et al., 2005; Zanette et al., 2006; Valdez Domingos et al., 2007; Torres et al., 2012; Chapter 1).

Nowadays, it is accepted that marine pollution monitoring cannot be sustained only by chemical data because these do not provide any indication of the deleterious effects exerted to biota and ecosystems by cocktails of pollutants in multiple stress scenarios (Cajaraville et al., 2000; Allan et al., 2006); consequently, it is recommended to include both chemical and biological effects endpoints in pollution monitoring programs (ICES, 2011; OSPAR Commission, 2013a). Amongst the most used biological effects endpoints, biomarkers are ‘‘early warning signals’’ useful to assess the biological effects exerted by mixtures of chemicals on sentinel species in complex environmental conditions (Cajaraville et al., 1993; Marigómez et al., 2006; 2013). Thus, biomarkers have recently become an integral component of environmental monitoring programmes in several countries (Schettino et al., 2012).

To our knowledge, "biological effects" monitoring based on the biomarker approach is not currently being carried out in the Caribbean coastal zone. Although information about the biological effects exerted by chemical pollutants on mangrove oysters is gaining attention (Chung, 1980; Alves et al., 2002; Rebelo et al., 2003; Zanette et al., 2006; 2008; Paixão et al., 2007; Maranho et al., 2012; Catharino et al., 2015), the monitoring capacities and facilities can be in many WCR countries far away from those required to conduct sophisticated and most-advanced biomarker-based monitoring using oysters as sentinels (Van Lavieren et al., 2011). However, as very recently stated by Blaise et al., (2016) the application of biomarkers is not necessarily inhibited by monetary and logistic constraints because effortless biomarkers can provide basic

knowledge on animal health and water quality and are technically achievable everywhere. Accordingly, and aware of the technical and logistic limitations (e.g., dealing with limited accessibility and difficulties for secure sample transportation and quality *in situ* processing), a toolbox of non-sophisticated and reliable biological effects endpoints was selected to assess the potential of the mangrove cupped oyster, *C. rhizophorae,* as sentinel for pollution monitoring in Caribbean mangroves and coastal zones. This toolbox included responses easy to measure at population and individual level and histopathological analyses; these latter allow scoring responses at systemic and tissue levels on the basis of cheap, solid and straightforward technology.

The Stress-on-Stress (SoS) response has been recommended by ICES (2012) for monitoring programmes as an indicator of mussel health status (Hellou and Law, 2003; Pampanin et al., 2005). Further on, the successful application of SoS response as biomarker for environmental monitoring has been extended to other bivalve species, especially in sub-Arctic and temperate regions (Blaise et al., 2016). SoS response is a cost-effective test, in which the capacity of bivalves to survive on air is scored as a measure of resilience (Smaal et al., 1991; Veldhuizen-Tsoerkan et al., 1991; Viarengo et al., 1995; 2007; de Zwaan and Eertman 1996). It has been applied in the field to detect effects of urban discharges to estuarine and coastal waters using both native (Hellou and Law, 2003; Pampanin et al., 2005) and transplanted mussels (Moles and Hale, 2003; de los Ríos et al., 2013), as well as for assessing oil spill impact (Thomas et al., 1999) and in laboratory experiments (Veldhuizen-Tsoerkan et al., 1991; Eertman et al., 1995; Viarengo et al., 1995). It is not as sensitive as some core biomarkers of general stress (e.g. lysosomal membrane stability) but is more sensitive than others and its methodology is simple, rapid and low-cost (Viarengo et al., 1995).

Flesh Condition Index (FCI) reflects the physiological status of bivalves (Orban et al., 2002; Strogyloudi et al., 2012) and it has been reported that it is reduced on exposure to metals and organic chemicals (Andral et al., 2004; Ivanković et al., 2005; Mubiana et al., 2006). SoS response and FCI were recognised amongst those effortless biomarkers easy to be applied without major monetary or logistic constrains (Blaise et al., 2016).

Gamete development and gonad histopathology were included in the toolbox of biological effects endpoints because the gametogenic cycle is a backbone reference to understand health condition and the biological effects of pollutants, and because changes in normal gametogenic cycle and reproduction disturbances (e.g. intersex) are well-known biological effects of chemical pollutants (Wintermyer and Cooper, 2007; Ortiz-Zarragoitia and Cajaraville, 2010; 2011; Baussant et al., 2011). Finally, digestive gland histopathology was also incorporated into the

toolbox, as a wide range of contaminants, including metals, pesticides and PAHs, is known to provoke histopathological alterations in this tissue (Au 2004; Usheva et al., 2006; Aarab et al., 2008; Kim and Powell, 2007; Kim et al., 2008; Bignell et al., 2011; Garmendia et al., 2011; Khan et al., 2015).

Aimed at contributing to develop a suitable multi-biomarker approach for pollution monitoring in Caribbean mangroves and coastal zones using as sentinel species the mangrove cupped oyster, *C. rhizophorae*, a pilot field study was carried out in 8 localities (3 in Nicaragua and 5 in Colombia) subjected to different levels and type of pollution (Chapter 1). Samples were collected in the rainy and dry seasons in 2012-2013. Results dealing with the tissue levels of pollutants were published in a preceding paper (Chapter 1), in which the suitability of mangrove cupped oysters as biomonitors for the Caribbean mangroves and coastal zones was confirmed. In the present investigation, the biological effects at different levels of biological complexity (stress on stress response, reproduction, condition index, tissue-level biomarkers and histopathological condition) were determined, integrated as IBR/n index (Belaieff and Burgeout, 2002; Broeg and Lehtonen, 2006; Marigómez et al., 2013), and compared with tissue burden of chemical pollutants (Chapter 1) in order to achieve an integrative biomonitoring approach.

2. Material and Methods

2.1. Sampling sites and sample collection

Different representative scenarios of mangrove ecosystems from the Caribbean were selected. In Nicaragua, subtidal (<1 m depth) oyster reefs were studied in two localities (Fig. 1): Bluefields (sampling sites: Punta Lora and Half Way Cay) and Pearl Lagoon (sampling site: Pigeon Cay). Punta Lora was considered as a prospective reference site (far away from urban settlements) whilst Half Way Cay and Pigeon Cay were selected as potentially polluted areas influenced by aquatic transport and urban discharges (GEF-REPCar, 2011; Ebanks-Mongalo et al., 2013). In Colombia, intertidal prop roots of mangrove trees were studied in Cartagena Bay and Barbacoas Bay (Fig. 2) and intertidal rocky shores in Santa Marta Bay (Fig. 3).

In Cartagena Bay, a mangle islet 200 m north of Isla Maparadita (0.5 Km offshore the Terminal of Cartagena Port) and Isla Brujas were selected as seemingly polluted sites, as shown in previous studies (Vivas-Aguas et al., 2010; 2014). Isla Brujas is an islet adjacent to the industrial zone of Mamonal (oil refineries, petrochemicals, and asphalt, cement and smelting plants) that

also receives the direct impact of the Dique Channel. This channel was open 5 centuries ago to connect the Magdalena River with the Cartagena Bay for navigation and constitutes a major source of sediments and chemical pollution in the Cartagena Bay (Vivas-Aguas et al., 2014). In addition, Isla Barú in Barbacoas Bay was selected as a potential reference site; however, it also may be influenced by the Magdalena River to which it communicates by smaller channels via the Dique Channel since the 1950's (Gómez-Giraldo et al., 2009).

In Santa Marta Bay, the Marina Santa Marta was selected as a sampling site subject to strong anthropogenic influence (Garcia et al., 2012), whereas the nearby Taganga Harbour was a priori considered as a reference site. Sampling was carried out over one year (2012-2013) in the rainy season (October 2012) and in the dry season (March 2013).

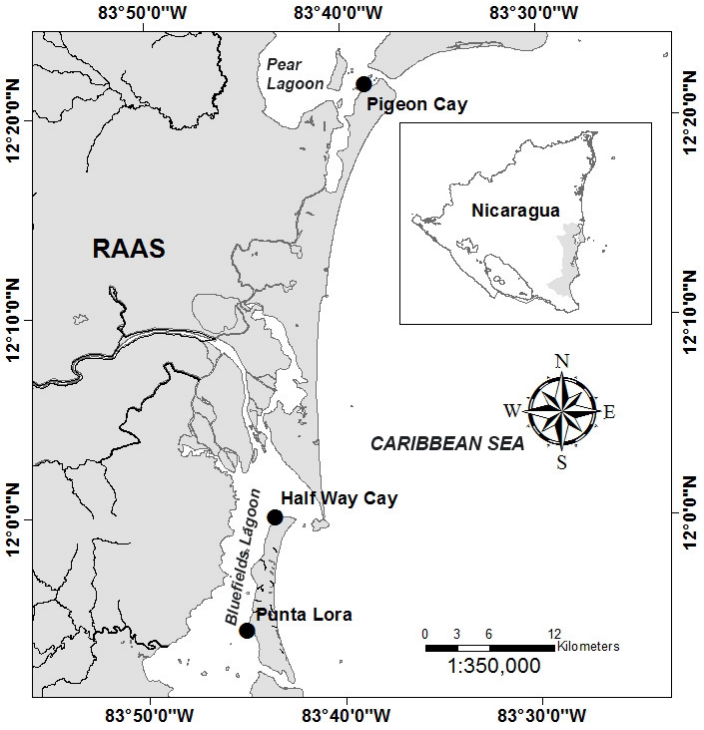


Fig. 1. Caribbean coastal maps from Nicaragua, showing the localities where oysters *Crassostrea rhizophorae* were collected in 2012-2013. Nicaraguan coast: Pigeon Cay (12°21'42.50"N-83°38'32.11"W); Half Way Cay (11°59'58.73"N-83°43'28.24"W) and Punta Lora (11°54'21.02"N-83°44'58.68"W).

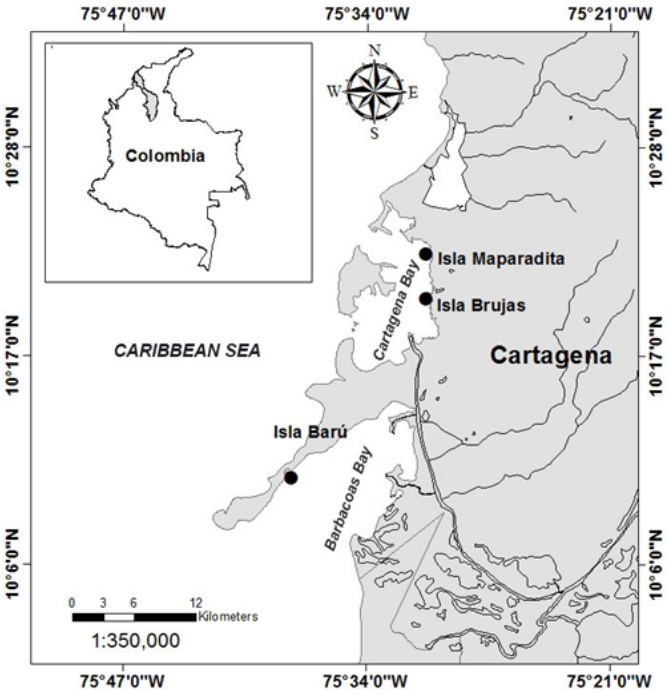


Fig. 2. Caribbean coastal maps from Cartagena-Colombia, showing the localities where oysters *Crassostrea rhizophorae* were collected in 2012-2013. Isla Barú (10°10'56.02"N-75°38'21.74"W); Isla Brujas (10°19'59.07"N-75°30'47.24"W) and Isla Maparadita (10°22'21.09"N-75°30'48.65"W) in Cartagena Bays.

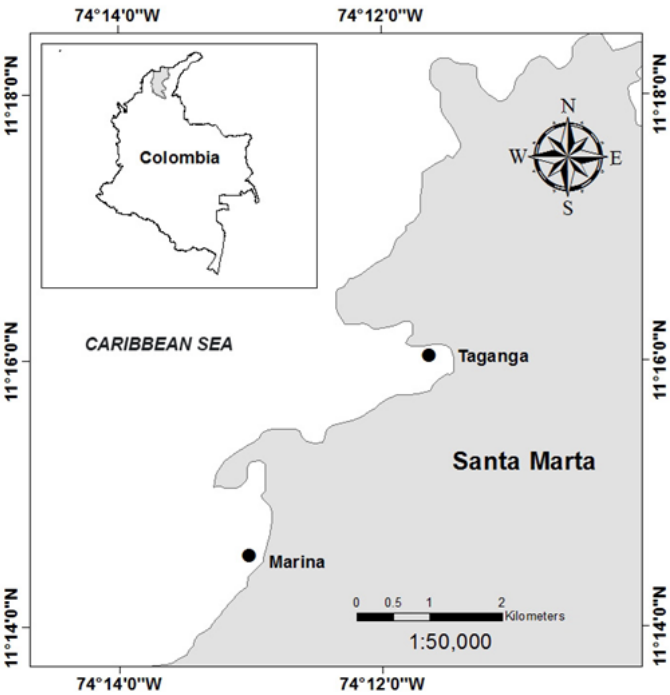


Fig. 3. Caribbean coastal maps from Santa Marta-Colombia, showing the localities where oysters *Crassostrea rhizophorae* were collected in 2012-2013. Marina Santa Marta (11°14'31.59"N-74°13'05.24"W) and Taganga (11°16'07.68"N-74°11'37.37"W) in Santa Marta Bay.

Environmental conditions and anthropogenic impact and contamination were different between Nicaraguan and Colombian coastal areas, as well as among localities within each study area (Table 1). Further on, the levels of pollutants in each locality were determined in a parallel study (Chapter 1). Briefly, low tissue concentrations of metals (other than Hg) and PAHs, moderate-to-high tissue concentrations of Hg, HCHs, DDTs, detectable levels of chlorpyrifos, PCBs and BDE85 (in Pigeon Cay) and negligible levels of musks were recorded in Nicaraguan oysters. The profile of POPs was different in the oysters from the Colombian localities where (a) the tissue concentration of HCHs, chlorpyrifos, PCBs and PBDEs were in the range of non-

detected to low; (b) noticeable tissue concentrations of musk fragrances were recorded in the dry season; and (c) the levels of Ag, As, Cd, Pb, and PAHs in several localities ranged from moderate to extremely high.

Up to 85 mangrove cupped oysters (*Crassostrea rhizophorae*) were collected per sampling site, of which 25 were used for the chemical assessment (Chapter 2) and the remaining 60 for biological effects analyses presented herein. Upon collection, oysters were placed in 15 L plastic boxes (2 individuals /L) in seawater at ambient temperature and transported to the laboratory (for 3-6 h) before processing. Due to logistic problems, sampling could not be conducted in Pigeon Cay during the dry season.

Table 1. Environmental conditions of Nicaraguan and Colombian coastal areas studied herein, including general traits of anthropogenic impact and pollution (Dumailo, 2003; Vivas-Aguas et al., 2013; Mancera-Pineda et al., 2013; Chapter 1). SST, seawater surface temperature; DO, dissolved oxygen; SOM, suspended organic matter; SSP Suspended Solid Particles; HDD, dissolved hydrocarbons; OCP, organochlorate pesticides; NDA, no data avilable; (sed), in sediments; (wb); waterborne.

		Pigeon Cay (1)	Half Way Cay (1)	Punta Lora (1)	Isla Barú (2)	Isla Brujas (2)	Isla Mapar. (2)	Marina (2) (3)	Taganga (2) (3)
SST (ºC)	RS	29	>30	>30	32	33	33	29-30	29-30
	DS	26	29	27	31	29	30	25-26	25-26
Salinity (‰)	RS	2	6	2	15	17	10	35	35
	DS	26	26	12	34	35	33	37	37
pH	RS	NDA	NDA	NDA	8.7	8.2	8.2	8.2	8.3
	DS	NDA	8.7	8.7	8.2	8.1	8.1	8.1	8.2
DO (mg/L)	RS	MIN	MIN	MIN	7	11	11	4.5	6.5
	DS	MAX	6	8	6	9	9	8	6
SOM/SSP (mg/L)	RS	HIGH	NDA	NDA	>250	<10	<10	20	10
	DS	LOW	NDA	NDA	>250	<10	<10	20	10
Turbidity (m)	RS	HIGH	HIGH (0.7)	HIGH (0.7)	HIGH+	HIGH	MID	MID+	MID+
	DS	LOW			LOW	LOW	LOW	LOW	LOW
NH_4 (µg/L)	RS	NDA	NDA	NDA	250	150	200	7	18
	DS	NDA	NDA	NDA	200	100	100	2	0
Faecal Bacteria	RS	NDA	++	+	++	++	++	++	+
	DS	NDA			+	-	-	-	-
HDD (µg/L)	RS	NDA	+ (sed)	-	8	13	4	4-6	<1
	DS	NDA		-	-	11	6	<1	<1
OCP (ng/L)	RS	NDA	>150 (sed)	>150 (sed)	<30 (wb)	<30 (wb)	<30 (wb)	<30 (wb)	<30 (wb)
	DS	NDA			-	-	-	-	-
Pb (wb)(µg/L)	RS	NDA	NDA	NDA	10	200	200	4.5	-
	DS	NDA	NDA	NDA	30	50	100	-	-

(1) Dumailo (2003), (2) Vivas-Aguas et al., (2013) and (3) Mancera-Pineda et al., (2013)

2.2. Condition and histopathology

2.2.1. Stress on Stress (SoS) test

LT_{50} was calculated after the "survival-in-air" SoS test (Veldhuizen-Tsoerkan et al., 1991; Viarengo et al., 1995) performed on 30 oysters from each site and sampling period that were placed over wet paper on plastic trays at constant room temperature (34ºC) and 100% humidity. Survival was assessed daily and oysters were considered to be dead when their valves gaped and failed to close when they were physically stimulated, or simply presented a bad smell (Pampanin et al., 2005).

2.2.2. Flesh Condition Index

Two different methods were carried out to estimate the flesh condition index (FCI). In Nicaraguan oysters, shell cavity volume (SCV) was determined according to Rebelo et al., (2005). Briefly, moulds of the shell internal cavity were made using plasticine and introduced into a water-filled glass column to verify water displacement. Displaced water dripped through a tap in the column and was collected in a Petri dish over a balance; through the water weight, SCV was calculated in ml. Soft parts from each individual were dried at 105ºC and weighed to the nearest 0.1 mg on an analytical balance in order to calculate flesh dry-wt (FDW; Lobel and Wright 1982). Then, FCI was calculated (Scott and Lawrence 1982) as FDW/SCV = 100 × FDW (g)/SCV (ml). In Colombian oysters, FCI was determined as FDW/SDW, where SDW is the shell dry weight (dried at room temperature) according to Crosby and Gale, (1990). SDW was measured in g and shell length (L) was determined as the length in mm of the largest valve (the long axis measured to the nearest 0.1 mm with Vernier callipers; Dame, 1972). SDW and L were not determined in Nicaraguan oysters. However, estimates of these parameters were calculated on the basis of SCV values upon applying model functions (Cabrera-Peña et al., 1983; Crosby and Gale, 1990) adjusted to local population traits, as derived from direct SDW and L measurements carried out only in oysters collected in the three Nicaraguan localities in October 2013. As FCI was calculated by distinct approaches in Nicaraguan and Colombian oysters, it was decided to calculate FDW/L as an oyster FCI suitable for inter-regional comparisons. Moreover, using L as the reference to construct condition indices in oysters (e.g. FDW/L or FDW/L^3; Hellou et al., 2003) overcomes uncertainties associated to unpredictable variations in SDW and difficulties resulting from inaccurate SCV determinations (Blaise et al., 2016).

2.2.3. Histology and histopathology

Upon dissection of their soft body (N=20 oysters/sample), central cross-sectioned slices (3–5 mm thick, including digestive gland, gonad (mantle) and additional tissues) were fixed in Davidson's fixative for 48 h and paraffin embedded. Microtome sections (5 µm) were obtained using an automated rotary Leica RM 2255 microtome (Leica Microsystems, Nussloch, Germany), stained with haematoxylin-eosin and examined under the light microscope (Olympus BX61; Tokyo, Japan) for gamete development determination and histopathological diagnosis (Kim et al., 2006).

Gamete development and reproductive disturbance. Gamete developmental stages were examined microscopically. Slides of 20 oysters per sample were examined individually under the light microscope using 10× and 20× objective lenses. The sex of each animal was recorded and the total number of female and male oysters was calculated for each sampling time and locality, and with these data, the sex ratio was calculated for each sampling time and locality (Ortiz-Zarragoitia et al., 2011). Further on, the sex ratio index (SRI) was calculated as the G value of females-to-males ratio (F:M) upon the gross assumption of theoretical F:M ratios (after Vélez, 1982) of 2.3:1 in Nicaragua rainy season, 1:1 in Nicaragua dry season and Colombia rainy season and 0.75 in Colombia dry season. Intersex index (IXI) was calculated as the prevalence of intersex individuals at each locality and season.

The prevalence of gamete developmental stages was determined upon scoring the maturity of the follicles and gametes according to Kim et al., (2006); and also a gonad index (GI) value was assigned (modified after Ortiz-Zarragoitia and Cajaraville 2010): 1 = undifferentiated (GI = 0); 2 = early development (GI = 1); 3 = mid development (GI = 2); 4 = late development (GI = 4); 5 = full development (GI = 5); 6 = spawning (GI = 3); 7 = spawned (GI = 2) and 8 = spawned (GI = 1). Gamete mass index (GMI) was calculated as the log ratio of the sum of the prevalence of developmental stages 2-5 (gonad tissue growth) to the sum of the prevalence of stages 6-8 (gonad tissue loss). Undifferentiated index (UDI) was estimated as the prevalence of stage 1. Oocyte atresia prevalence was recorded in female oysters and its intensity was scored (0-4) individually according to the scale for abnormal gonad development proposed by Kim et al., (2006) for mussels. Oocyte atresia index (OAI) was calculated by multiplying prevalence × intensity. Reproductive Anomalies Index (RAI) was calculated as weighted average of the deviation from the theoretical maximum anomalies of the specific indices of reproductive disturbance, according to the following formula:

$$RAI = \frac{1}{5} \times \left(\frac{CF \times SRI}{SRI_{50}} + \frac{CF \times IXI}{IXI_{50}} + \frac{CF \times GMI}{GMI_{max}} + \frac{CF \times UDI}{UDI_{max}} + \frac{CF \times OAI}{OAI_{50}} \right)$$

where CF=×100/1.6 (thus RAI changes between 0 and 100); SRI_{50}=27.7 for a 50% deviation from the theoretical sex ratio; IXI_{50}=50, when a 50% prevalence of intersex individuals occurs; (c) where GMI_{max}=2, when 100% of the gametes are mature in absence of any sign of spawning; (d) UDI_{max}=100, when 100% of the individuals do not present differentiated gametes; and OAI_{50}=2, the median value of the scale for atresia scoring after Kim et al., (2006).

Digestive gland histopathology. Slides of 20 oysters per sample were examined individually under the light microscope using 10×, 20× or 40× objective lenses. Parasites and histopathological alterations were scored using either quantitative or presence/absence scales (Kim et al., 2006). Intracellular ciliates, unidentified intracellular protists, *Nematopsis* sp., metazoans (trematodes, cestodes), *Rickettsia/Clamydia*-like organisms (R/CLO) and granulocytomas were recorded quantitatively following procedures previously described (Kim et al., 2006; Kim and Powell, 2007; Garmendia et al., 2010; 2011). Quantitative scores were made by keeping a running count of the incidences as the slide was scanned to avoid re-examination of each slide multiple times for each category. Haemocytic infiltration (without distinction between focal and diffuse), brown cell aggregates and disseminated neoplasia were scored as present/absent for each individual oyster (Zaroogian and Yevich, 1993; Kim and Powell, 2004). Prevalence and intensity of histopahological lesions were determined in Nicaragua, and prevalence in Colombia, according to Kim and Powell (2007) and Garmendia et al., (2011), as follows:

$$Prevalence = \frac{N.affected\ individuals\ \times 100}{N.examined\ individuals}$$

$$Intensity = \frac{\sum_{i=1}^{n} (N.occurrences\ of\ parasite\ or\ pathology\ per\ individual)}{N.affected\ individuals}$$

The inflammatory response index (IRI) was calculated as the sum of the prevalence values of oedema, granulocytomas and disseminated neoplasia. The parasitic infestation index (PII) was calculated as the sum of the prevalence values calculated individually for each of the 5 groups of parasites aforementioned. The digestive gland atrophy index (DGAI) was estimated according to Kim and Powell (2007).

2.3. Integrated Biological Response (IBR)

IBR index was based on the integration of five biological responses from cellular to community levels (DGAI, IRI, FDW/L, RAI and $1/LT_{50}$) according to Beliaeff and Burgeot (2002), Broeg and Lehtonen (2006), and Marigómez et al., (2013). Calculations were based on a multivariate graphic method, according to the following procedure: (1) calculation of the mean and standard deviation for each sample; (2) standardization of data for each sample: $x_i' = (x_i-x)/s$; where, x_i' = standardised value of the biomarker; x_i = mean value of a biomarker from each sample; x = general mean value of x_i calculated from all compared samples (data set); s = standard deviation of x_i calculated from all samples; (3) addition of the standardised value obtained for each sample to the absolute standardised value of the minimum value in the data set: $y_i = x_i'+|x_{min}'|$; (4) calculation of the radar plot triangular areas as $A_i = (y_i \times y_{i+1})/2$, where ''$y_i$'' and ''$y_{i+1}$'' are the standardised values of each biomarker and its next one in the radar plot, respectively; and (5) calculation of the IBR index which is the summing-up of all the radar plot triangular areas ($IBR=\sum A_i$) (Beliaeff and Burgeot, 2002). Since the IBR value is directly dependent on the number of biomarker in the data set, the obtained IBR value was divided by the number of biomarkers used to calculate IBR/n (Broeg and Lehtonen, 2006).

2.4. Statistical analyses

Statistical analyses were carried out with the aid of SPSS version 22 statistical package (IBM SPSS, Armonk, NY, USA). The normality distribution (Shapiro-Wilk's test) and homogeneity of variance (Levene's test) of data were determined before proceeding with subsequent analyses. Due to the high variability between individuals, even after normal distribution transformation of the data, non-parametric analyses were carried out. Significant differences in the prevalence of inflammatory responses and parasitic lesions, and in health disturbance (anomalies) indices were analysed by the Z score test. Survival values of SoS test were analysed with the non-parametric Kaplan-Meier analysis, followed by the Tarone-Ware post hoc test for comparisons between localities for each season and between seasons for each locality. Differences in SCV, L, SDW, FCI (FDW/SCV and FDW/SDW), DGAI and GI were determined by applying Kruskal–Wallis followed by Dunn's post hoc test. Sex ratio bias was studied using the G test of association, comparing total number of female and male mussels and normalizing for theoretical gender bias. In all the tests used herein, significant differences were established at $p<0.05$.

3. Results

3.1. Nicaragua

Shell L, FDW, SCV and SDW were smaller ($p<0.05$) in Pigeon Cay than in Half Way Cay and Punta Lora in the rainy season; likewise, all these biometric parameters presented lower values ($p<0.05$) in the dry season than in the rainy season in the latter two localities (Table 2). Moreover, FDW was smaller in Half Way Cay than in Punta Lora at both seasons. A similar pattern was observed regarding condition indices. FSW/SCV, SDW/L and FDW/L values were lower in Pigeon Cay than in Half Way Cay and Punta Lora during the rainy season, lower in the dry season than in the rainy season in the latter two localities and smaller in Half Way Cay than in Punta Lora in the rainy season (Figs. 4a to 4c).

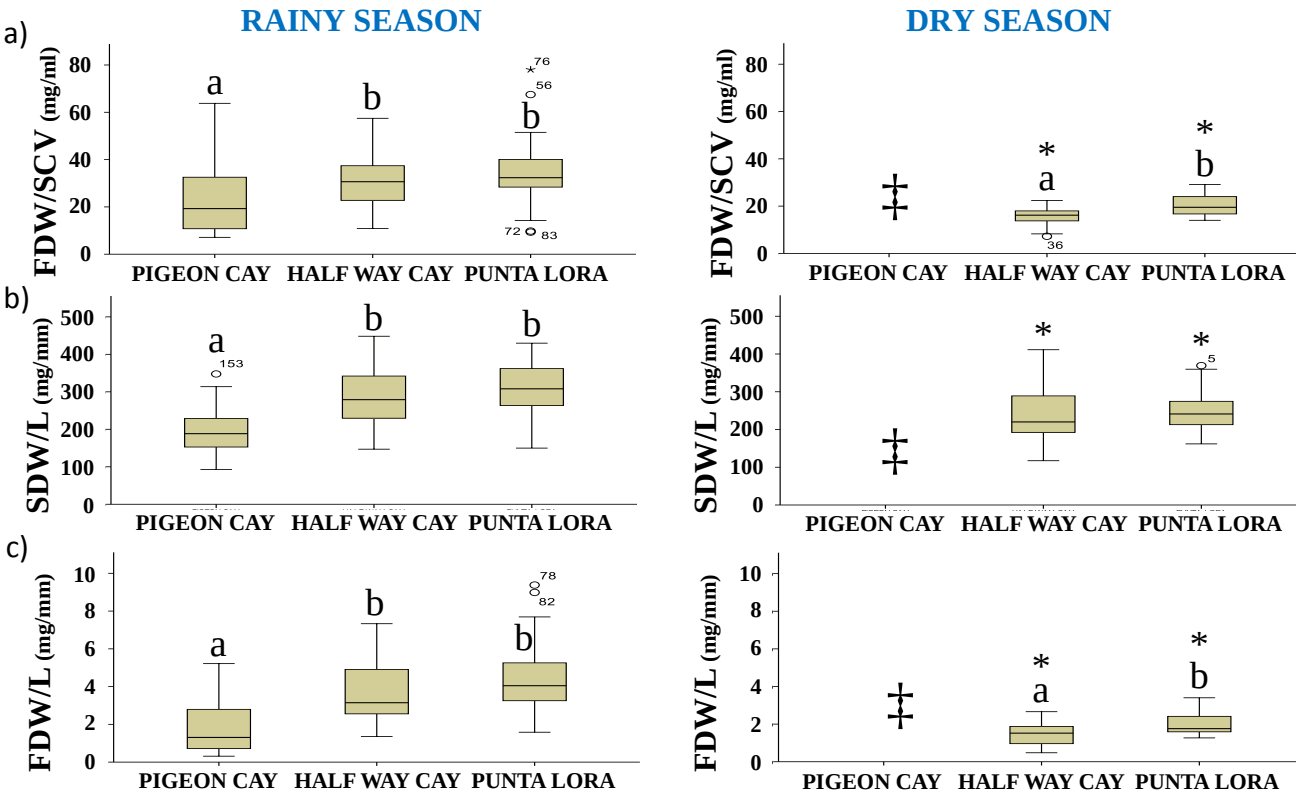


Fig. 4. Biomarkers of anomalies in oyster health indicative of disturbance in ecosystem health for the rainy and the dry seasons in Nicaraguan mangrove lagoons (Pigeon Cay, Half Way Cay and Punta Lora). Different letters denote statistically significant differences between localities and asterisk (*) indicate seasonal differences for a locality ($p<0.05$). SDW/L, shell growth index; FDW/SCV and FDW/L, condition indices. ‡, no data.

Table 2. Condition and histopathology of *Crassostrea rhizophorae* from shallow subtidal oyster reefs of Nicaraguan mangrove lagoons. Shell cavity volume, condition index and atrophy their values are mean ± standard error (n = 20). Sex Ratio Index = SRI's G value. The superscript letters (a, and b) indicate significant differences between groups ($p<0.05$). Asterisks (*) indicate seasonal difference for a locality ($p<0.05$); ♯, significantly different from the theoretical gender bias (Vélez, 1982) according to the G test ($p<0.05$). [1], estimated based on model functions.

	RAINY SEASON			DRY SEASON	
	Pigeon Cay	**Half Way Cay**	**Punta Lora**	**Half Way Cay**	**Punta Lora**
Shell Length (L; mm)[1]	59.7±2.1[a]	79.9±2.6[b]	84.8±2.4[b]	70.4±4.0*	72.4±2.6*
Flesh dry-wt (FDW; mg)	118.4±16.3[a]	296.1±22.6[b]	370.3±26.1[b]	113.3±16.3[a]*	149.5±13.9[b]*
Shell Cavity Vol (SCV; ml)	4.9±2.5[a]	9.6±4.4[b]	10.9±4.3[b]	7.3±4.4*	7.4±2.8*
Shell dry-wt (SDW; g)[1]	11.2±1.1[a]	24.0±1.8[b]	27.2±1.7[b]	18.4±2.5[a]*	18.6±1.6[a]*
Sex ratio (Sex Ratio Index)	5.2:1♯ (3.91)	6.4:1♯ (5.74)	2.1:1♯ (0.09)	1:1.4* (0.01)	1.7:1* (2.16)
Intersex Index	0	0	0	0	0
Gonad Index	3.5±0.2[b]	3.8±0.2[b]	3.3±0.1[a]	2.1±0.4[a]*	3.7±0.3[b]
Gametogenic Mass Index	0.77	0.70	0.10	0.10	0.34
Undifferentiated Index	0.05	0.08	0.00	0.00	0.00
Oocyte Atresia Prevalence	0.387	0.406	0.222	0.000	0.333
Oocyte Atresia Intensity	0.806±1.327	0.594±0.979	0.407±0.971	0.000[a]*	0.417±0.669[b]
Oocyte Atresia Index	0.312	0.241	0.090	0.000	0.139
Prevalence (oedema) %	18.5	35	2.5	89	44
Prevalence (BCA) %	95	100	97.5	89	100
Preval. (granulocytomas) %	5	0	0	0	0
Preval. (diss. neoplasia) %	5	5	0	0	0
***Prevalence** (ciliates) %*	*29*	*23.5*	*0*	*17*	*33*
***Preval.** (Underted. Prot.) %*	*2.5*	*10*	*0*	*11*	*17*
Prevalence (Total Prot.) %	*31.5*	*33.5*	*0*	*28*	*50*
Preval. (*Nematopsis*) % [(1)]	97.5	76	25.5	89	39
Prevalence (Metazoan) %	0	0	0	11	0
Prevalence (R/CLO) %	0	0	0	0	10

[(1)] Intensity *(individuals per section)* = 62.5 (PC-RS); 12.5 (HC-RS); 9 (HC-DS); 2.5 (PL- RS); 2 (PL-DS)

Sex ratio values for each sampling location at each sampling time are shown in Table 2. Statistically significant bias in the sex ratio towards female condition (2.82:1; G value=8.65, *p<0.05*) was detected for the whole Nicaraguan population of mangrove cupped oysters in comparison with the theoretical sex ratio of 1.65:1 (resulting from the average between 2.3:1 (rainy season) and 1:1 (dry season) sex ratios). This bias towards female condition was also found in the rainy season (3.75:1; G value = 4.95, *p=0.03*) but not in the dry season (1.21:1; G value = 5.08, *p=0.18*), when a 22.5% of the oysters presented undifferentiated gonad. Indeed, the three studied localities showed a significant bias to females in the rainy season (Table 2). No intersex was recorded. Different gamete development stages were observed depending on the season and the locality (Figs. 5 and 6). In the rainy season, gamete development was delayed in Pigeon Cay in comparison with the other two localities, and most advanced in Punta Lora (Fig. 5). In the dry season, gamete development went more ahead in Half Way Cay than in Punta Lora (Fig. 5). Accordingly, significant differences (*p<0.05)* were observed in GI between seasons in Half Way Cay and between Half Way Cay and Punta Lora in both seasons (Table 2). In contrast, no differences in GMI and UDI were recorded (Table 2). The levels of oocyte atresia (Fig. 6c) recorded in both seasons in all the localities were similar except in Half Way Cay in the dry season, when no oocyte atresia was recorded (Table 2). As a whole, RAI reflects only a low-to-moderate (<10) reproductive disturbance in Pigeon Cay and Half Way Cay, mainly featured by deviations in the expected SRI and GMI values (Fig. 7a).

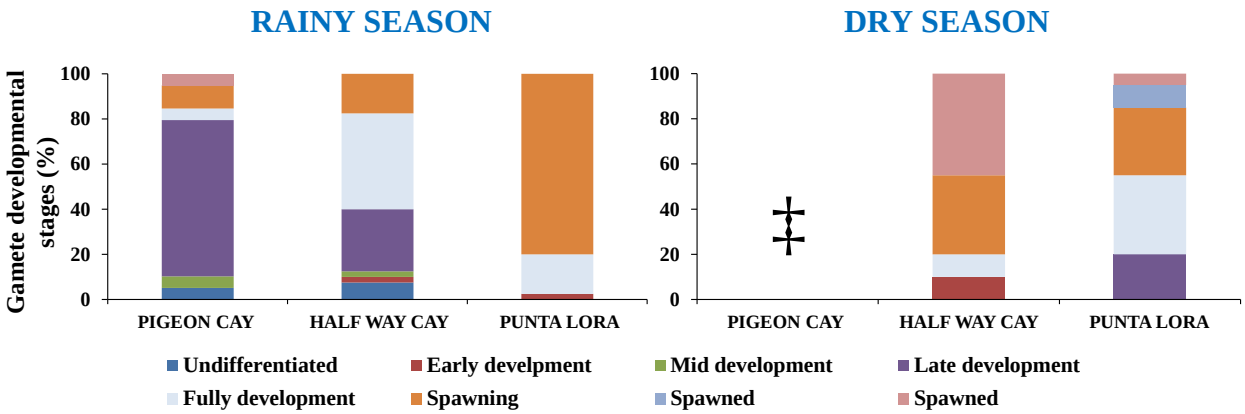


Fig. 5. Gamete development stages of oyster *Crassostrea rhizophorae* collected from Nicaragua at the rainy and the dry seasons. ‡, no data.

Histopathological examination revealed the presence of a variety of parasites such as intracellular ciliates, undetermined intracellular protists, *Nematopsis* sp., trematodes and *Rickettsia/Clamydia*-like organisms (R/CLO) (Fig. 8). Some parasites had a local impact such as

metazoans (Half Cay in dry season) and R/CLO (Punta Lora in dry season) (Fig. 8g and i). Other parasites such as the unidentified intracellular protists were found sporadically (Fig. 8e). Ostensibly, parasite prevalence was low in Punta Lora in the rainy season and PI was higher in Half Way Cay than in Punta Lora all over the year (Table 2).

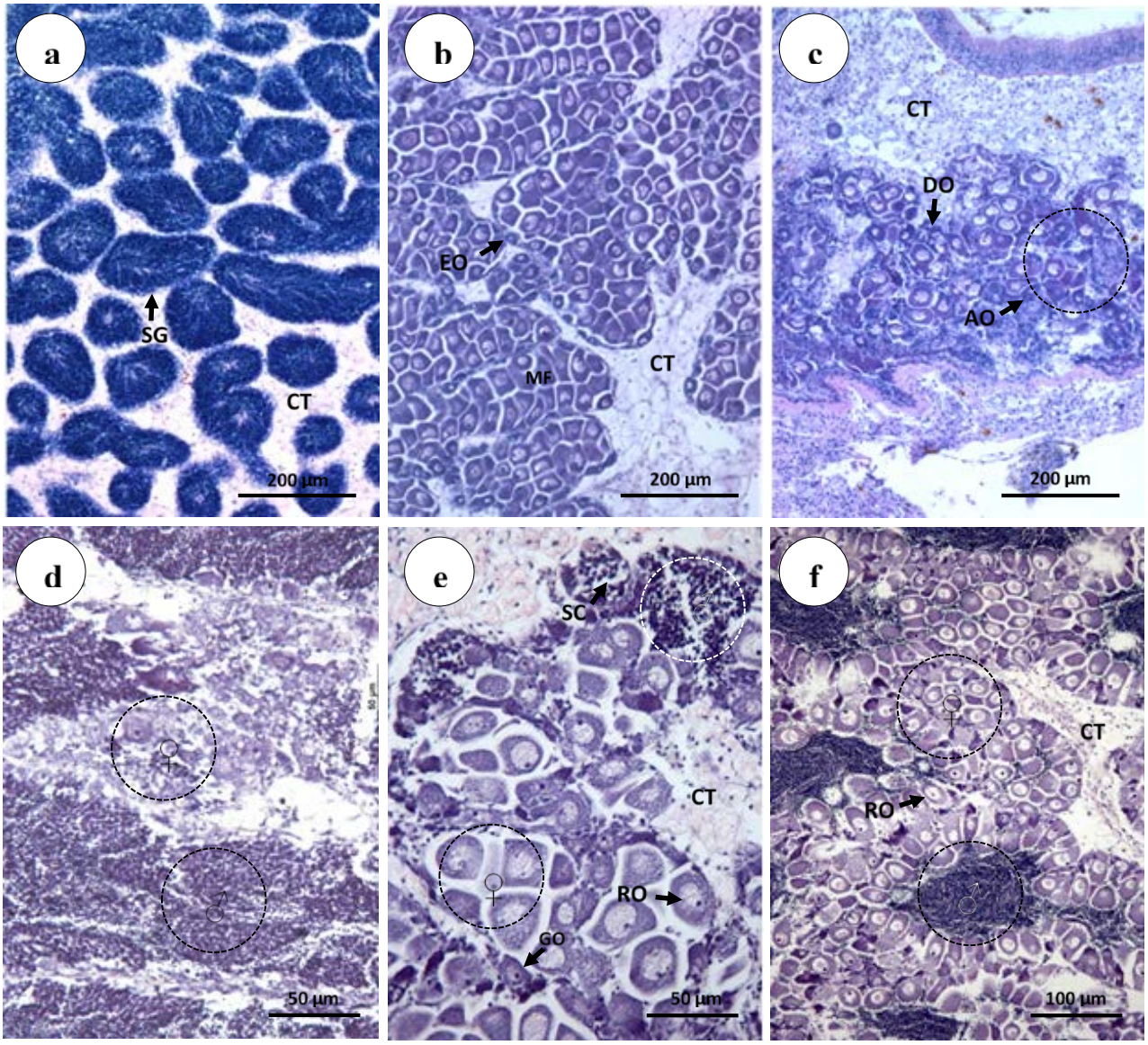


Fig. 6. Light micrographs of gonad sections and intersex individuals of *Crassostrea rhizophorae*. a) normal gonad of a male; b) normal gonad of a female; c) female from Half Way Cay with atretic oocytes; d) male from Brujas with intersex; e) female from Marina with intersex; f) female from Barú with intersex. AO, atretic oocytes; EO, early oocyte; GO, growing oocyte; DO, degenerated ovum; RO, ripe ovum; SG, spermatogonia; SC, spermatocytes; CT, connective tissue; MF, mature follicles; ♀, female germ cells; ♂, male germ cells.

Nevertheless the intensity of parasitic infestations was generally low. It is worth noting that intracellular ciliates (Fig. 8f) and *Nematopsis* sp. (Fig. 8h) presented the highest prevalence values

all along the study period; whilst the highest intensities were seemingly recorded for *Nematopsis* sp. and unidentified intracellular protists (Table 2) in the rainy season. PII values are higher during the rainy season in Pigeon Cay and Half Way Cay than in Punta Lora, where PII values raised in the dry season (Fig. 7b). Unlike in the dry season, the histological integrity of the digestive gland tissue was seemingly altered in all the studied localities in the rainy season (Figs. 8a and b). A large part of the digestive gland tissue was occupied by disorganized ICT with haemocytic infiltration (Fig. 8b and d) and the caliper of digestive ducts was particularly undersized (Fig. 8b and f). Inflammatory responses such as massive haemocytic infiltrations of ICT (oedema), brown cell aggregates, granulocytomas and disseminated neoplasia were observed (Fig. 8b to 8d). Prevalence of inflammatory responses was apparently higher in the dry season than in the rainy season and in Half Way Cay than in Punta Lora; however, significant differences after applying the Z score test could only be established at $p<0.1$ (Table 2). Prevalence of brown cell aggregates was always high whereas only one granulocytoma was found in one oyster from Pigeon Cay in the rainy season (Fig. 8d). Two isolated cases of oysters with disseminated neoplasia were recorded in the rainy season (Table 2; Fig. 8c). Unlike for the case of PII, IRI was higher in the dry season than in the rainy season (Fig. 7b and c); however, its values were also always higher in Pigeon Cay and Half Way Cay than in Punta Lora.

Digestive alveoli presenting severe atrophy (with a wide lumen and thin epithelium) and separated by ample areas of ICT (Figs. 8b, d and f) were observed all over the year but their incidence was particularly high in the rainy season. In addition, digestive cells vacuolisation (Fig. 8e) was recorded in a few specimens (<10%) from half way Cay at both seasons. Thus, in the rainy season DGAI was similar in the three localities and always higher than in the dry season ($p<0.05$; Fig. 7d). In contrast, in the dry season DGAI was different between localities, with higher values in Half Way Cay than in Punta Lora ($p<0.05$; Fig. 7d).

LT_{50} was significantly different between seasons for Half Way Cay and Punta Lora (Tarone-Ware test, $p<0.05$) but not between localities at each season (Fig. 7e). The highest LT_{50} values were observed in the rainy season, whilst 2-3 fold lower values were recorded in the dry season (Fig. 7e).

Five indices of biological response (DGAI, IRI, FDW/L, RAI, LT50) were represented in radar plots (Fig. 9). The depicted profiles revealed that in the rainy season low levels of biological complexity (DGAI and IRI) and reproduction (RAI) were responsive in Half Way Cay and Punta

Lora whilst all the indices but LT_{50} (SoS) responded in Pigeon Cay. Conversely, quite different profiles were envisaged in the dry season, with responses at more complex biological levels and especially in SoS.

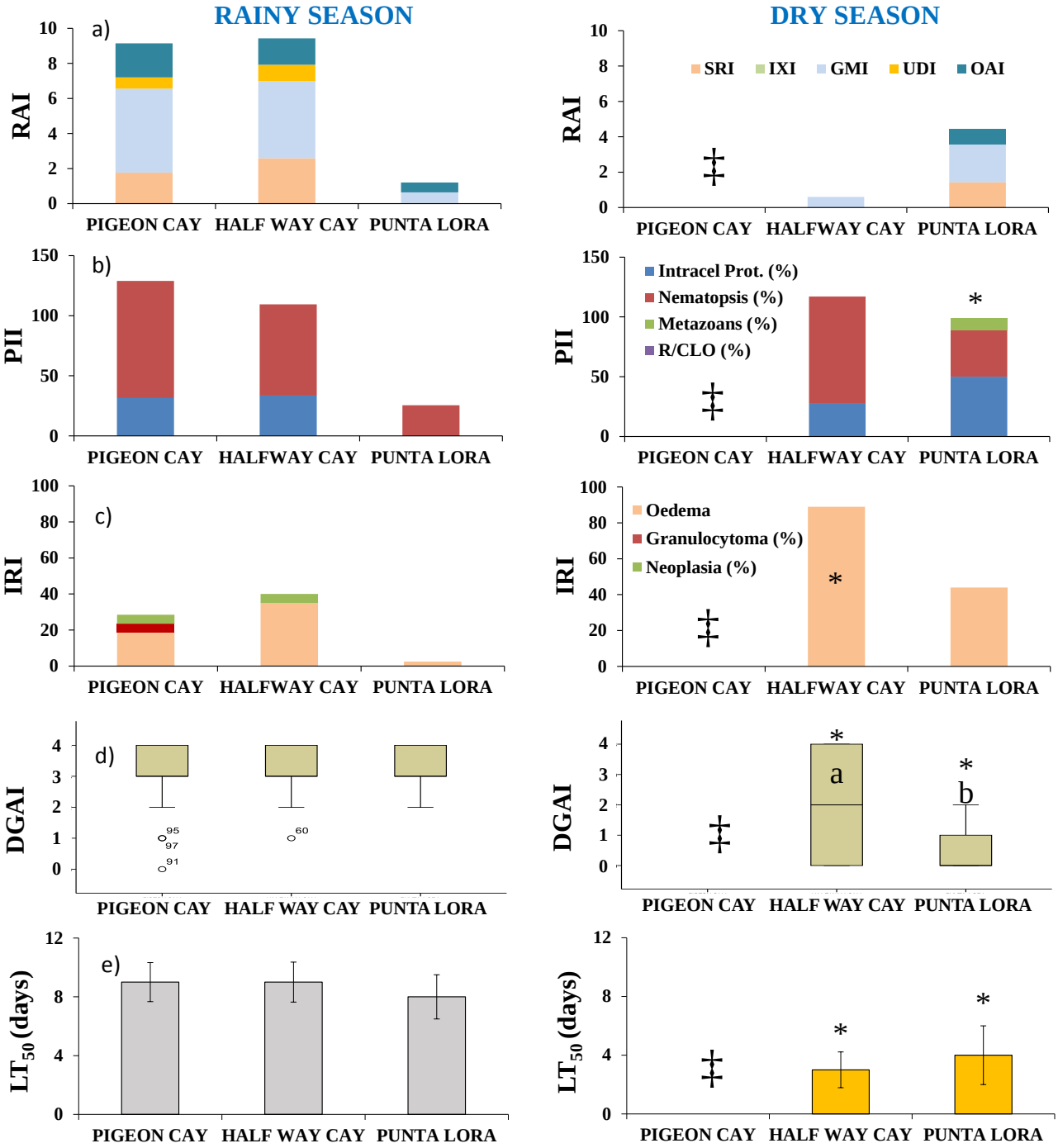


Fig. 7. Biomarkers of anomalies in oyster health indicative of disturbance in ecosystem health for the rainy and the dry seasons in Nicaraguan mangrove lagoons (Pigeon Cay, Half Way Cay and Punta Lora). Different letters denote statistically significant differences between localities and asterisk (*) indicate seasonal differences for a locality (p<0.05). RAI, reproductive anomalies index; PII, parasitic infestation index; IRI, inflammatory response index; DGAI, digestive gland atrophy index; LT_{50}, median survival time after SOS test. ‡, no data.

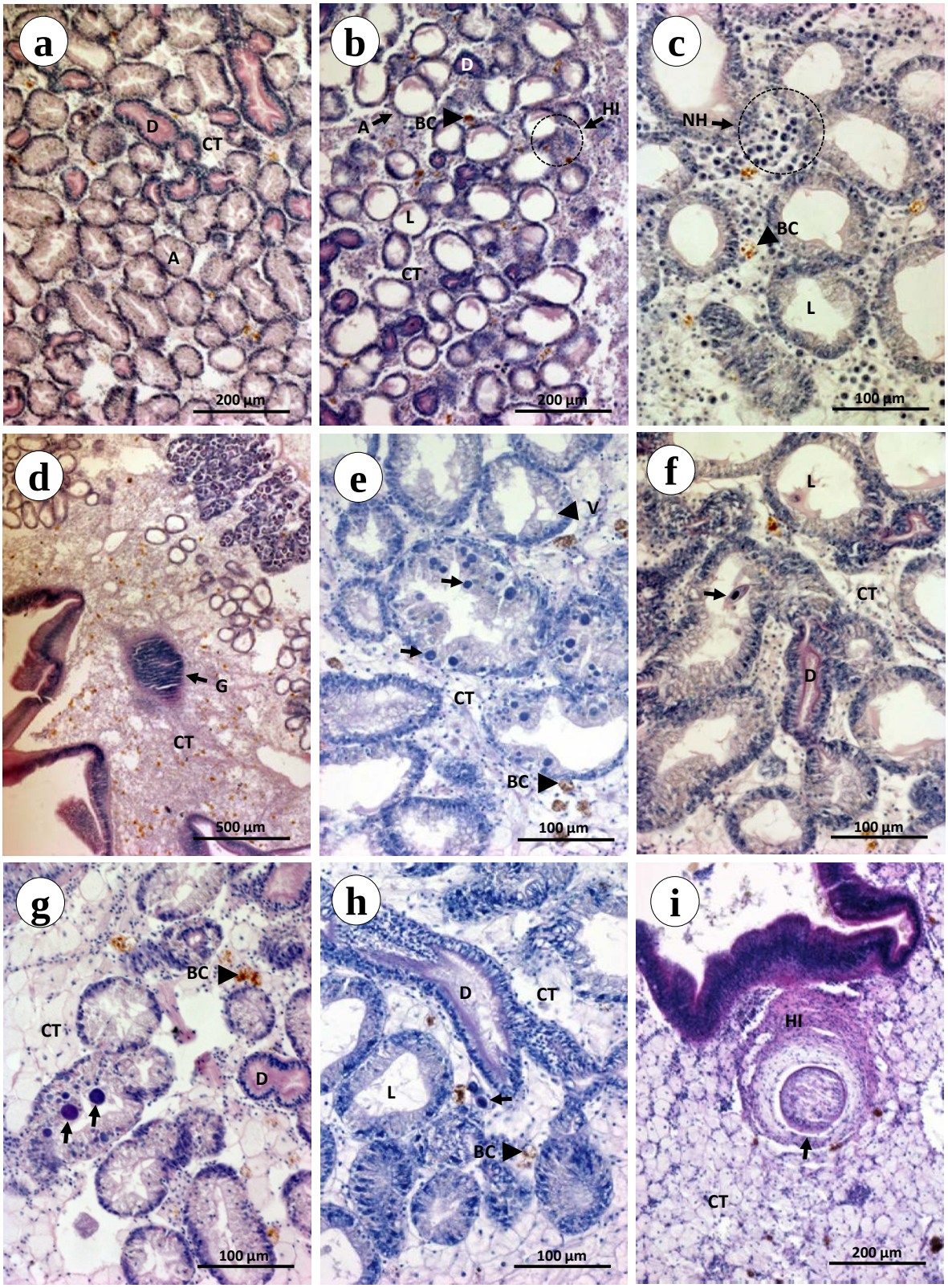


Fig.8. Paraffin sections of the digestive gland of *Crassotrea rhizophorae* stained with haematoxylin-eosin: a) normal digestive gland b) digestive gland with diffuse infiltration haemocyte and atrophied alveoli; c) disseminated neoplasia; d) granulocytoma and diffuse infiltration haemocytic; e) unidentified protists; f) ciliate; g) R/CLO; h) *Nematopsis* sp.; i) metazoan. Abbreviations: A, alveoli; D, duct; CT, connective tissue; HI, haemocytic infiltration; L, lumen; NH, neoplastic haemocytes. Arrows, tissue alterations; arrowheads indicate the presence of brown cell (BC) aggregates and vacuolization (V).

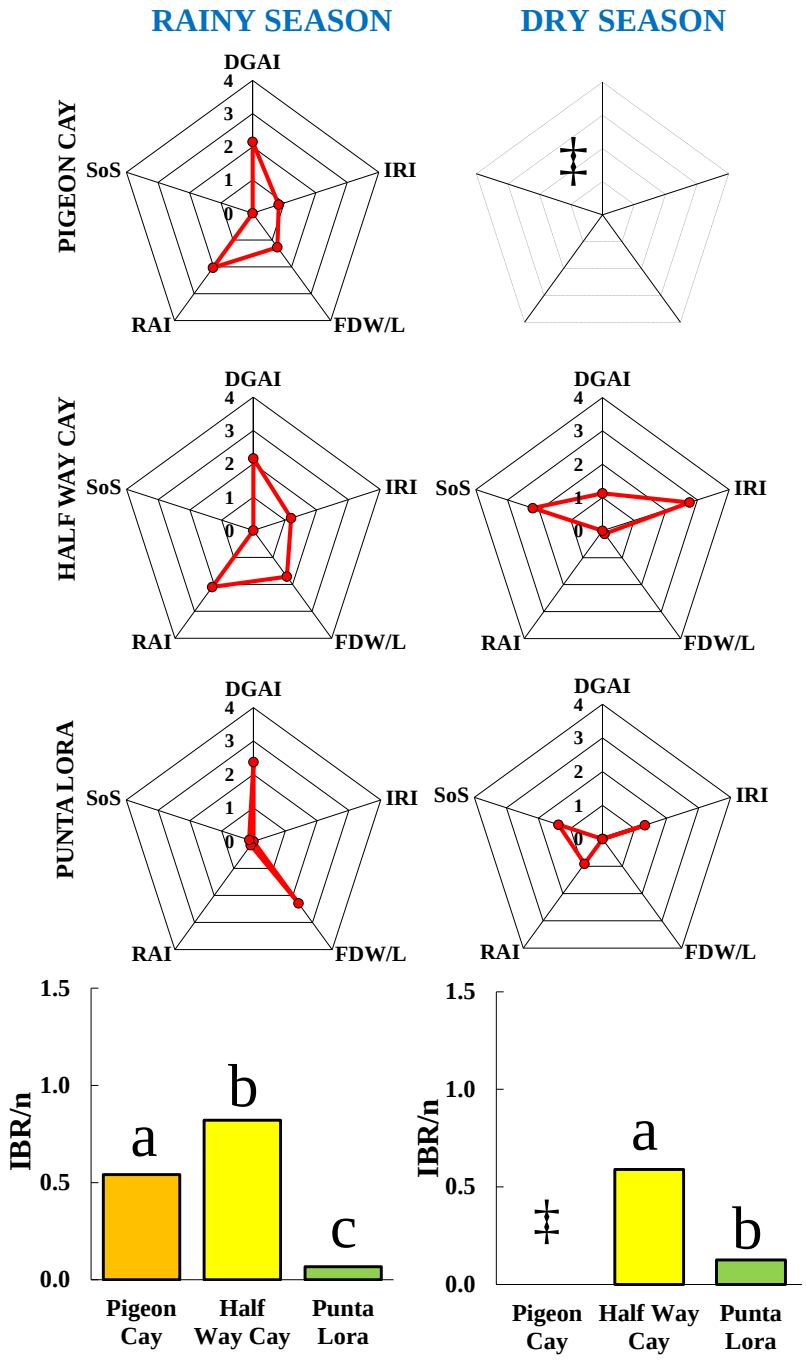


Fig. 9. Radar plots for biomarkers and the corresponding IBR/n index for the rainy and the dry seasons in Nicaraguan mangrove lagoons (A). Different letters denote statistically significant differences between pairs of means according to the Z-score test ($p< 0.05$). DGAI, digestive gland atrophy index; IRI, inflammation index; FDW/L, condition index; RAI, reproductive anomalies index; LT_{50}, median survival time after SOS test. ‡, no data.

Thus, significant differences were found between localities in the IBR/n index in the rainy season after applying the Z score test, IBR/n values being lower in Punta Lora than in Half Way Cay and in this latter lower than in Pigeon Cay (Fig. 9). In the dry season, IBR/n values in Punta Lora and Half Way Cay were higher than the rainy season, without differences between localities.

3.2. Colombia

Shell L showed the highest values in the Marina Santa Marta and the lowest in Isla Brujas in both seasons, and no seasonal variability was observed (Table 3). The highest FDW values were

recorded in Isla Maparadita and Marina Santa Marta in the rainy season, whilst values were much lower in all the localities except Marina Santa Marta in the dry season (Table 3). The highest SDW values were found in Marina Santa Marta in both seasons, while SDW was significantly lower in the dry season than in the rainy season in Isla Brujas, Isla Maparadita and Taganga ($p<0.05$, Table 3). The highest FDW/SDW was recorded in Isla Maparadita followed by values in Taganga and Isla Barú, with lower values in the dry season than in the rainy season in Isla Maparadita and Isla Barú ($p<0.05$; Fig. 10a).

The highest SDW/L was recorded in Marina Santa Marta in both seasons, with lower values in the dry season than in the rainy season in Isla Brujas, Isla Maparadita and Taganga, and higher values in the dry season than in the rainy season in Isla Barú ($p<0.05$, Fig. 10b). The highest FDW/L values were recorded in Isla Maparadita and Marina Santa Marta in the rainy season and in Isla Barú and in Marina Santa Marta in the dry season ($p<0.05$, Fig. 10c).

Sex ratio values for each sampling location at each sampling time are shown in Table 3. No statistically significant bias in the sex ratio of the whole studied population (1.14:1; G value=2.55, *p=0.11*) was detected in comparison with the theoretical sex ratio of 1:1.14 resulting from the average between 1:1 (rainy season) and 1:1.33 (dry season) sex ratios. Accordingly, no bias was found in any season (rainy season: 1.16:1; G value = 0.80, *p=0.37*; dry season: 1:1.06; G value = 0.925, *p=0.34*). Among the studied localities, only Taganga showed a significant bias to female condition in both seasons (Table 3). Intersex cases were sporadically found: one female from Marina Santa Marta in the rainy season, and one female from Isla Barú and one from Marina Santa Marta and one male from Isla Brujas in the dry season (Table 3). All cases showed separate male and female gonad follicles (Figs. 6d-f).

Most oysters were in advanced gamete development stages (e.g., spawning and spawned; Fig. 11). In the rainy season, Marina Santa Marta and Taganga showed the most advanced gamete development stages; however, the opposite was found in the dry season. Significant differences in GI were found between seasons in Isla Brujas and Taganga and between localities for both seasons (Fig. 11). Likewise, differences in GMI and UDI were recorded between localities but no between seasons (Table 3). Atretic oocytes were only observed in one oyster from Marina Santa Marta in the rainy season. As a whole, RAI reflects only a low-to-moderate (<10) reproductive disturbance, mainly featured by deviations in the expected SRI, GMI and UDI values (Fig. 12a).

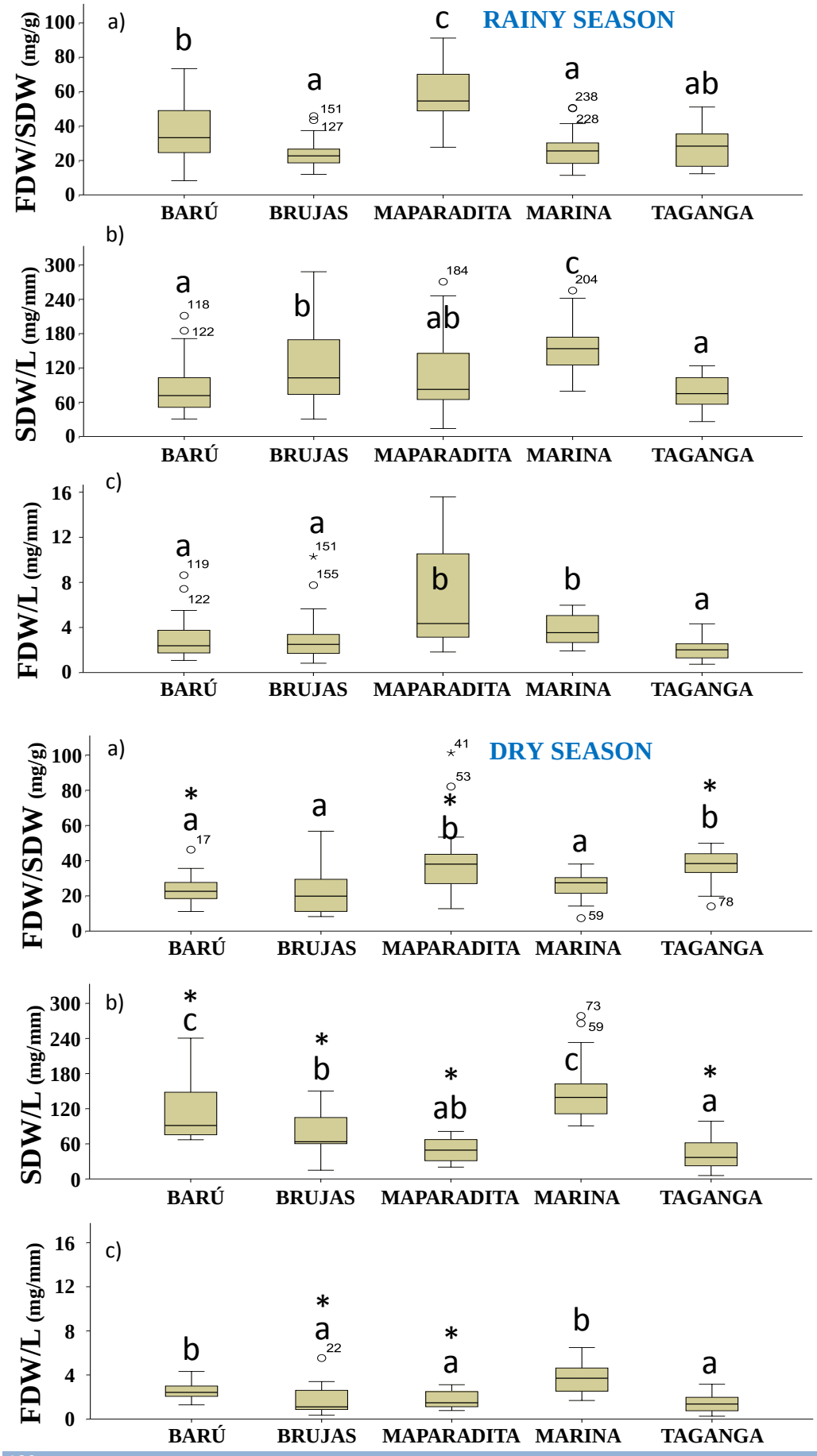


Fig. 10. Biomarkers of anomalies in oyster health indicative of disturbance in ecosystem health for the rainy and the dry seasons in Colombian mangrove swamps (Isla Barú, Isla Brujas, Isla Maparadita, Marina and Taganga). Different letters denote statistically significant differences between localities and asterisk (*) indicate seasonal differences for a locality ($p<0.05$). SDW/L, shell growth index; FDW/SDW and FDW/L, condition indices.

Table 3. Condition and histopathology of *Crassostrea rhizophorae* from intertidal roots/docks of Colombian mangrove swamps. Shell cavity volume, condition index and atrophy their values are mean ± standard error (n=20). Sex Ratio Index = SRI's G value. The superscript letters (a, b and c) indicate significant differences between pairs of localities ($p < 0.05$). Asterisk (*) indicate seasonal difference for a locality ($p< 0.05$); ♯, significantly different from the theoretical gender bias (Vélez, 1982) according to the G test ($p<0.05$).

	RAINY SEASON					DRY SEASON				
	I. Barú	**I. Brujas**	**I. Mapar.**	**Marina**	**Taganga**	**I. Barú**	**I. Brujas**	**I. Mapar.**	**Marina**	**Taganga**
Shell Length (L; mm)	39.3 ±1.7^{bc}	33.3 ±1.1^{a}	34.1 ±0.8^{ab}	41.5 ±1.9^{c}	33.7 ±1.3^{a}	35.6 ±1.6^{a}	34.1 ±1.9^{a}	35.7 ±0.9^{a}	41.1 ±1.6^{b}	39.3 ±3.1^{ab}
Flesh dry-wt (FDW; mg)	103.1 ±7.6^{a}	95.3 ±8.3^{a}	231.1 ±26.1^{b}	153.7 ±9.6^{b}	70.7 ±6.0^{a}	91.7 ±8.1^{b}	56.7 ±8.9^{a}*	62.7 ±6.6^{a}*	155.4 ±17.3^{c}	53.5 ±6.3^{a}
Shell dry-wt (SDW; g)	3.2 ±1.7^{a}	4.3 ±2.5^{b}	3.7 ±2.2^{ab}	6.5 ±2.9^{c}	2.7 ±1.2^{a}	4.1 ±2.0^{c}	2.6 ±1.2^{b}*	1.7 ±0.6^{ab}*	6.3 ±2.8^{d}	1.4 ±0.7^{a}*
Sex ratio (Sex Ratio Ind)	1.2:1 (0.40)	1:1.1 (0.11)	1.2:1 (0.4)	1.4:1 (0.76)	2:1$^{♯}$ (4.08)	1.3:1 (1.17)	1:2.3$^{(1)}$ (0.80)*	1.5:1 (2.37)	1:1.4 (0.00)	2.5:1$^{(1)}$ (4.67)
Intersex Index	0	0	0	2.5	0	5	5	0	5	0
Gonad Index	3.6 ±0.2^{b}	3.3 ±0.2^{b}	3.4 ±0.2^{b}	2.6 ±0.2^{a}	2.4 ±0.2^{a}	3.2 ±0.3^{b}	1.6 ±0.3^{a}*	3.0 ±0.3^{b}	3.3 ±0.3^{b}	3.4 ±0.4^{b}*
Gamet. Mass Index	0.26$^{(a)}$	0.40$^{(b)}$	0.19$^{(a)}$	0.07$^{(a)}$	0.02$^{(a)}$	0.16$^{(a)}$	0.00$^{(b)}$	0.10$^{(a)}$	0.30$^{(a)}$	0.30$^{(a)}$
Undifferent. Index	0.00	0.05	0.0	0.03	0.05	0.05^{a}	0.35^{b}	0.00^{a}	0.00^{a}	0.07^{a}
Oocyte Atresia Prevalence	0^{a}	0^{a}	0^{a}	0.053^{b}	0^{a}	0	0	0	0	0
Oocyte Atresia Intensity	0^{a}	0^{a}	0^{a}	0.053± 0.101^{b}	0^{a}	0	0	0	0	0
Oocyte Atresia Index	0^{a}	0^{a}	0^{a}	0.003^{b}	0^{a}	0	0	0	0	0
Prevalence (oedema) %	2.5$^{(a)}$	15$^{(a)}$	30$^{(b)}$	0$^{(a)}$	0$^{(a)}$	85$^{(b)}$	55$^{(a)}$	45$^{(a)}$	70$^{(a)}$	40$^{(a)}$
Prevalence (BCA) %	0^{a}	0^{a}	2.5^{a}	22.5^{b}	2.5^{a}	10^{a}	5^{a}	0^{a}	45^{b}	5^{a}
Prevalence (granuloc) %	0	0	0	0	0	0	0	0	0	0
Preval. (diss neoplasia) %	0	0	0	0	0	0	0	0	0	0
Prevalence (ciliates) %	*2.5*$^{(a)}$	*7.5*$^{(b)}$	*2.5*$^{(a)}$	*0*$^{(a)}$	*0*$^{(a)}$	*10*$^{(a)}$	*15*$^{(b)}$	*0*$^{(a)}$	*5*$^{(a)}$	*5*$^{(a)}$
Preval (Undert. Prot.) %	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Preval. (Total Prot.) %	*2.5*$^{(a)}$	*7.5*$^{(b)}$	*2.5*$^{(a)}$	*0*$^{(a)}$	*0*$^{(a)}$	*10*$^{(a)}$	*15*$^{(b)}$	*0*$^{(a)}$	*5*$^{(a)}$	*5*$^{(a)}$
Prevalence (*Nematopsis*)%	0	2.5	2.5	0	0	0^{a}	30^{b}	0^{a}	0^{a}	0^{a}
Prevalence (Metazoan)%	0	2.5	0	0	2.5	0^{a}	0^{a}	10^{b}	0^{a}	0^{a}
Prevalence (R/CLO) %	0^{a}	5^{b}	0^{a}	0^{a}	0^{a}	10^{b}	0^{a}	10^{b}	0^{a}	5^{a}

$^{(1)}$ >30% of oysters with undifferentiated gonad.

A variety of parasites such as intracellular ciliates, *Nematopsis* sp., metazoan and R/CLO were found although, in general terms, both prevalence and intensity were low all along the study

(Table 3; Figs. 8 and 12b). The highest PII values were found in Isla Brujas, where in addition PII was significantly higher in the dry season than in the rainy season (most likely due to the moderate-to-high prevalence of *Nematopsis* sp.); likewise, PII tended to be higher in the dry season than in the rainy season in all the localities (Fig. 12b).

Overall, no major alteration in the histological integrity of the digestive gland tissue was recorded. However, in the dry season the prevalence of oedema in digestive gland tissue in all the localities was high, especially in Isla Barú and Marina Santa Marta, and the prevalence of brown cell aggregates in Marina Santa Marta was moderately high (Table 3). Granulocytomas and disseminated neoplasia were not recorded. Prevalence of inflammatory responses were apparently higher in the dry season than in the rainy season (Table 3). IRI values were higher in Isla Barú than in the other localities in the dry season, when they were seemingly higher in all the localities; although seasonal differences were not significant in Isla Maparadita and Isla Brujas because IRI values were also conspicuous in the rainy season (Fig. 12c). Medium values of DGAI (score ~1-2) were recorded in all the localities all along the study. Nevertheless, DGAI values were significantly higher in the dry season than in the rainy season in Isla Brujas and, *vice versa*, higher in the rainy season than in the dry season in Taganga (Fig. 12d). Besides, DGAI was significantly different amongst localities in the dry season, with highest values in Isla Brujas followed by Isla Maparadita (Fig. 12d).

LT_{50} values were significantly different between seasons, especially in Barú, Isla Brujas and Isla Maparadita ($p<0.05$, Fig. 12e); the lowest LT_{50} values being recorded in these localities in the rainy season and less markedly in the dry season, although still LT_{50} values recorded in Isla Brujas were the lowest (Fig. 12e).

The profiles depicted by the 5 indices of biological response varied with season (radar plots in Fig. 13), except in Marina and Taranga where FDW/L and RAI were equally responsive at both seasons. In the rainy season, LT_{50} was particularly sensitive in Isla Barú, Isla Brujas and Isla Maparadita, whilst in the dry season responses at mid-to-high level of biological complexity (FDW/L and RAI) were elicited in Isla Barú and at low-to-mid level (DGAI, IRI and FDW/L) in Isla Maparadita and, especially, in Isla Brujas (Fig. 13). As a result, IBR/n index showed significant differences in both seasons (Fig. 13): Isla Brujas and Isla Maparadita showed higher IBR/n values than Isla Barú, Marina Santa Marta and Taganga in the rainy season, Isla Brujas being the locality with the highest IBR/n values in the dry season.

4. Discussion

The habitats and environmental conditions of Nicaragua and Colombia were different, so the anthropogenic impact and the profile and levels of pollutants were as well (Dumailo, 2003; Vivas-Aguas et al., 2013; Mancera-Pineda et al., 2013; Chapter 1). Thus, shallow subtidal oyster reefs were investigated in Nicaragua whilst the study in Colombia was addressed to intertidal prop roots of mangrove trees and intertidal rocky shores. Moreover, SST and salinity were higher in Colombia than in Nicaragua, with extreme high SST (>33ºC) in the former and extreme low salinities (<2‰) in the latter in the rainy season (Table 1).

As a result, a main variable to keep in mind for inter-regional comparisons is that oysters from Nicaragua (Shell L_{max}=7.3±1.7 cm) and Colombia (Shell L_{max}=3.7±0.9 cm) corresponded to different size classes, which were the most abundant ones at each corresponding sampling site.

4.1. Nicaragua

Size varied with season, with the largest oysters being collected in Half Way Cay and Punta Lora in the rainy season. Accordingly, FDW, L and SCV were higher in Half Way Cay and Punta Lora than in Pigeon Cay in the rainy season, which suggests that growth was reduced in Pigeon Cay oysters.

Generally speaking, stress is associated with a decrease in flesh condition, whereas a higher abundance of food and hormonally active substances will increase flesh condition (Smaal and Van Stralen, 1990). Different indices are available to determine flesh condition in oysters (Crosby and Gale, 1990). In Nicaragua, FDW/SCV and FDW/L were clearly lower in the dry season than in the rainy season, and lower in Pigeon Cay than in the other two localities. Seasonal changes in FCI have been previously reported in mangrove oysters (Nascimento and Pereira, 1980; Nascimento et al., 1980; Meyer et al., 1998; Rebelo et al., 2005). However, the seasonal pattern may vary depending on locally relevant differences in nutritional and reproductive status, as well as on environmental constrains (e.g. the presence of pollutants). Thus, unlike in the present study, in *C. gigas* and *C. corteziensis* from the Gulf of California lagoons, FDW/SCV was higher in the dry season than in the rainy season (Osuna-Martínez et al., 2010). Likewise, high FDW/SCV and FDW/L values have been reported in *C. rhizophorae* both in the dry season, associated to high levels of nutrients in the water (Meyer et al., 1998; Rebelo et al., 2005), but also in the rainy season, concomitantly with full gonad maturation (Nascimento and Pereira, 1980; Nascimento et al.,

1980). Presently, high FDW/L values would be expected together with not spawning advanced gametogenic stages; this seems to apply when the rainy and the dry seasons are compared in Half Way Cay, and to a lesser extent in Punta Lora, where a higher incidence of spawning stages in the dry season than in the rainy season coincides with lower FDW/L values. However, the lowest FDW/L was recorded in Pigeon Cay, where gamete development was seemingly arrested in the rainy season (late gamete development stage was dominant resulting in high GI values) and the highest in Punta Lora in the rainy season were spawning stages were dominant. Therefore, reproduction was not the main factor influencing FDW/L in the rainy season.

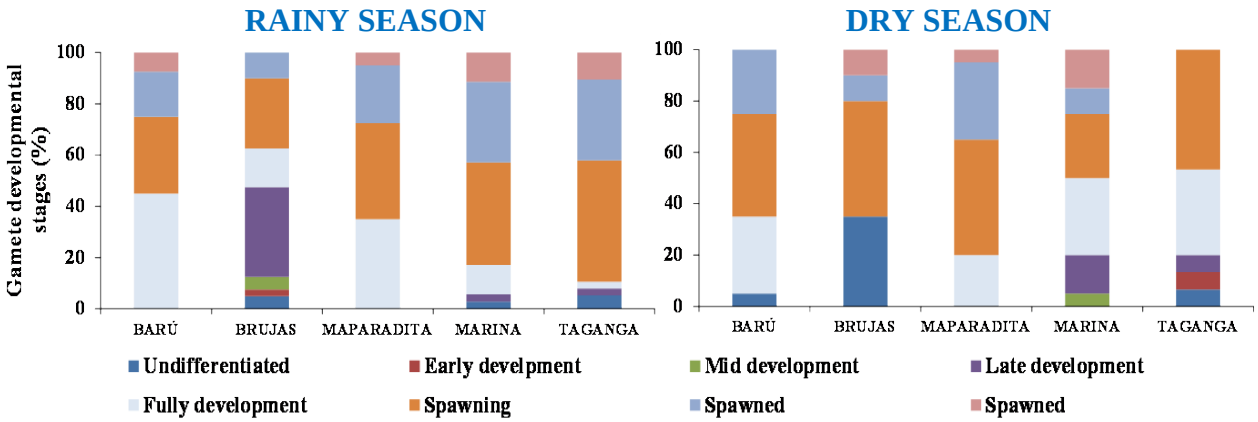
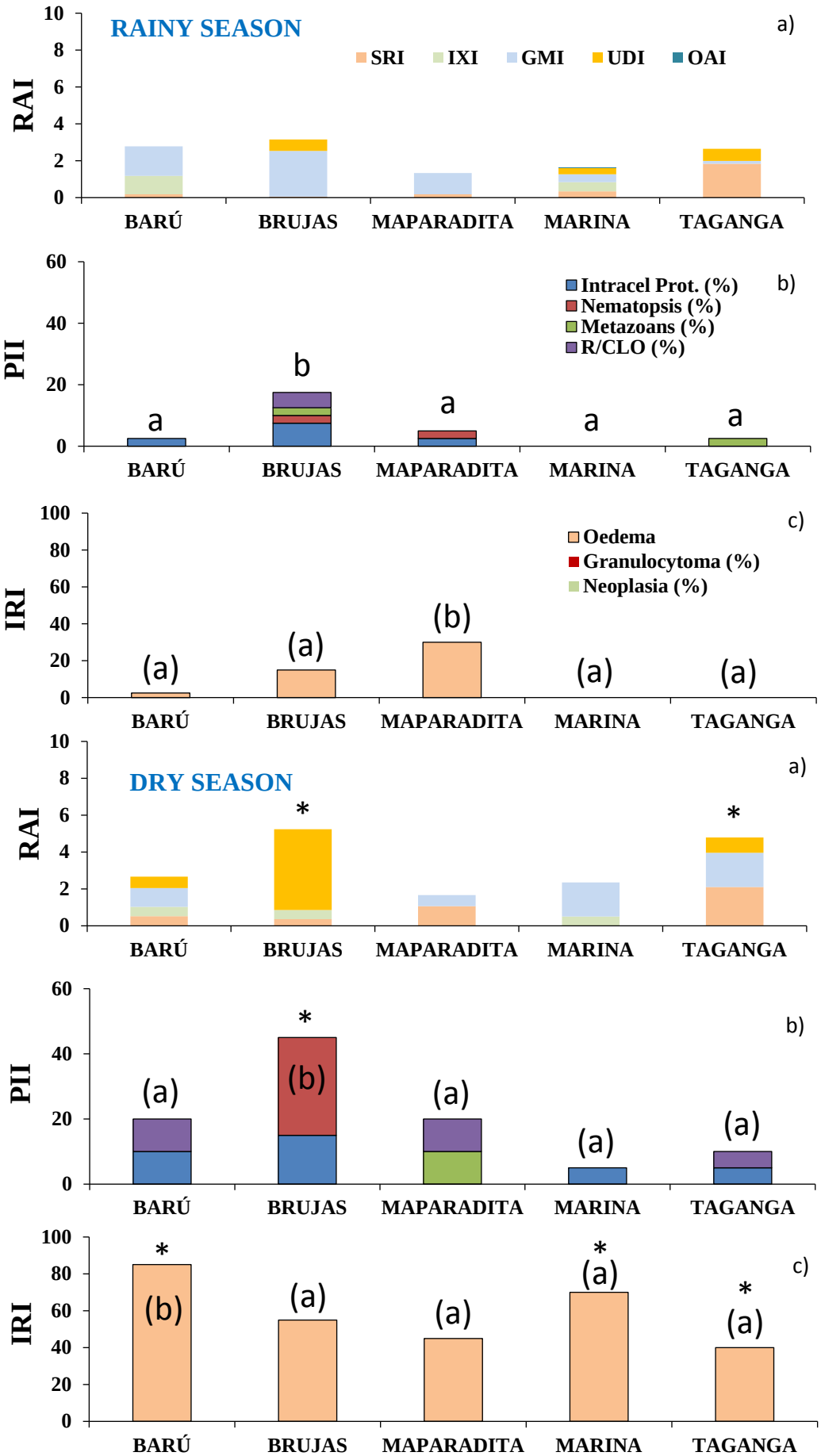


Fig. 11. Gamete development stages of oyster *Crassostrea rhizophorae* collected from Colombia at the rainy and the dry seasons.

Conversely, the low FDW/SCV and FDW/L values found in Pigeon Cay in comparison with Half Way Cay and Punta Lora might be attributed to the presence of pollutants (e.g., PAHs, PCBs and PBDEs), as reflected in pollution indices such as the Pollution Load Index (PLI; Tomlinson et al., 1980) and the Chemical pollution index (CPI; Bellas et al., 2011), according to Chapter 1. Indeed, FDW/SCV was proposed as an inexpensive, quick, representative and responsive tool for monitoring pollution (Scott and Lawrence 1982), commonly used to estimate growth differences among oysters living in different environmental conditions (Austin et al., 1993). Likewise, FDW/SCV provides indication of the nutritive status of oysters and of whether they are subject to stress conditions (Crosby and Gale, 1990). For example, in *C. virginica*, exposure to metals such as Cu and Cr is directly related with a decrease in FDW/SCV (Aguilar et al., 2012); and in a field study in Brazil, FDW/SCV was found to be different in *C. rhizophorae* from estuaries with different levels of PAHs and PCBs (Valdez Domingos et al., 2007).

Reproduction anomalies were recorded and scored and were also more relevant in the rainy season than in the dry season and more marked in Pigeon Cay and Half Way Cay than in Punta Lora where, conversely, anomalies were most evident in the dry season. It is worth noting that the so-called reproductive anomalies do not necessarily mean deleterious or pathological effects but simply deviations from the expected values that might be indicating potential disturbances in oysters' reproduction. The main anomalies recorded in this study included alterations in sex ratio, gamete development delay/arrest and oocyte atresia. *Crassostrea* oysters are protandrous hermaphrodites and sex ratio values can vary depending on the species, age, size and local conditions (Dove and O'Connor, 2012; Park et al., 2012). Sex appears to be determined by a single gene with a dominant male allele M and a recessive protandrous allele F, such that FF animals are protandrous and MF animals are permanent males (Powell et al., 2011). In *C. rhizophorae*, females predominate and can reach up to 90% of the population although their prevalence may vary with size and season (Vélez, 1982). In raft cultivated *C. rhizophorae* (>20 mm shell length), females were predominant (male/female ratio = 0.2-0.6) during the rainy season and especially in the largest size classes (Gordon, 1988). Accordingly, sex ratio indicated a dominance of females in Nicaragua; however, feminisation went beyond the expected values (according to criteria by Vélez, 1982) in the rainy season, especially in Pigeon Cay and Half Way Cay but also in Punta Lora. Sex ratio in Punta Lora oysters was comparable to those reported for *C. rhizophorae* in the Laguna Grande de Obispo, in Venezuela (F:M, 2.42:1; Montes et al., 2007), and in Guaratuba Bay (F:M, 1.6:1) and in Camanu Bay (F:M, 1.9:1 y 2.1:1) in Brazil (Christo and Absher, 2006; Lenz and Boehs, 2011). A predominance of females was also observed in *Crassostrea brasiliana* from Brazil (Castilho-Westphal et al., 2015). Sex ratio values in Pigeon Cay and Half Way Cay, however, were much higher. This apparent anomaly could be due to the large size of the oysters (L_{max}>80 mm), or caused by exposure to oestrogenic compounds in the rainy season. Skewed sex ratios have been reported in bivalve populations from sites polluted with tributyltin, metals and PAHs (Gagne et al., 2002; 2003; Gauthier- Clerc et al., 2002). Likewise, oestrogenic effects have been reported in bivalves exposed to sewage effluents and to urban and agricultural runoff waters (Blaise et al., 1999; 2003; Gagne et al., 2001; Quinn et al., 2004; Ortiz-Zarragoitia and Cajaraville 2010). Their exposure would be favoured by either the enhanced input of chemicals with endocrine disruption capacity (e.g. pesticides) in the rainy season. In contrast, sex ratio skew towards males was recorded in Half Way Cay in the dry season. In agreement, a similar masculinisation was reported in summer for *C. rhizophorae* by Lenz and Boehs, (2011).


RAINY SEASON
a)
RAI
SRI
IXI
GMI
UDI
OAI
BARÚ
BRUJAS
MAPARADITA
MARINA
TAGANGA
b)
PII
Intracel Prot. (%)
Nematopsis (%)
Metazoans (%)
R/CLO (%)
c)
IRI
Oedema
Granulocytoma (%)
Neoplasia (%)
DRY SEASON

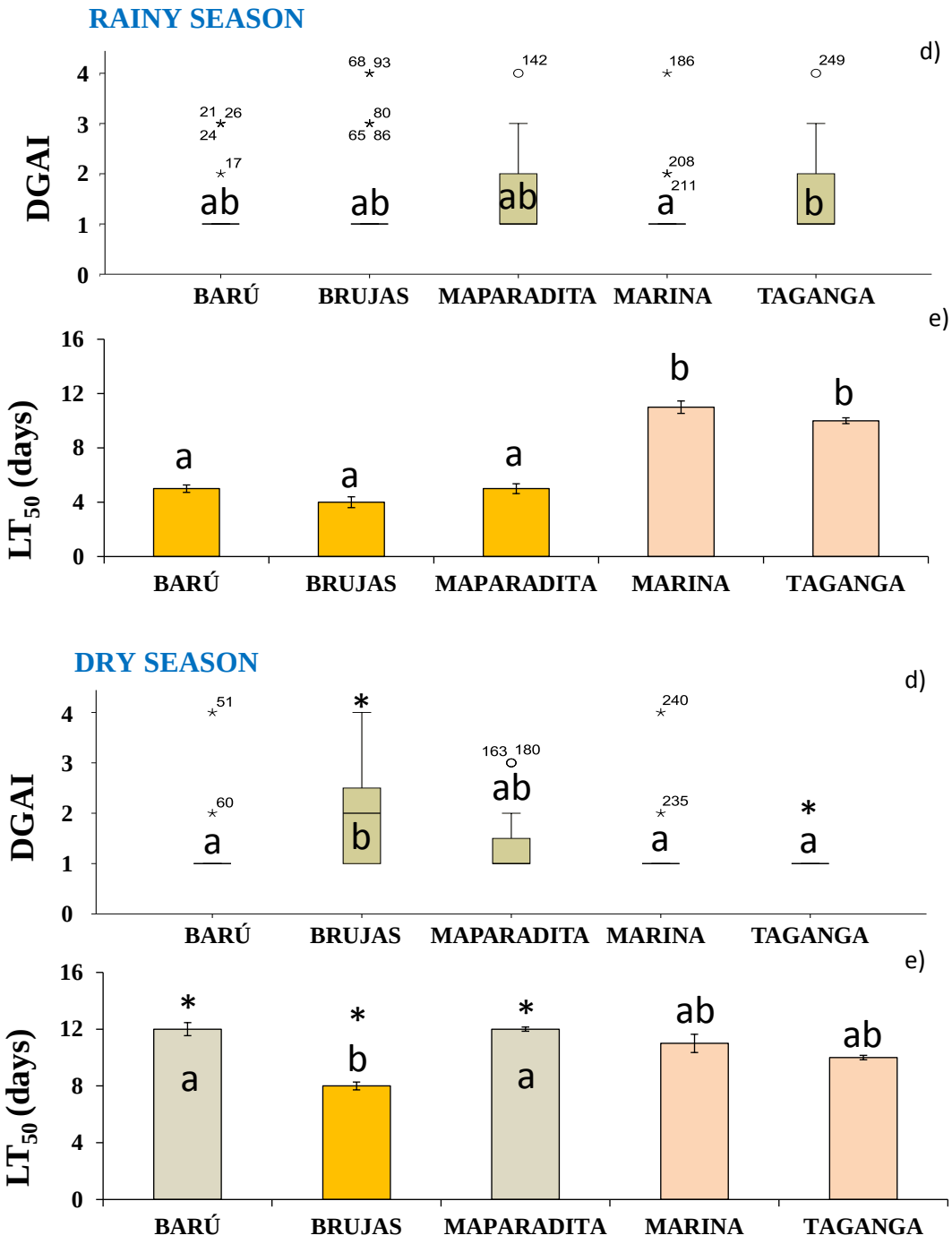


Fig. 12. Biomarkers of anomalies in oyster health indicative of disturbance in ecosystem health for the rainy and the dry seasons in Colombian mangrove swamps (Barú, Brujas, Maparadita, Marina and Taganga). Different letters denote statistically significant differences between localities and asterisk (*) indicate seasonal differences for a locality (p<0.05; p<0.1, when shown between brackets). RAI, reproductive anomalies index; PII, parasitic infestation index; IRI, inflammatory response index; DGAI, digestive gland athrophy index; LT_{50}, median survival time after SOS test.

Mangrove cupped oysters reproduce continuously throughout the year with peaks of seasonal spawning associated to temperature and salinity; however, spawning and spatfall are continuous in lagoons with temperatures higher than 25ºC (Vélez, 1982), like Bluefields and Pearl Lagoon. As seen in diverse species of *Crassostrea* from different regions of the world, GI is correlated with seawater temperature, salinity and quantity and quality of the food supply (Ren et al., 2003; Christo and Absher, 2006; Paixão et al., 2013). At least 2-3 spawning peaks per reproductive cycle have been reported in *C. rhizophorae* from different regions, but the main one seems to be in the rainy season (Nascimento et al., 1978; Villarroel et al., 2004; Lenz and Boehs, 2011). However, our results suggest that, as reported for many other tropical bivalves (Pouvreau et al., 2000), wild mangrove oysters from Nicaraguan Caribbean seem to follow a continuous reproductive cycle, albeit certain peaks of more intense spawning activity could not be disregarded. Similarly, *C. virginica* from Mecoacan was reported to spawn all along the year (George-Zamora et al., 2003). More detailed studies on wild mangrove oyster populations would be required in this respect. Nevertheless, gamete development was seemingly delayed in Pigeon Cay in comparison with Punta Lora in the rainy season, with Half Way Cay in between. In the dry season, however, gamete development was delayed in Punta Lora in comparison with Half Way Cay. In bivalves, exposure to chemicals such as oils, PAHs, DDTs, and alkylphenols causes alterations in gamete development and enhances oocyte atresia (Aarab et al., 2004; Binelli et al., 2001; Lowe, 1988; Lowe and Pipe, 1987; Ortiz-Zarragoitia and Cajaraville, 2006; 2011). Delayed gametogenesis have been reported in bivalve populations from sites polluted with tributyltin, metals and PAHs (Gagne et al., 2002; 2003; Gauthier- Clerc et al., 2002). The occurrence of gamete development arrest in Pigeon Cay and Half Way Cay but not in Punta Lora may be therefore due to pollutant specific effects. Interestingly, the highest tissue levels of PAHs were recorded in oysters from Pigeon Cay, followed by oysters from Half Way Cay; as well as the highest levels of PBDEs (Chapter 1). Conversely, OCPs do not seem to be related to effects on gamete development, as the highest tissue levels were recorded in oysters from Punta Lora in the rainy season (Chapter 1) in absence of apparent effects on gametogenesis.

Oocyte atresia is a normal process in advanced gametogenesis and post spawning stages, as described in e.g. *C. brasiliana* (Castilho-Westphal et al., 2015). Herein, atresia was recorded at higher levels in Pigeon Cay and Half Way Cay than in Punta Lora in the rainy season and its intensity values were particularly high in the two former localities resulting in significantly higher OAI values. Enhanced oocyte atresia could be a sign of gonad resorption and spawning abortion

aimed at re-directing energy resources from reproduction to counteract stressors (e.g. pollutants). Exposure to chemical pollutants is known to enhance oocyte atresia (Aarab et al., 2004; Binelli et al., 2001; Lowe, 1988; Lowe and Pipe, 1987; Ortiz-Zarragoitia and Cajaraville, 2006; 2011). In agreement, the levels of oocyte atresia can be related to the PLI and CPI values recorded in the studied localities (Chapter 1).

RAI incorporated the above discussed reproductive anomalies and showed that these were most relevant in Pigeon Cay and Half Way Cay in the rainy season and to a lesser extent in Punta Lora in the dry season. Though these reproductive anomalies might have result from "natural causes" (e.g. old large sized oysters might tend to be female with atretic oocytes) the observed inter-site differences suggested that additional stress sources might be acting (e.g., pollutants, nutrient availability, hypoxia or extreme salinity).

Although the intensity of parasitic infestations was generally low, it was higher in Half Way Cay than in Punta Lora all over the year; parasite prevalence being especially low in the rainy season in Punta Lora. Most frequently recorded parasites were *Nematopsis* sp. and ciliates, whilst neither metazoans (except a few in Half Way Cay in the rainy season) nor R/CLO were recorded. *Nematopsis* sp. seems to be a common parasite in Caribbean *C. rhizophorae* (Nascimento et al., 1986; Sabry et al., 2007; 2011, 2013; Brandão et al., 2013), mussels (Boehs et al., 2010; Garmendia et al., 2011; Ceuta and Boehs, 2012) and other oyster species (Aguirre-Macedo et al., 2007; Afsar et al., 2014). No seasonal trend was envisaged in the prevalence of *Nematopsis* sp., which was much lower in Punta Lora than in Half Way Cay in both seasons and in Pigeon Cay. *Nematopsis* has been recorded all throughout the year in *Mytella guyanensis* (Boehs et al., 2010; Ceuta and Boehs, 2012) but a marked seasonality in the prevalence of *Nematopsis* in *C. rhizophorae* has also been reported (Nascimento et al., 1986; Sabry et al., 2007; Brandão et al., 2013). The prevalence values recorded in Pigeon Cay, and Half Way Cay can be considered high according to previous criteria (Brandão et al., 2013). At low infection intensities this protist only causes inflammatory responses although at high intensities can be harmful, especially in the gills (Bower et al., 1994). Likewise, ciliates were recorded all over the year at similarly moderate prevalence values in all the localities except in Punta Lora in the rainy season where it was absent.

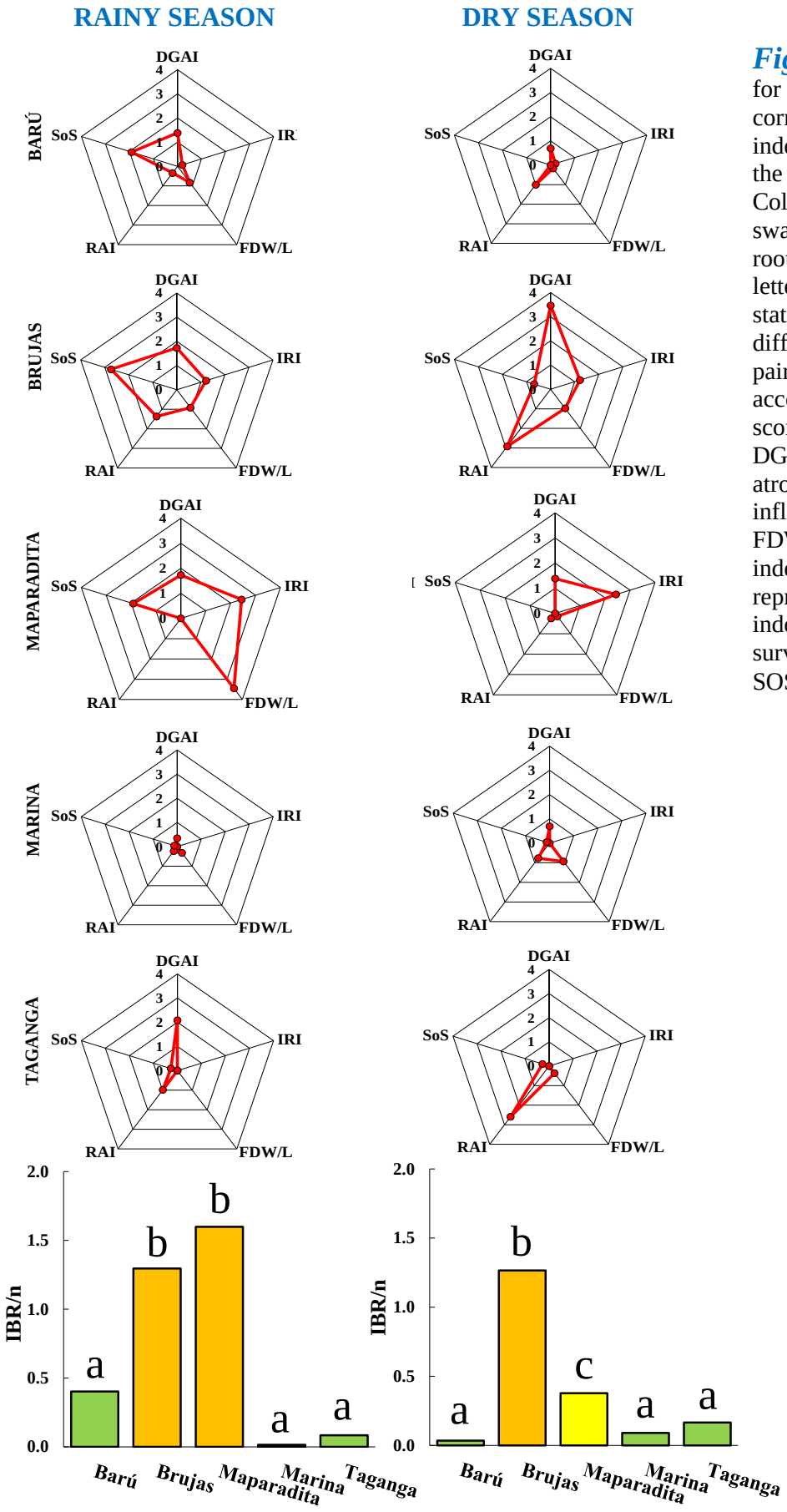


Fig. 13. Radar plots for biomarkers and the corresponding IBR/n index for the rainy and the dry seasons in Colombian mangrove swamps at intertidal roots/docks. Different letters denote statistically significant differences between pairs of means according to the Z-score test ($p < 0.05$). DGAI, digestive gland atrophy index; IRI, inflammation index; FDW/L, condition index; RAI, reproductive anomalies index; LT_{50}, median survival time after SOS test.

As a result, PII was high in Half Way Cay in both seasons and in Pigeon Cay, and moderate in Punta Lora in the dry season. The differences do not seem to be related to size (or age) because oysters from Pigeon Cay are the smallest but present the highest PII value; whilst usually large and aged individuals exhibit more severe parasitisation. Likewise, FCI does not seem to be relevant because oysters with similar FDW/L values show different parasite load. It might happen that environmental stressors or pollution exposure provokes immunodeficiency or that high turbidity and levels of suspended particulate or organic matter in the water column might facilitate parasitic infestation. In presence of high levels of suspended organic matter, growth of bacteria and algae and then of protozoans, their predators, is enhanced (La Rosa et al., 2001). Thus, the abundance of ciliates has been proposed as an indicator or the host health and the level of eutrophication (Palm and Dobberstein, 1999). The presence of ciliates depends on environmental factors such as temperature, salinity and suspended matter (e.g., turbidity) and has been suggested to be indicative of organic pollution resulting from e.g., domestic sewage (Marcogliese and Cone, 1997; Brandão et al., 2013). They are believed to be commensals (Lauckner, 1983) that only may result harmful at high intensities and not always, as reported in *C. rhizophorae* in Brazil (Nascimento et al., 1986; Boehs et al., 2009; da Silva et al., 2011; Brandão et al., 2013)

Disorganized ICT with haemocyte infiltration and shrinkage of digestive diverticula, digestive alveoli atrophy and digestive cell vacuolisation were observed in all the studied Nicaraguan localities, mainly in the rainy season. Comparable histopathological alterations were interpreted as a stress response to pollution enhancement in the rainy season (Valdez Domingos et al., 2007). Granulocytomas and disseminated neoplasia were only found, at low prevalence, in Pigeon Cay and Half Way Cay. These inflammatory lesions have been associated to the presence of pollutants but also to pathogens (Villalba et al., 2001; Kim et al., 2008; Bignell et al., 2011; Garmendia et al., 2011; De Vico and Carella, 2012). Oedema and brown cell aggregates were observed all along the year. Whilst he prevalence of brown cell accumulates was close to 100% in all the cases, the prevalence of oedema was higher in the dry season than in the rainy one and higher in Half Way Cay than in Punta Lora and Pigeon Cay, with highest values in Half Way Cay in the dry season. However, intensity values were low except in Pigeon Cay that exceeded the scores in other localities by a factor of 5 to 30 times. Elevated levels of haemocytic infiltration and oedema are general responses to chemical pollutants in molluscs (Au, 2004). These inflammatory responses were shown to be related to the tissue concentration of Cd and hydrocarbons in *C. virginica* from Mexican coastal ecosystem (Gold-Bouchot et al., 1995) and therefore they were suggested as indicators of pollution-induced stress for native oysters in coastal lagoons (Gold-Bouchot et al.,

2007). The prevalence of oedema seems to be related to PII and was especially high in Half Way Cay in the dry season, when the prevalence of intracellular parasites was highest.

Accordingly, IRI runs in parallel with PII suggesting that most inflammatory responses were associated to the presence of parasites and pathogens, in agreement with Carella et al., (2015). Nevertheless other aetiologies cannot be fully disregarded because granulocytomas, disseminated neoplasia, induced immunodepression can be caused by e.g. chemical agents (Au, 2004), and because the prevalence of the two former and the intensity of oedemas were higher in the Pigeon cay and Half Way cay in the rain season, where oysters presented the highest pollutant tissue levels (Chapter 1). Moreover, according to the recorded DGAI values (Couch 1985; Bodin et al., 2004; Kim and Powell, 2007; Garmendia et al., 2011; Apeti et al., 2014; Cuevas et al., 2015), general stress seems to be higher in the rainy season than in the dry one, with some signals of moderate stress in Half Way Cay in the dry season. Pollution by chemicals in mixture is a potential factor activating latent infection in oysters (Chu et al., 2002). Thus, in mussels collected from the Lanesundfjord in Norway following a contaminant gradient, non-parasitic tissue abnormalities were related to chemical contamination; however, dose-response relationships could not be directly defined (Auffret, 1988).

LT_{50} showed a very different profile in comparison with other general stress biomarkers such as DGAI. It was in the range of 3-9 d, the lowest being recorded in the dry season. This might indicate that oysters are more resistant to additional stress in the rainy season than in the dry season, which would not be expected from a stressed organism. Certainly, a better individual condition could explain these inconsistent results which corresponds to the cases of Half Way Cay and Punta Lora in the rainy season (high FDW/L and high LT_{50}) but unfortunately it does not apply for the case of Pigeon Cay (low FDW/L and high LT_{50}). On the other hand, the LT_{50} recorded in Nicaragua are not really high in comparison with e.g. Colombia oysters and therefore the resilience of oysters could be considered affected at regional scale and especially in the dry season. According to our records there is no previous data on SoS response in *C. rhizophorae* so that LT_{50} values can be only interpreted in comparative terms. Thus, LT_{50} in Nicaragua (~8 d) does not reach the values of 12 d recorded in some localities in Colombia in this study, which a more marked decrease than those reported in other species to discriminate polluted and reference sites (Blaise et al., 2016). In contrast, the low LT_{50} values recorded in Nicaragua in comparison with Colombian oysters could also be explained because Nicaraguan oysters were larger, as concluded for other species. For instance, small mussels present higher LT_{50} values than large mussels both under

welfare and stressed conditions (Thomas et al., 1999). More detailed research is needed to characterise SoS response in mangrove cupped oysters in the Caribbean region, though the present results are challenging. In any case, the same reasoning could be applied locally for the case of Nicaraguan oysters, with expected higher LT_{50} in small than in large oysters if environmental conditions are not dissimilar. However, Pigeon Cay oysters are smaller than in Half Way Cay and Punta Lorain the rainy season but LT_{50} values are not higher than in the two latter localities, which might suggest some effect on SoS in this locality. The low LT_{50} values recorded in the dry season can be explained by different, not necessarily opposing, causes know to lead to reduced survival-in air in bivalves (Viarengo et al., 1995; Thomas et al., 1999; Hellou and Law, 2003; Pampanin et al., 2005), say: poor individual condition (low CI and FDW/L), reproductive stress during spawning, unfavourable environmental conditions (hypoxia and high turbidity and salinity) and seasonal sources of pollutants (i.e., pesticides, etc.). In mussels from the Halifax Harbor low LT_{50} values were associated with bioaccumulation of polycyclic aromatic compounds (Hellou and Law, 2003). In mussels caged in contaminated estuaries for 6 wk, survival-in-air (ranging between 5.5-8 d, depending on the pollution levels) was correlated with the sum of CB44 and CD52 but not with other pollutants present (Smaal et al., 1991). Moreover, SoS response has been proposed to be an excellent means of valuating the effect of long-term chronic exposures (e.g. to crude oil; Thomas et al., 1999). For example, 4 years after the Exxon Valdez oil spill, *M. trossulus* from impacted sites survived in air only for 10–14 d whilst neighbouring reference mussels survived for 13–24 d; accordingly, they still presented high body burdens of PAHs (Thomas et al., 1999).

The present approach thus suggests that oysters are not equally healthy in the different localities and seasons investigated herein, which is directly evidenced by the IBR index; this provides an integrated view on the health status of sentinel organisms even though seasonal variability could act as a confounding factor, as previously suggested for bivalves from temperate regions subject to pollution (Leiniö and Lehtonen, 2005; Pytharopoulou et al., 2008; Serafim et al., 2012; Marigómez et al., 2013). Integrating these anomalies in the oysters' health status into the IBR/n index provided a general indication of the ecosystem health disturbance. The worst situation was that recorded in Half Way Cay followed by Pigeon Cay, both with comparable health profiles, whilst ecosystem health was less disturbed in Punta Lora, where the signal provided by DGAI in the rainy season might be indicative of a recent distressing episode, unlike the situation in other localities that seems to be more pervasive. In summary, complex multi-factorial sources of health disturbance seem to co-occur over a regional background of low-to-moderate pollution (e.g. by pesticides and domestic sewage; e.g. as shown in Table 1) in Nicaraguan Caribbean coast and

therefore correlation between IBR/n and pollution indices such as PLI and CPI was not significant. Though individual biological responses at each locality and season was to a large extent consistent, the lack of correlation may be due to the strong influence of the physiological state (modulated by multi-factorial drivers and seasonal variation under natural field conditions) of the oysters on these measurements.

4.2. Colombia

Shell length, in the range of 33-42 mm, was quite variable amongst localities, oysters from Marina Santa Marta being the largest. Moreover, SDW values were exceptionally high in Marina Santa Marta in both seasons, and SDW/L in Marina Santa Marta (both seasons), Isla Brujas (rainy season) and, to a lesser extent, in Isla Barú (dry season). Abnormal shell growth, including e.g. hypertrophy and chambering, has been associated to endocrine disruptors such as TBTs, which are characteristic of marinas and dockyards (Chagot et al., 1990); which could be the case of Marina Santa Marta.

The highest FDW/SDW was recorded in Isla Maparadita, with lower values in the dry season than in the rainy season. Although it is commonly used in the case of mussels as an indicator of individual condition (Lobel and Wright 1982; Smaal and van Stralen, 1990), FDW/SDW is known to have a limited value as index of nutritive status or recent stress in oysters. On the one hand, both flesh and shell growth are disparately variable throughout the year, especially in tropical oysters; on the other hand, unpredictable variations in SDW result from natural mineral incrustations and normally occurring shell break-offs (Crosby and Gale, 1990; Blaise et al., 2016). Indeed, the aforementioned differences in SDW (e.g. potential alterations in shell growth) may render FDW/SDW even less valuable in this particular study. In contrast, FDW/L overcomes uncertainties associated to unpredictable variations in SDW (Hellou et al., 2003; Blaise et al., 2016). Herein, together with high FDW/L values recorded in Isla Maparadita during the rainy season and to a lesser extent in Marina Santa Marta in both seasons the lowest were recorded in Taganga in both seasons, with overall higher values in all the localities in the rainy season than in the dry season. Cyclicity in condition index has been related to periods of post-spawning occurring towards the end of each rainy season (Gordon, 1988); which does not seem to be the present case, as spawning is not discontinued and, in any case, the productive cycle effects should have been the other way round and no relationship was perceived between FDW/L and gamete development. For instance, as they were at a non-spawning stage high FDW/L values would be expected in Isla

Brujas and Taganga oysters in the dry season but, quite the reverse, FDW/L was minimal. On the other hand, the high FDW/L values recorded in the rainy season can be also attributed to seasonal increases in suspended organic matter. Interestingly, seasonal increase in FCI in oysters is positively correlated with in microbial load whereas decreases in FCI have been related with high microbial tissue loads due to sewage pollution, which causes lower assimilation efficiency (Jana et al., 2014). Interestingly, in the present study, the faecal bacteria load is higher in the rainy season than in the dry season, which will coincide with high FWD/L values, and Taganga, with the lowest FDW/L values, receives sewage directly from the Santa Marta marine outfall; especially during the dry season, which is the high touristic season in Santa Marta. Finally, seasonal cyclic fluctuations in FCI could be explained partially by changes in salinity and number of days within various temperature regimes, which may result in decreased feeding activity or diet quality abundance (Austin et al., 1993). The salinity in Isla Maparadita during the rainy season is more extremely reduced than in any other locality studied. As a whole, in Santa Marta Bay, upwelling in the dry season leads to increased salinity, decreased temperature and turbidity and enrichment of inorganic nutrients due to the contribution of deep marine waters. In the rainy season, outwelling occurs, mediated by tropical rainfall and relevant river loads of sediment, organic matter and domestic sewage to the Santa Marta Bay; this results in decreased salinity and dissolved oxygen, and augmented temperature, turbidity and phosphorus and chlorophyll concentrations (Mancera-Pineda et al., 2013). The Gaira and Manzanares rivers contribute with freshwater and sediments to the bay during the rainy season, together with inorganic nutrients, domestic sewage and litter. Besides, mineral coal transportation, a major port activity, contributes to enhanced sedimentation and sediment re-suspension thus leading to elevated levels of suspended particulate matter. Therefore, FDW/L would be governed by the nutritional status of by the influence of stressors or specific contamination sources (e.g. domestic sewage) rather than by seasonality in gamete development and spawning.

According to the SRI, a spot feminisation could be envisaged in Taganga oysters in both seasons. This locality is influenced by discharges from the nearby marine outfall of Santa Marta and could therefore be recipient of potentially oestrogenic chemical pollutants. In contrast, intersex oysters were found in Marina Santa Marta (both seasons) and Isla Brujas (dry season). Intersex has been reported in the vicinity of sewage discharge locations in a variety of species from copepods to fish (Moore and Stevenson, 1994; Ortiz-Zarragoitia and Cajaraville, 2010; 2011; Puy-Azurmendi et al., 2010; 2013); however, intersex gonads are normally found at small numbers

(0.3-1.6%) in *C. rhizophorae* (Vélez, 1982) and herein intersex cases, with separate male and female gonad follicles, were only sporadically. Nevertheless, the intersex cases presently recorded coincide with the cases of oversized shell, which has been related to the presence of anti-oestrogenic compounds such as TBT (Chagot et al., 1990). In any case, the presence of endocrine disrupting chemicals seems to be a question of concern; these chemicals include oestrogens, anti-oestrogens, anti-androgens, toxicants that reduce steroid hormone levels, toxicants that affect the CNS, and toxicants that affect hormonal status (Depledge and Billinghurst, 1999), which typically occur in mixtures in domestic sewage effluents and industrial wastes. Moreover, a certain arrest in gametogenesis was envisaged in Isla Brujas in the dry season, with a high prevalence of oysters with undifferentiated follicles. In the rainy season, Marina Santa Marta and Taganga showed the most advanced gamete development stages; however, the opposite was found in the dry season. Oocyte atresia was practically negligible and therefore RAI was mainly influenced by altered sex ratios and arrest/delay of the gametogenic cycle, with the highest scores recorded in Isla Brujas and Taganga, followed by Isla Barú. The observed low-to-moderate reproduction anomalies seem to be related to pollutant exposure rather than to nutritional or environmental conditions. Components of domestic sewage and a wide range of industrial chemicals are capable of disrupting the endocrine systems of a wide range of wildlife species (Depledge and Billinghurst, 1999).

The prevalence of parasites (such as *Nematopsis* sp, ciliates, intracellular protists, RCL/Os, and metazoans) was low in all the samples; though seemingly higher in the dry season than in the rainy season. These parasites are common in *C. rhizophorae* and related species and their seasonal variations and their relation to environmental conditions have been previously reported. The presence of *Nematopsis* sp and ciliates seems to be indicative of organic pollution (Marcogliese and Cone, 1997; Brandão et al., 2013), and unlike in Colombia, it has been recorded at high prevalence in Nicaraguan mangrove cupped oysters (present study) and, overall, in tropical bivalves (Nascimento et al., 1986; Aguirre-Macedo et al., 2007; Sabry et al., 2007; 2011, 2013; Boehs et al., 2010; Ceuta and Boehs, 2012; Brandão et al., 2013). RCL/Os have been previously recorded in the digestive gland cells of *C. rhizophorae* (da Silva et al., 2011; Zeidan et al., 2012; Brandão et al., 2013; Sabry et al., 2013) with low prevalence usually below 30% and absence of pathological effects others than hypertrophy of infected cells. In *C. rhizophorae* from Maraú River in Brazil, low prevalence (3.3%) of metazoan and R/CLO was also reported in the rainy season in comparison with the dry season (10-13.3%; Brandão et al., 2013). Likewise, low prevalence (1.76%) of the metazoan *Tylocephalum* was found in *C. rhizophorae* in the dry season, associated

with high water temperature (Sabry et al., 2007), as well as for R/CLO in the dry season in *Mytella guyanesis* (Ceuta and Boehs, 2012). Isla Brujas, especially in the dry season, exhibited moderate prevalence values for *Nematopsis* sp. and ciliates. As a result, PII was higher in Isla Brujas (both seasons) than in the other localities, followed by Isla Barú and Isla Maparadita in the dry season. Pollution by chemicals in mixture is a potential factor activating latent infection in oysters (Chu et al., 2002). Isla Brujas received the input from the Dique Channel, which is a main source of sediments and chemical pollution in the Cartagena Bay, both in the rainy and the dry seasons; indeed, metals and PAHs in sediments are at concentrations that pose environmental risk, according to international policies (Vivas-Aguas et al., 2010). Thus, some degree of immunodepression might be envisaged in Isla Brujas oysters but this cannot be confirmed with the present data and in any case it seems to be of minor entity.

Regarding inflammatory responses only oedema and brown cell accumulation (this latter at much lower levels than in Nicaraguan oysters) were recorded, particularly high in the dry season. At this season there might be some common causing agent everywhere, but most especially in Barú and Marina, be this a particular pollutant, sewage contamination or environmental conditions. Inflammatory responses in bivalves are generally considered to be indicative of stress, linked to severe lesions caused by parasitosis (Cochennec-Laureau et al., 2003; Garmendia et al., 2011; Comesaña et al., 2012) or xenobiotics compounds (Rojas. et al., 1999; Villalba et al., 2001; Sheir and Handy, 2010). Elevated levels of haemocytic infiltration and oedema are general responses to chemical pollutants in molluscs (Au, 2004). These inflammatory responses were shown to be related to the tissue concentration of Cd and hydrocarbons in *C. virginica* from Mexican coastal ecosystem (Gold-Bouchot et al., 1995) and therefore they were suggested as indicators of pollution-induced stress for native oysters in coastal lagoons (Gold-Bouchot et al., 2007). Thus, IRI exhibited the same trends than PII in the rainy season but not at all in the dry season. DGAI, which represents a fast and enduring biological response to environmental stressors (Garmendia et al., 2011; Marigómez et al., 2013), only exhibited a low-to-moderate raise in Isla Brujas in the dry season and therefore it did not provide any clear evidence of health impairment.

LT_{50} was in the range of 4-12 d. Oysters from Isla Brujas in the dry season and, most markedly, those from Isla Barú, Isla Brujas and Isla Maparadita in the rainy season exhibited very low LT_{50} values. Unlike in Nicaraguan mangrove cupped oysters, in which the lowest LT_{50} values were recorded in the dry season, Colombian oysters seem to be more resistant to additional stress in the dry season than in the rainy season, as it would be expected from a stressed organism. Moreover,

LT_{50} values were in general terms higher than in Nicaragua, which might be related to the smaller size of the Colombian oysters or to the poorer health condition of the Nicaraguan oysters at regional scale, as above discussed. Overall, the SoS response profile in the dry season resembled the one recorded for DGAI but, conversely, these biological responses were apparently not at all related in the rainy season, nor related to other observed health and growth parameters, neither with gamete development. Likewise, LT_{50} values could not be related to the pollutant tissue burdens reported in the oysters (Chapter 1). In the case of intertidal mussels from temperate regions, LT_{50} values around 3-6 d have been reported on exposure to Cu, Cd, PCB congeners, Aroclor 1254, individual PAHs (e.g., 9,10-dimethyl 1,2-benzanthracene, fluoranthene or benzo[a]pyrene), in comparison with >10 d recorded in control and reference mussels (Veldhuizen-Tsoerkan et al., 1991; Eertman et al., 1995; Viarengo et al., 1995). Thus, as a general rule, LT_{50} values >10 d are indicative of healthy condition whereas values <6 d are found in stressed mussels (Viarengo et al., 1995; Davies and Vethaak, 2012; Martínez-Gómez et al., 2017). These critical values, however, can vary depending on the nutritional, reproductive and physiological status of the organisms and on environmental factors; thus, for instance, small mussels seem to be more tolerant to survival-in-air than larger ones, both in the polluted and non-polluted sites (Thomas et al., 1999). Likewise they can be different for different species, e.g. even if they are sympatric *Mytilus* spp. (Hellou and Law, 2003), and therefore the present results obtained for mangrove cupped oysters must be interpreted cautiously. It seems that SoS response if the result of multiple agents interacting (pollution, environmental factors).

As previously reported in mussels from temperate regions (Hellou and Law, 2003; Koukousika and Dimitriadis, 2005; Nesto et al., 2007; Pampanin et al., 2005), a strong seasonal variability in SoS, which followed opposite patterns in Nicaraguan and Colombian oysters, was envisaged. This intricate response pattern may be attributed to region-specific factors that may influence SoS response, such as ambient temperature, reproductive stage and size (Martínez-Gómez et al., 2017). Moreover, survival in air is known to be more limited for subtidal bivalves than for intertidal ones (Babarro and de Zwaan, 2008), and oysters from Nicaragua inhabited shallow subtidal reefs in mangrove lagoons whilst those from Colombia were collected from intertidal roots/docks in mangrove swamps. Thus, we must be cautious to interpret the present LT_{50} values alone, though they can be useful for comparative purposes and to understand the battery of biomarkers in an integrative manner.

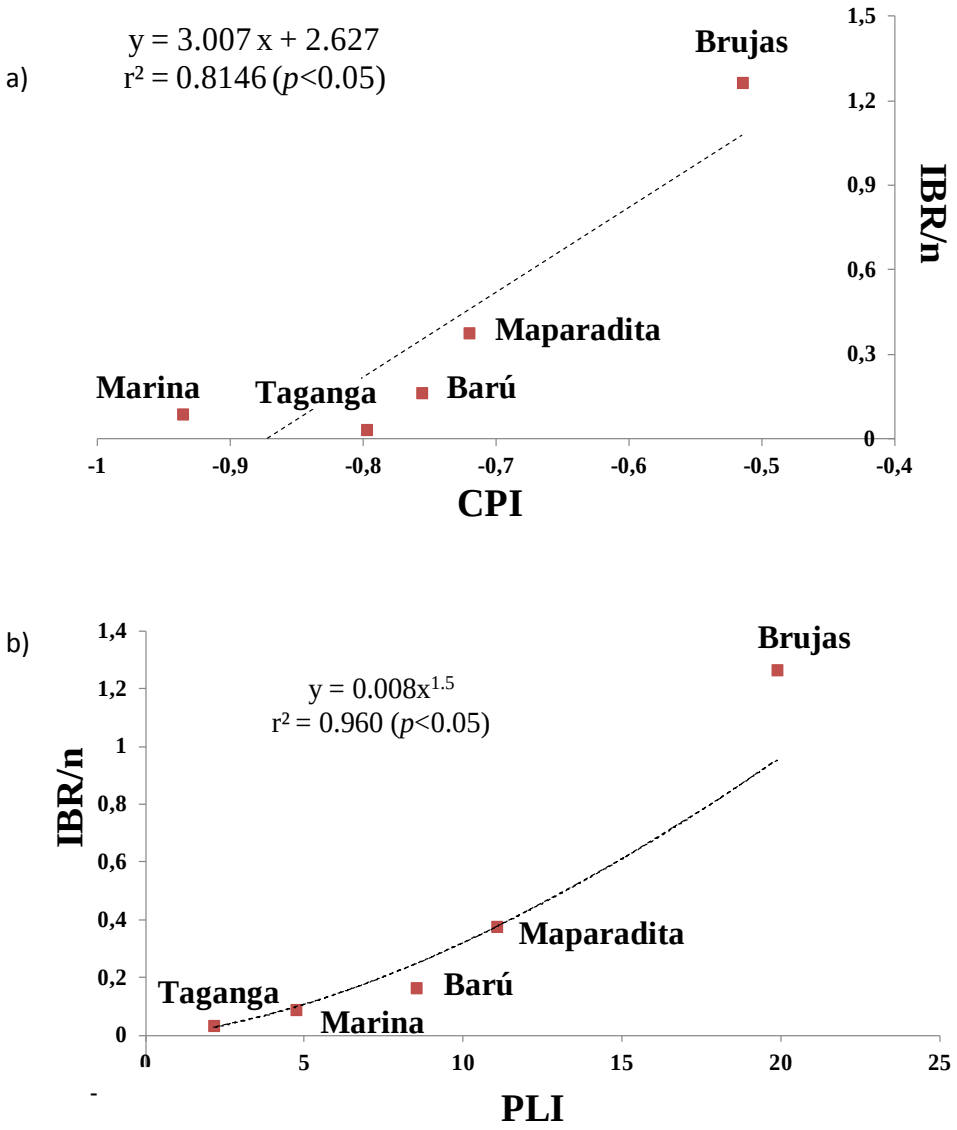


Fig. 14. Regression models and correlation between IBR/n and CPI (a) and PLI (b) in Colombian mangrove swamps in the dry season. CPI and PLI data obtained in Chapter 1. CPI, Chemical Pollution Index (Bellas et al., 2011; 2014); PLI, Pollution Load Index (Tomlinson et al., 1980).

Though modulated by natural variables and confounding factors, different indicators of health disturbance alone and in combination were related to the presence of different profiles and levels of chemical pollutants present at low-to-moderate levels. Thus, IBR/n discriminated Isla Brujas and Isla Maparadita from the other localities in both seasons as well as Isla Brujas from Isla Maparadita in the dry season. Considering altogether the present results and the pollutant tissue levels reported for these mangrove cupped oysters (Chapter 1), it can be concluded that different mixtures of a variety of persistent (e.g., As, Cd, PAHs) and emerging chemical pollutants (e.g. musk fragrances) in combination with different levels of organic and particulate matter resulting from upwelling and sewage discharges, and environmental factors (salinity, temperature) elicit a different degree of disturbance in ecosystem health condition in mangrove-lined Caribbean coastal

ecosystems, as reflected in sentinel *C. rhizophorae*. As a result, IBR/n was correlated with pollution indices such as PLI and CPI (Fig. 14), even though the levels of biological anomalies used as indicators of health disturbance and the levels of pollutants were in general terms low-to-moderate in a real field situation influenced and modulated by biological variables and environmental factors that varied seasonally.

In conclusion, integrative approaches for marine and coastal pollution monitoring involve the use of methodological standards in both sampling, analysis of samples and interpretation of results (ICES 2011; OSPAR Commission 2013b). Assessment of ecosystem health disturbance for the monitoring of pollution and its biological consequences in tropical and subtropical coastal regions is constrained by (a) difficulties for systematic routine sampling (e.g. accessibility to sampling sites is expensive and complicated, and may be hampered by natural phenomena such as hurricanes, cyclones and earthquakes); (b) the limited knowledge on the biology and ecology of local/regional target species; (c) the lack of adequate sample transportation to the laboratory (this may take long time under non-optimal conditions for sample quality preservation); and (d) technological hurdles (e.g. cryo-processing and cryo-sample transportation) that impede the application of core endpoints elsewhere applied in coastal biomonitoring. Moreover, quantifying a suite of biomarkers often requires purchasing dedicated equipment, special wares, and expensive reagents not always easy to afford by developing countries (Blaise et al., 2016). As previously established by Blaise et al. (2016) for mussels of sub-Arctic and temperate regions, our study supports the use of simple methodological approaches to diagnose anomalies in the health status of mangrove cupped oysters from different localities and to identify potential causing agents. These anomalies may reflect disturbances in ecosystem health that can be associated to chemical pollutants, eutrophication (e.g. from domestic sewage) as well as to naturally or human-driven anomalies and extremes in environmental factors such as salinity, dissolved oxygen, suspended matter, etc. Consequently, the easy methodological approach used herein is useful for the assessment of health disturbance in a variety Caribbean coastal ecosystems using mangrove cupped oysters as sentinel species. Likewise, this approach is useful to identify presumptive sources of health disturbance, be these chemical pollutants or other stress sources, which can further on be identified following specific and more complicated and expensive analytical procedures.

5. References

Aarab N, Minier C, Lemaire S, Unruh E, Hansen PD, Larsen BK, Andersen OK, Narbonne JF (2004) Biochemical and histological responses in mussel (*Mytilus edulis*) exposed to North Sea oil and to a mixture of North Sea oil and alkylphenols. Mar Environ Res 58: 437–441.

Aarab N, Pampanin DM, Naevdal A, et al (2008) Histopathology alterations and histochemistry measurements in mussel, *Mytilus edulis* collected offshore from an aluminium smelter industry (Norway). Mar Pollut Bull 57: 569–74.

Afsar N, Siddiqui G, Roberts D (2014) Parasite inspection in five commercially important oyster species (Mollusca : Bivalvia) of Pakistan. J Basic Applied Sci 10: 220–225.

Aguilar CA, Montalvo C, Rodríguez L, Cerón JG, Cerón RM (2012) American oyster (*Crassostrea virginica*) and sediments as a coastal zone pollution monitor by heavy metals. Int J Environ Sci Technol 9: 579–586.

Aguirre-Macedo ML, Simá-Álvarez RA, Román-Magaña MK, Güemez-Ricalde JI (2007) Parasite survey of the eastern oyster *Crassostrea virginica* in coastal lagoons of the southern Gulf of Mexico. J Aquat Anim Health 19: 270–279.

Alfonso JA, Handt H, Mora A, Vásquez Y, Azocar J, Marcano E (2013) Temporal distribution of heavy metal concentrations in oysters *Crassostrea rhizophorae* from the central Venezuelan coast. Mar Pollut Bull 73: 394–398.

Allan I, Vrana B, Greenwood R, Mills G, Roig B, Gonzalez C (2006) A “toolbox” for biological and chemical monitoring requirements for the European Union’s Water Framework Directive. Talanta 69: 302–322.

Alves SRC, Severino PC, Ibbotsom DP, Silva AZX, Lopes FRAS, Saenz LA, Bainy ACD (2002) Effects of furadan in the brown mussel *Perna perna* and in the mangrove oyster *Crassostrea rhizophorae*. Mar Environ Res 54: 241–245.

Andral B, Stanisiere JY, Sauzade D, Damier E, Thebault H, Galgani F, Boissery P (2004) Monitoring chemical contamination levels in the Mediterranean based on the use of mussel caging. Mar Pollut Bull 49: 704–712.

Apeti DA, Kim Y, Lauenstein G, Tull J, Warner R (2014) Occurrence of Parasites and Diseases in Oysters and Mussels of the U.S. Coastal Waters. National Status and Trends, the Mussel Watch monitoring program. NOAA Technical Memorandum NOSS/NCCOS 182. Silver Spring, MD, 51 pp.

Au DWT (2004) The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. Mar Pollut Bull 48: 817–834.

Auffret M (1988) Histopathological changes related to chemical contamination in *Mytilus edulis* from field and experimental conditions. Mar Ecol Prog Ser 46: 101–107.

Austin H, Haven DS, Moustafa MS (1993) The relationship between trends in a condition index of American oysters *Crassotrea virginica*, and environmental parameters in three Virginia estuaries. Estuaries 16: 362–374.

Babarro JMF, de Zwaan A (2008) Anaerobic survival potential of four bivalves from different habitats. A comparative survey. Comp Biochem Physiol 151A: 108–113.

Baussant T, Ortiz-Zarragoitia M, Cajaraville MP, Bechmann RK, Taban IC, Sanni S (2011) Effects of chronic exposure to dispersed oil on selected reproductive processes in adult blue mussels (*Mytilus edulis*) and the consequences for the early life stages of their larvae. Mar Pollut Bull 62: 1437–45.

Bayen S (2012) Occurrence, bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems: A review. Environ Int 48: 84–101.

Beliaeff B, Burgeot T (2002) Integrated biomarker response: a useful tool for ecological risk assessment. Environ Toxicol Chem 21: 1316–1322.

Bellas J, Albentosa M, Vidal-Liñán L, Besada V, Franco MA, Fumega J, González-Quijano A, Viñas L, Beiras R (2014) Combined use of chemical, biochemical and physiological variables in mussels for the assessment of marine pollution along the N-NW Spanish coast. Mar Environ Res 96: 105–117.

Bellas J, Nieto O, Beiras R. 2011. Integrative assessment of coastal pollution: development and evaluation of sediment quality criteria from chemical contamination and ecotoxicological data. Cont Shelf Res 31: 448–456.

Bignell JP, Stentiford GD, Taylor NGH, Lyons BP (2011) Histopathology of mussels (*Mytilus* sp.) from the Tamar estuary, UK. Mar Environ Res 72: 25–32.

Binelli A, Bacchetta R,Vailati G, Galassi S, Privini A (2001) DDT contamination in Lake Maggiore (N. Italy) and effects on zebra mussel spawning. Chemosphere 45: 409–415.

Blaise C, Gagné F, Burgeot T (2016) Three simple biomarkers useful in conducting water quality assessments with bivalve mollusks. Environ Sci Pollut Res doi:10.1007/s11356-016-6908-6

Blaise C, Gagne F, Pellerin J, Hansen PD (1999) Determination of vitellogenin-like properties in *Mya arenaria* hemolymph (Saguenay Fjord, Canada): a potential biomarker for endocrine disruption. Environ Toxicol 14: 455–465.

Blaise C, Gagné F, Salazar M, Salazar S, Trottier S, Hansen P-D (2003) Experimentally-induced feminisation of freshwater mussels after long-term exposure to a municipal effluent. Fresenius Environ Bull 12: 865–870

Bodin N, Burgeot T, Stanisiere JY, Boquene G, Menard D, Minier C, Boutet I, Amat A, Cherel Y, Budzinski H (2004) Seasonal variations of a battery of biomarkers and physiological indices for the mussel *Mytilus galloprovincialis* transplanted into the north- west Mediterranean Sea. Comp Biochem Physiol 138C: 411–427.

Boehs G, Lenz TM, Villalba A (2009) Xenomas in *Crassostrea rhizophorae* (Ostreidae) from Camamu Bay, Bahia, Brazil. Braz J Biol 69: 457–458.

Boehs G, Villalba A, Ceuta LO, Luz JR (2010) Parasites of three commercially exploited bivalve mollusc species of the estuarine region of the Cachoeira river (Ilhéus, Bahia, Brazil). J Invertebr Pathol 103: 43–7.

Bower SM, McGladdery SE, Price IM (1994) Synopsis of infectious diseases and parasites of commercially exploited shellfish. Annu Rev Fish Dis 4: 1–199.

Brandão RP, Boehs G, Silva PM Da (2013) Health assessment of the oyster *Crassostrea rhizophorae* on the southern coast of Bahia, northeastern Brazil. Rev Bras Parasitol Veterinária 22: 84–91.

Broeg K, Lehtonen KK (2006) Indices for the assessment of environmental pollution of the Baltic Sea coasts: integrated assessment of a multi-biomarker approach. Mar Pollut Bull 53: 508–22.

Cabrera-Peña J, Zamora-Madriz E, Pacheco-Urpí O (1983) Determinación del tamaño comercial de la ostra de manglar, *Crassostrea rhizophorae* (Guilding, 1828) en sistema de cultivo suspendido en Estero Viscaya, Limón, Costa Rica. Rev Biol Trop 31: 257-261.

Cajaraville MP, Bebianno MJ, Blasco J, Porte C, Sarasquete C, Viarengo A (2000) The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. Sci Total Environ 247: 295–311.

Cajaraville MP, Marigómez I, Angulo E (1993) Correlation between cellular and organismic responses to oil-induced environmental stress in mussels. Sci Total Environ 134, Suppl: 1353–1371.

Carella F, Feist S, Bignell JP, De Vico G (2015) Comparative pathology in bivalves: aetiological agents and disease processes. J Invert Pathol 131: 107–120.

Carvalho FP, Villeneuve JP, Cattini C, Rendón J, de Oliveira JM (2009) Pesticide and PCB residues in the aquatic ecosystems of Laguna de Terminos, a protected area of the coast of Campeche, Mexico. Chemosphere 74: 988–995.

Castañeda-Chávez MR (2011) DDT in *Crassostrea virginica* (Gmelin, 1791) of Coastal Lagoons in the Gulf of Mexico. J Agric Sci 3: 183–193.

Castilho-Westphal GG, Magnani FP, Ostrensky A (2015) Gonad morphology and reproductive cycle of the mangrove oyster *Crassostrea brasiliana* (Lamarck, 1819) in the baía baía de Guaratuba, Paraná, Brazil. Acta Zool 96: 99–107.

Catharino MGM, Vasconcellos MBA, Kirschbaum AA, Gasparro MR, de Sousa ECPM, Minei CC, Moreira EG (2015) Passive biomonitoring study and effect biomarker in oysters *Crassostrea brasiliana* (Lamark, 1819: Mollusca, Bivalvia) in Santos and Cananéia Estuaries in São Paulo State, Brazil. J Radioanal Nucl Chem 303: 2297–2302.

Ceuta LO, Boehs G (2012) Parasites of the mangrove mussel *Mytella guyanensis* (Bivalvia: Mytilidae) in Camamu Bay, Bahia, Brazil. Braz J Biol 72: 421–7.

Chagot D, Alzieu C, Sanjuan J, Grizel H (1990) Sublethal and histopathological effects of trace levels of tributyltin fluoride on adult oysters *Crassostrea gigas*. Aquat Living Resour 3: 121–130.

Christo SW, Absher TM (2006) Reproductive Period of *Crassostrea rhizophorae* (Guilding, 1828) and *Crassostrea Brasiliana* (Lamark, 1819) (Bivalvia : Ostreidae) in Guaratuba Bay, Paraná, Brazil. J Coast Res 1215–1218.

Chu FLE, Volety AK, Hale RC, Huang Y (2002) Cellular responses and disease expression in oysters (*Crassostrea virginica*) exposed to suspended field — contaminated sediments. Mar Environ Res 53: 17–35.

Chung KS (1980) Acute toxicity of selected heavy metals to mangrove oyster *Crassostrea rhizophorae*. Bull Jap Soc Sci Fisheries 46: 777–780.

Cochennec-Laureau N, Auffret M, Renault T, Langlade A (2003) Changes in circulating and tissue-infiltrating hemocyte parameters of European flat oysters, *Ostrea edulis*, naturally infected with Bonamia ostreae. J Invertebr Pathol 83: 23–30.

Comesaña P, Casas SM, Cao A, Abollo E, Arzul I, Morgan B, Villalba A (2012) Comparison of haemocytic parameters among flat oyster *Ostrea edulis* stocks with different susceptibility to bonamiosis and the Pacific oyster *Crassostrea gigas*. J Invertebr Pathol 109: 274–286.

Couch JA (1985) Prospective study of infectious and non-infectious diseases in oysters and fishes in three Gulf of Mexico estuaries. Dis Aquat Org 1: 59–82.

Crosby M, Gale L (1990) A review and evaluation of bivalve condition index methodologies with a suggested standard method. J Shellfish Res 9: 233–237.

Cuevas N, Zorita I, Costa PM, Franco J, Larreta J (2015) Development of histopathological indices in the digestive gland and gonad of mussels: integration with contamination levels and effects of confounding factors. Aquat Toxicol 162: 152–164.

da Silva AZ, Zanette J, Ferreira JB, Guzenski J, Freire Marques MR, Bainy ACD (2005) Effects of salinity on biomarker responses in *Crassostrea rhizophorae* (Mollusca, Bivalvia) exposed to diesel oil Ecotox Envir Safety 62: 376–382.

da Silva PM, Magalhães ARM, Barracco MA (2011) Pathologies in commercial bivalve species from Santa Catarina State, southern Brazil. J Mar Biol Assoc UK 92: 571–579.

Dame RF (1972) Comparison of various allometric relationships in intertidal and subtidal American oysters. Fishery Bulletin 70: 1121–1126.

Davies IM, Vethaak AD (2012) Integrated marine environmental monitoring of chemicals and their effects. ICES Coop Res Rep No 315, 277 pp.

de los Ríos A, Pérez L, Ortiz-Zarragoitia M, Serrano T, Barbero MC, Echavarri-Erasun B, Juanes J a, Orbea A, Cajaraville MP (2013) Assessing the effects of treated and untreated urban discharges to estuarine and coastal waters applying selected biomarkers on caged mussels. Mar Pollut Bull 77: 251–265.

De Vico G, Carella F (2012) Morphological features of the inflammatory response in molluscs. Res Vet Sci 93: 1109–1115.

de Zwaan A, Eertman R (1996) Anoxic or aerial survival of bivalves and other euryoxic invertebrates as a useful response to environmental stress—a comprehensive review. Comp Biochem Physiol 113C:299–312.

Defew LH, Mair JM, Guzman HM (2005) An assessment of metal contamination in mangrove sediments and leaves from Punta Mala Bay, Pacific Panama. Mar Pollut Bull 50: 547–552.

Depledge MH, Billinghurst Z (1999) Ecological significance of endocrine disruption in marine invertebrates. Mar Pollut Bull 39: 32–38.

Dove MC, O'Connor WA (2012) Reproductive cycle of Sydney rock oysters, *Saccostrea glomerata* (Gould 1850) selectively bred for faster growth. Aquaculture 324–325: 218–225.

Dumailo S (2003) Evaluación de la problemática ambiental por medio del estudio de algunos aspectos de sedimentación y contaminación en la laguna de Bluefields, RAAS, Nicaragua. MSc Thesis. CIRA-UNAN, 242 pp.

Ebanks-Mongalo BF, Suárez-Sánchez J, Siu-Estrada E, Montoya-Arguello JJ, Mairena-Valdivia DA, Flores-Pacheco A, Van der Wal JC, Valencia-Quintana P (2013) Concentración de plaguicidas en agua, sedimentos y ostiones (*Crassostrea rizophorae*) de la Laguna de Bluefields, RAAS, Nicaragua. WANI: 49-54.

Eertman RHM, Groenink CLFMG, Sandee B, Hummel H, Smaal AC (1995) Response of the blue mussel *Mytilus edulis* L. following exposure to PAHs or contaminated sediment. Mar Environ Res 39: 169–173.

Ellison AM (2004) Wetlands of Central America. Wetl Ecol Manag 12: 3–55.

Fernandez A, Singh A, Jaffé R (2007) A literature review on trace metals and organic compounds of anthropogenic origin in the Wider Caribbean Region. Mar Pollut Bull 54: 1681–1691.

Gagne F, Blaise C, Lachance B, Sunahara GI, Sabik H (2001) Evidence of coprostanol estrogenicity to the freshwater mussel *Elliptio complanata*. Environ Pollut 115: 97–106.

Gagne F, Blaise C, Pellerin J, Gauthier-Clerc S (2002) Alteration of the biochemical properties of female gonad and vitellin in the clam *Mya arenaria* at contaminated sites in the Saguenay Fjord. Mar Environ Res 53: 295–310

Gagne F, Blaise C, Pellerin J, Pelletier E, Douville M, Gauthier-Clerc S, Viglino L (2003) Sex alteration in soft-shell clams (*Mya arenaria*) in an intertidal zone of the Saint Lawrence River (Quebec, Canada). Comp Biochem Physiol 134C: 189–198.

García F, Palacio C, Garcia U (2012) Water quality at Santa Marta coastal area (Colombia). Dyna 79: 85–94.

Garmendia L, Soto M, Cajaraville M, Marigómez I (2010) Seasonality in cell and tissue-level biomarkers in *Mytilus galloprovincialis*: relevance for long-term pollution monitoring. Aquat Biol 9: 203–219.

Garmendia L, Soto M, Vicario U, Kim Y, Cajaraville M, Marigómez I (2011) Application of a battery of biomarkers in mussel digestive gland to assess long-term effects of the Prestige oil spill in Galicia and Bay of Biscay: tissue-level biomarkers and histopathology. J Environ Monit 13: 915–932.

Gauthier-Clerc S, Pellerin J, Blaise C, Gagne F (2002) Delayed gametogenesis of *Mya arenaria* in the Saguenay fjord (Canada): a consequence of endocrine disruptors? Comp Biochem Physiol 131C: 457–467.

GEF-REPCar (2011) Agricultural pesticide residues in Caribbean coastal waters. Colombia, Costa Rica and Nicaragua 2008-2011. GEF-REPCar Rpt UNEP, 63 pp.

George-zamora A, Sevilla-Hernández LM, Aldana-Aranda D (2003) Ciclo gonádico del ostión americano *Crassostrea virginica* (Lamellibranchia: Ostreidae) en Mecoacán, Tabasco, México Arturo. Rev Biol Trop 51: 109–117.

Gold-Bouchot G, Simá R, Zapata-Pérez O, Güemez J (1995) Histopathological effects of petroleum hydrocarbons and heavy metals on the American oyster, *Crassostrea virginica*, from Tabasco, Mexico. Mar Pollut Bull 31: 439–445.

Gold-Bouchot G, Zapata-Pérez O, Ceja-Moreno V, Rodríguez-Fuentes G, Simá-Alvarez R, Aguirre-Macedo ML, Vidal-Martínez VM, Da Ros L, Nasci C (2007) Biological effects of environmental pollutants in American oyster, *Crassostrea virginica*: a field study in Laguna de Terminos, Mexico. Int J Environ Hlth 1: 171–184.

Gómez-Giraldo A, Osorio A, Toro FM, Osorio JD, Alvarez OA, Arrieta A (2009) Circulation pattern in the "Barbacoas" bay and its influence on sediment transport to the "Rosario" islands. Avances Rec Hidraul 20: 21–40.

Gordon CM (1988) Sex ratio condition and glycogen content of raft cultivated mangrove oysters *Crassostrea rhizophorae*. J Shellfish Res 72: 395–400

Hellou J, Law RJ (2003) Stress on stress response of wild mussels, *Mytilus edulis* and *Mytilus trossulus*, as an indicator of ecosystem health. Environ Pollut 126: 407–416.

Hellou J, Yeats P, Steller S, Gagne F (2003) Chemical contaminants and biological indicators of mussel health during gametogenesis. Envir Toxicol Chem 22: 2080–2087.

ICES (2011) Report of the Study Group on Integrated Monitoring of Contaminants and Biological Effects (SGIMC), 14-18 March 2011. Copenhagen, Denmark. ICES CM 2011/ACOM: 30, 265 pp.

ICES (2012) ICES WGBEC REPORT 2012 Report of the Working Group on Biological Effects of Contaminants (WGBEC), 12-16 March 2012, Porto, Portugal. ICES C. 2012/SSGHIE04, 131 pp.

Ivanković D, Pavičić J, Erk M, Filipović-Marijić V, Raspor B (2005) Evaluation of the *Mytilus galloprovincialis* Lam. digestive gland metallothionein as a biomarker in a long-term field study: Seasonal and spatial variability. Mar Pollut Bull 50: 1303–1313.

Jana H, Mondal KC, Maity C, Das A, Halder SK, Mitra A, Pati BR (2014) Effect of microbial load on the condition index of the edible oyster, *Saccostrea cucullata*, in the Sundarbans, India. Internat J Aquat Biol 2: 238–245.

Kanhai LDK, Gobin JF, Beckles DM, Lauckner B, Mohammed A (2014) Metals in sediments and mangrove oysters (*Crassostrea rhizophorae*) from the Caroni Swamp, Trinidad. Environ Monit Assess 186: 1961–1976.

Kanhai LDK, Gobin JF, Beckles DM, Lauckner B, Mohammed A (2015) Polycyclic aromatic hydrocarbons (PAHs) in *Crassostrea rhizophorae* and *Cathorops spixii* from the Caroni Swamp, Trinidad, West Indies. Environ Sci Pollut Res 22: 1366–1379.

Khan MI, Ayub Z, Siddiqui G (2015) Impact of marine pollution in green mussel *Perna viridis* from four coastal sites in Karachi, Pakistan, North Arabian Sea: histopathological observations. Indian J Exp Biol 53: 222–227.

Kim Y, Ashton-Alcox KA, Powell EN (2006) Histological techniques for marine bivalve molluscs : update. Silver

Spring, MD. NOAA Technical Memorandum NOS NCCOS 27.

Kim Y, Powell EN (2004) Surfclam histopathology survey along the Delmarva mortality line. J Shellfish Res 23: 429–441.

Kim Y, Powell EN (2007) Distribution of parasites and pathologies in sentinel bivalves: NOAA status and trends "Mussel Watch" program. J Shellfish Res 26: 1115–1151.

Kim Y, Powell EN, Wade TL, Presley BJ (2008) Relationship of parasites and pathologies to contaminant body burden in sentinel bivalves: NOAA Status and Trends "Mussel Watch" Program. Mar Environ Res 65: 101–127.

Koukouzika N, Dimitriadis VK (2005) Multiple biomarker comparison in *Mytilus galloprovincialis* from the Greece coast: "lysosomal membrane stability, neutral red retention, micronucleus frequency and stress on stress". Ecotoxicology 14: 449–463.

La Rosa T, Mirto S, Marino A, Alonzo V, Maugeri TL, Mazzola A (2001) Heterotrophic bacteria community and pollution indicators of mussel-farm impact in the Gulf of Gaeta (Tyrrhenian Sea). Mar Environ Res 52(4): 301–21

Lauckner G (1983) Diseases of Mollusca: Bivalvia. In: Kinne O (ed.), Diseases of Marine Animals. Hamburg: Biologisshe Anstalt Helgoland, pp 477–970.

Leiniö S, Lehtonen KK (2005) Seasonal variability in biomarkers in the bivalves *Mytilus edulis* and *Macoma balthica* from the northern Baltic Sea. Comp Biochem Physiol 140C: 408–421.

Lenz T, Boehs G (2011) (Bivalvia : Ostreidae) en la Bahía de Camamu, Bahia, Brasil. Rev Biol Trop 59: 137–149.

Lewis M, Pryor R, Wilking L (2011) Fate and effect of anthropogenic chemicals in mangrove ecosystems: a review. Envir Pollut 159: 2328–2346.

Lobel PB, Wright DA (1982) Relationship between body zinc concentration and allometric growth measurements in the mussel *Mytilus edulis*. Mar Biol 66: 145–150.

Lowe DM (1988) Alterations in cellular structure of *Mytilus edulis* resulting from exposure to environmental contaminants under field and experimental conditions. Mar Ecol Prog Ser 46: 91–100.

Lowe DM, Pipe RK (1987) Mortality and quantitative aspects of storage cell utilization in mussels, *Mytilus edulis*, following exposure to diesel oil hydrocarbons. Mar Environ Res 22: 243–251.

Mancera-Pineda JE, Pinto G, Vilardy S (2013) Seasonal patterns of water masses distribution in Santa Marta bay, Colombian Caribbean: relative importance of upwelling and outwelling. Bol Invest Mar Cost 42: 329-360.

Maranho LA, Pereira CDS, Choueri RB, Cesar A, Gusso-Choueri PK, Torres RJ, Abessa DMDS, Morais RD, Mozeto AA, DelValls TA, Martín-Díaz ML (2012) The application of biochemical responses to assess environmental quality of tropical estuaries: field surveys. J Environ Monit 14: 2608-2615.

Marcogliese DJ, Cone DK (1997) Parasite communities as indicators of ecosystem stress. Parassitologia 39: 227–232.

Marigómez I, Garmendia L, Soto M, Orbea A, Izagirre U, Cajaraville MP (2013) Marine ecosystem health status assessment through integrative biomarker indices: a comparative study after the Prestige oil spill "Mussel Watch". Ecotoxicology 22: 486–505.

Marigómez I, Soto M, Cancio I, Orbea A, Garmendia L, Cajaraville MP (2006) Cell and tissue biomarkers in mussel, and histopathology in hake and anchovy from Bay of Biscay after the Prestige oil spill (Monitoring Campaign 2003). Mar Pollut Bull 53: 287–304.

Martínez-Gómez C, Robinson CD, Burgeot T, Gubbins M, Halldórson HP, Albentosa M, Bignell JP, Hylland K, Vethaak D (2017) Biomarkers of general stress in mussels as common indicators for marine biomonitoring programmes in Europe: The ICON experience. Mar Environ Res 124: 70–80.

Meyer U, Hagen W, Medeiros C (1998) Mercury in a northeastern Brazilian mangrove area, a case study: potential of the mangrove oyster Crassostrea rhizophorae as bioindicator for mercury. Mar Biol 131: 113–121.

Moles A, Hale N (2003) Use of physiological responses in *Mytilus trossulus* as integrative bioindicators of sewage pollution. Mar Pollut Bull 46: 954–958.

Montes-M A, Prieto-Arcas A, Ruiz LJ (2007) Abundancia, biomasa y proporción sexual en una población natural de la ostra (*Crassostrea rhizophorae*) en la Laguna grande de Obispo, Estado Sucre, Venezuela. Boletín del Cent Investig Biológicas 41: 485–501.

Moore CG, Stevenson JM. 1994. Intersexuality in benthic harpacticoid copepods in the Firth of Forth, Scotland. J Nat History 28: 1213–1230.

Mubiana VK, Vercauteren K, Blust R (2006) The influence of body size, condition index and tidal exposure on the variability in metal bioaccumulation in *Mytilus edulis*. Environ Pollut 144: 272–279.

Nascimento IA (1978) Reprodução da ostra do mangue, Crassostrea rhizophorae (Guilding, 1828): um subsídio ao cultivo. São Paulo, Brasil. USP, 200 pp.

Nascimento IA, Pereira SA (1980) Changes in the condition index for mangrove oysters (*Crassostrea rhizophorae*) from Todos os Santos Bay, Salvador, Brazil. Aquaculture 20: 9–15.

Nascimento IA, Pereira SA, Souza RCE (1980) Determination of the optimum commercial size for the mangrove oyster (*Crassostrea rhizophorae*) in Todos os Santos Bay, Brazil. Aquaculture 20: 1–8.

Nascimento IA, Smith DH, Kern F, Pereira SA (1986) Pathological findings in *Crassostrea rhizophorae* from Todos os Santos Bay, Bahia, Brazil. J Invertebr Pathol 47: 340–349.

Nascimento, IA, Leite, MBN, Sansone, G, Pereira, SA, Smith, DH (1998) Stress protein accumulation as an indicator of impact by the petroleum industry in Todos os Santos Bay, Brazil. J Aquat Ecosyst Hlth Manag 1: 101–108.

Nesto N, Romano S, Moschino V, Mauri M, Da Ros L (2007) Bioaccumulation and biomarker responses of trace metals and micro-organic pollutants in mussels and fish from the Lagoon of Venice, Italy. Mar Pollut Bull 55: 469–84.

Orban E, Di Lena G, Nevigato T, Casini I, Marzetti A, Caproni R (2002) Seasonal changes in meat content, condition index

and chemical composition of mussels (*Mytilus galloprovincialis*) cultured in two different Italian. Food Chem 77: 57–65.

Ortiz-Zarragoitia M, Cajaraville M P (2006) Biomarkers of exposure and reproduction-related effects in mussels exposed to endocrine disruptors. Arch Environ Contam Toxicol 50: 361–369.

Ortiz-Zarragoitia M, Cajaraville MP (2010) Intersex and oocyte atresia in a mussel population from the Biosphere's Reserve of Urdaibai (Bay of Biscay). Ecotoxicol Environ Safety 73: 693–701.

Ortiz-Zarragoitia M, Garmendia L, Barbero MC, Serrano T, Marigómez I, Cajaraville MP (2011) Effects of the fuel oil spilled by the Prestige tanker on reproduction parameters of wild mussel populations. J Environ Monit 13:84–94.

OSPAR Commission (2013a) Levels and trends in marine contaminants and their biological effects – CEMP Assessment report 2012.

OSPAR Commission (2013b) Background document and technical annexes for biological effects monitoring, Update 2013.

Paixão JF, Nascimento IA, Pereira SA, Leite MBL, Carvalho GC, Silveira JSC, Rebouças M, Matias GRA, Rodrigues ILP (2007) Estimating the gasoline components and formulations toxicity to microalgae (*Tetraselmis chuii*) and oyster (*Crassostrea rhizophorae*) embryos: An approach to minimize environmental pollution risk. Environ Res 103: 365–374.

Paixão L, Ferreira MA, Nunes Z, Fonseca-Sizo F, Rocha R (2013) Effects of salinity and rainfall on the reproductive biology of the mangrove oyster (*Crassostrea gasar*): Implications for the collection of brood stock oysters. Aquaculture 380–383: 6–12.

Palm HW, Dobberstein RC (1999) Occurence of trichodinid ciliates (Peritricha: Urceolariidae) in the Kiel Fjord, Baltic Sea, and its possible use as a biological indicator. Parasitol Res 85: 726–732

Pampanin DM, Volpato E, Marangon I, Nasci C (2005) Physiological measurements from native and transplanted mussel (*Mytilus galloprovincialis*) in the canals of Venice: survival in air and condition index. Comp Biochem Physiol 140A: 41–52.

Park JJ, Kim H, Kang SW, An CM, Lee SH, Gye MC, Lee JS (2012) Sex ratio and sex reversal in two-year-old class of oyster, *Crassostrea gigas* (Bivalvia: Ostreidae). Dev Reprod. 16: 385–388.

Polidoro B a., Carpenter KE, Collins L, Duke NC, Ellison AM, Ellison JC, Farnsworth EJ, Fernando ES, Kathiresan K, Koedam NE, Livingstone SR, Miyagi T, Moore GE, Nam VN, Ong JE, Primavera JH, Salmo SG, Sanciangco JC, Sukardjo S, Wang Y, Yong JWH (2010) The loss of species: Mangrove extinction risk and geographic areas of global concern. PLoS One 5: e10095.

Pouvreau S, Gangnery A, Tiapari J, Lagarde F, Garnier M, Bodoy A (2000) Gametogenic cycle and reproductive effort of the tropical blacklip pearl oyster, *Pinctada margaritifera* (Bivalvia: Pteriidae), cultivated in Takapoto atoll (French Polynesia). Aquat Living Resour 13: 37–48.

Powell EN, Klinck JM, Hofmann EE (2011) Generation time and the stability of sex-determining alleles in oyster populations as deduced using a gene-based population dynamics model. J Theor Biol 271: 27–43.

Puy-Azurmendi E, Ortiz-Zarragoitia M, Kuster M, Martínez E, Guillamón M, Domínguez C, Serrano T, Barbero MC, López de Alda M, Bayona JM, Barceló D, Cajaraville MP (2010) An integrated study of endocrine disruptors in sediments and reproduction-related parameters in bivalve molluscs from the Biosphere's Reserve of Urdaibai (Bay of Biscay). Mar Environ Res 69: S63–66.

Puy-Azurmendi E, Ortiz-Zarragoitia M, Villagrasa M, Kuster M, Aragón P, Atienza J, Puchades R, Maquieira A, DomínguezC, López de Alda M, Fernandes D, Porte C, Bayona JM, Barceló D, Cajaraville MP (2013) Endocrine disruption in thicklip grey mullet (*Chelon labrosus*) from the Urdaibai Biosphere Reserve (Bay of Biscay, Southwestern Europe). Sci Tot Envir 443: 233–244.

Pytharopoulou S, Sazakli E, Grintzalis K, Georgiou CD, Leotsinidis M, Kalpaxis DL (2008) Translational responses of *Mytilus galloprovincialis* to environmental pollution: integrating the responses to oxidative stress and other biomarker responses into a general stress index. Aquat Toxicol 89: 18–27.

Quinn B, Gagne F, Costello M, McKenzie C, Wilson J, Mothersill C (2004) The endocrine disrupting effect of municipal effluent on the zebra mussel (*Dreissena polymorpha*). Aquat Toxicol 66: 279–292.

Rebelo MF, Amaral MCR, Pfeiffer WC (2005) Oyster condition index in *Crassostrea rhizophorae* (Guilding 1828) from a heavy-metal polluted coastal lagoon. Braz J Biol 65: 345–51.

Rebelo MF, Pfeiffer WC, da Silva H, Moraes MO (2003) Cloning and detection of metallothionein mRNA by RT-PCR in mangrove oysters (*Crassostrea rhizophorae*). Aquat Toxicol 64: 359–362.

Ren JS, Marsden ID, Ross AH, Schiel DR (2003) Seasonal variation in the reproductive activity and biochemical composition of the Pacific oyster (*Crassostrea gigas*) from the Marlborough Sounds , New Zealand. New Zel J Mar Freshw Res 37: 171–182.

Rojas de Astudillo L, Yen IC, Bekele I (2005) Heavy metals in sediments, mussels and oysters from Trinidad and Venezuela. Rev Biol Trop 53: 41–51.

Rojas ZP, Campalans BM, González AM (1999) Hemocytic neoplasia in the Chilean oyster (*Tiostrea chilensis*) cultured in the south of Chile: New record. Investig Mar 27:15–18.

Sabry RC, da Silva PM, Gesteira TCV, Pontinha VA, Magalhães ARM (2011) Pathological study of oysters *Crassostrea gigas* from culture and *C. rhizophorae* from natural stock of Santa Catarina Island, SC, Brazil. Aquaculture 320: 43–50.

Sabry RC, Gesteira TCV, Boehs G (2007) First record of parasitism in the mangrove oyster *Crassostrea rhizophorae* (Bivalvia: Ostreidae) at Jaguaribe River estuary - Ceará, Brazil. Braz J Biol 67: 755 758.

Sabry RC, Gesteira TCV, Magalhães ARM, Barracco MA, Guertler C, Ferreira LP, Vianna, RT, da Silva PM (2013) Parasitological survey of mangrove oyster, *Crassostrea*

rhizophorae, in the Pacoti river estuary, Ceará State, Brazil. J Invert. Pathol 112: 24–32.

Schettino T, Caricato R, Calisi A, Giordano ME, Lionetto MG (2012) Biomarker approach in marine monitoring and assessment : new insights and perspectives. Open Environ Sci 6: 20–27.

Scott GI, Lawrence DR, 1982, The American oyster as a coastal zone pollution monitor: a pilot study. Estuaries, 5: 40-46.

Serafim A, Company R, Lopes B, Fonseca VF, França S, Vasconcelos RP, Bebianno MJ, Cabral HN (2012) Application of an integrated biomarker response index (IBR) to assess temporal variation of environmental quality in two Portuguese aquatic systems. Ecol Indic 19: 215–225.

Sericano JL, Wade TL, Jackson TJ, Brooks JM, Tripp BW, Farrington JW, Mee LD, Readmann JW, Villeneuve JP, Goldberg ED (1995) Trace organic contamination in the Americas: An overview of the US National Status & Trends and the International "Mussel Watch" programmes. Mar Pollut Bull 31: 214–225.

Sheir SK, Handy RD (2010) Tissue injury and cellular immune responses to cadmium chloride exposure in the common mussel *Mytilus edulis*: Modulation by lipopolysaccharide. Arch Environ Contam Toxicol 59: 602–613.

Silva CAR, Rainbow PS, Smith BD (2003) Biomonitoring of trace metal contamination in mangrove-lined Brazilian coastal systems using the oyster *Crassostrea rhizophorae*: comparative study of regions. Hydrobiologia 501: 199–206.

Silva CAR, Smith BD, Rainbow PS. (2006). Comparative biomonitors of coastal trace metal contamination in tropical South America (N. Brazil). Mar Envir Res 61: 439–455.

Smaal AC, van Stralen MR (1990) Average annual growth and condition of mussels as a function of food source. Hydrobiologia 195: 179–188.

Smaal AC, Wagenvoort A, Hemelraad J, Akkerman I (1991) Response to stress of mussels (*Mytilus edulis*) exposed in dutch tidal waters. Comp Biochem Physiol 100C: 197–200.

Strogyloudi E, Angelidis MO, Christides A, Papathanassiou E (2012) Metal concentrations and metallothionein levels in *Mytilus galloprovincialis* from Elefsis bay (Saronikos gulf, Greece). Environ Monit Assess 184: 7189–7205.

Thomas RE, Harris PM, Rice SD (1999) Survival in air of *Mytilus trossulus* following long-term exposure to spilled Exxon Valdez crude oil in Prince William sound. Comp Biochem Physiol 122C: 147–152.

Tomlinson DL, Wilson JG, Harris CR, Jeffrey DW (1980) Problems in assessment of heavy metals in estuaries and the formation of Pollution Index. Helgol Meeresunters 33: 566–575.

Torres RJ, Cesar A, Pereira CDS, Choueri RB, Abessa DMS, do Nascimento MRL, Fadini PS, Mozeto AA (2012) Bioaccumulation of polycyclic aromatic hydrocarbons and mercury in oysters (*Crassostrea rhizophorae*) from Two Brazilian estuarine zones. Int J Oceanogr 2012: 1–8.

Usheva LN, Vaschenko MA, Durkina VB (2006) Histopathology of the digestive gland of the bivalve mollusk *Crenomytilus grayanus* (Dunker, 1853) from southwestern Peter the Great Bay, Sea of Japan. Russ J Mar 32: 166–172.

Valdez Domingos FX, Azevedo M, Silva MD, Randi MAF, Freire CA, Silva de Assis HC, Oliveira Ribeiro CA (2007) Multibiomarker assessment of three Brazilian estuaries using oysters as bioindicators. Environ Res 105: 350–363.

Van Lavieren H, Metcalfe CD, Drouillard K, Sale PF, Bouchot GG, Reid R, Vermeulen L (2011) Strengthening coastal pollution management in the wider Caribbean region. Proc 64th Gulf Caribb Fish Inst 5: 1–11.

Veldhuizen-Tsoerkan MB, Holwerda DA, Zandee DI (1991) Anoxic survival time and metabolic parameters as stress indices in sea mussels exposed to cadmium or polychlorinated biphenyls. Arch Environ Contam Toxicol 20:259-265.

Vélez A (1982) Hermafroditismo en la ostra de mangle *Crassostrea rhizophorae*. Bol Inst Oceanogr Univ Oriente 21: 129-132.

Viarengo A, Canesi L, Pertica M, Mancinelli G, Accomando R, Smaal AC, Orunesu M (1995) Stress on stress response: a simple monitoring tool in the assessment of general stress syndrome in mussels. Mar Environ Res 39: 245–248.

Viarengo A, Lowe D, Bolognesi C, Fabbri E, Koehler A (2007) The use of biomarkers in biomonitoring: a 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. Comp Biochem Physiol 146C: 281–300.

Villalba A, Carballal MJ, López C (2001) Disseminated neoplasia and large foci indicating heavy haemocytic infiltration in cockles *Cerastoderma edule* from Galicia (NW Spain). Dis Aquat Organ 46: 213–216.

Villarroel E, Buitrago E, Lodeiros C (2004) Identification of environmental factors affecting growth and survival of the tropical oyster *Crassostrea rhizophorae* in suspended culture in the Golfo de Cariaco, Venezuela. Rev Cient Ciencias Vet 14: 28–35.

Vivas-Aguas L, Sánchez J, Betancourt J, Quintero M, Moreno Y, Santana C, Cuadrado I, Ibarra K, Rios M, Obando P, Sánchez D (2013) Diagnóstico y evaluación de la calidad ambiental marina en el Caribe y Pacifico colombiano. REDCAM Inf Téc 2014. INVEMAR. Santa Marta, 314 pp.

Vivas-Aguas L, Tosic M, Sánchez J, Narváez S, Cadavid B, Bautista P, Betancourt J, Parra J, Echeverri L, Espinosa L (2010) Diagnóstico y evaluación de la calidad ambiental marina en el Caribe y Pacifico colombiano. REDCAM Inf Téc 2010. INVEMAR. Santa Marta, 208 pp.

Vivas–Aguas LJ, Sánchez J, Cadavid B, Betancourt J, Quintero M, Moreno Y, Santana C, Cuadrado I, Ibarra K, Ríos M, Obando P, Sánchez D (2014) Diagnóstico y evaluación de la calidad de las aguas marinas y costeras del Caribe y Pacífico colombianos. Serie de publicaciones del Invemar No. 4. Red de vigilancia para la conservación y protección de las aguas marinas y costeras de Colombia (REDCAM). Informe Técnico 2013, INVEMAR, Santa Marta, 314 p.

Wallner-Kersanach M, Theede H, Eversberg U, Lobo S (2000) Accumulation and elimination of trace metals in a transplantation experiment with *Crassostrea rhizophorae*. Arch Environ Contam Toxicol 38: 40–45.

Wintermyer ML, Cooper KR (2007) The development of an aquatic bivalve model: Evaluating the toxic effects on gametogenesis following 2,3,7,8-tetrachlorodibenzo-p-

dioxin (2,3,7,8-TCDD) exposure in the eastern oyster (*Crassostrea virginica*). Aquat Toxicol 81: 10–26.

Zanette J, Flores Nunes F, Dias Medeiros I, Siebert MN, Mattos JJ, Lüchmann KH, Rodrigues de Melo CM, Dias Bainy AC (2008) Comparison of the antioxidant defense system in *Crassostrea rhizophorae* and *Crassostrea gigas* exposed to domestic sewage discharges. Mar Environ Res 66: 196–198.

Zanette J, Monserrat JM, Bianchini A (2006) Biochemical biomarkers in gills of mangrove oyster *Crassostrea rhizophorae* from three Brazilian estuaries. Comp Biochem Physiol 143C: 187–95.

Zaroogian G, Yevich P (1993) Cytology and biochemistry of brown cells in *Crassostrea virginica* collected at clean and contaminated stations. Environ Pollut 79: 191–197.

Zeidan GC, Luz MSA, Boehs G. (2012) Parasites of economically important bivalves from the southern coast of Bahia State, Brazil. Rev Bras Parasitol Vet 21: 391–398.

Chapter 3. Comparison of potential biomonitor and sentinel bivalve species suitable for biomonitoring of pollutants and ecosystem health disturbance in mangrove–lined Nicaraguan coastal systems

Scientific Contributions:

Aguirre-Rubí J, Ortiz-Zarragoitia M, Zaldibar B, Izagirre U, Etxebarria N, Espinoza F, Marigómez I. in prep. Comparison of potential biomonitor and sentinel bivalve species suitable for biomonitoring of pollutants and ecosystem health disturbance in mangrove–lined Nicaraguan coastal systems.

Keywords: mangrove, bioaccumulation, biomarkers, ecosystem health, monitoring

Abstract: This investigation aims at contributing to the use of the slender marsh clams (*Polymesoda arctata*), pustulose arks (*Anadara tuberculosa*), and ark shell clams (*Larkinia grandis*) as potential biomonitors and sentinels for pollution biomonitoring in mangrove-lined coastal systems in Nicaragua, based on previous experience acquired with mangrove cupped oysters, *Crassostrea rhizophorae* (Chapter 1 and 2). For this purpose, localities with different contaminant sources were selected in the Nicaraguan coastline. In the Caribbean, *P. arctata* was collected in Pigeon Cay (Pearl Lagoon) and Punta San Gabriel (Bluefields Lagoon). In the Pacific, *A. tuberculosa* was collected in Padre Ramos Reserve and Corinto, and L. grandis in Padre Ramos Reserve. Samples were collected in the rainy and dry seasons during 2012-2013. The tissue concentration of metals, PAHs and POPs were determined and integrated into pollution indices (CPI and PLI). In parallel, biological endpoints at different levels of biological complexity (condition indices, reproduction parameters, histopathology, and stress-on-stress response) were determined as biomarkers of ecosystem health disturbance. In the Caribbean, the tissue concentration of contaminants in *P. arctata* was, in general terms, low with some exceptions. Moreover, Ag, As, Cd, Hg, Ni and V were mainly recorded during the dry season, whilst PAHs and POPs, such as HCHs, DDTs, AHTN, PCBs and BDE85, were mainly recorded during the rainy season. As whole, the highest CPI and PLI values were recorded in Punta San Gabriel, which is consistent because this locality is located at the sediment deposition zone of Bluefields Lagoon. Nevertheless, it seems that metals and PAHs are not a matter of major concern in these Caribbean lagoons; in contrast, high levels of HCHs and DDTs and low-to-moderate levels of musk fragrances and PBDEs were recorded. Although only minor differences were found in biological parameters between localities and between seasons, low LT_{50} values were recorded in Punta San Gabriel during the rainy season, associated to the highest PLI and CPI values. In the Pacific coast, the main pollutants recorded in *A. tuberculosa* and *L. grandis* were HCHs, DDTs, AHTN and PDBEs in the rainy season and Cd in the dry season. In these two Pacific species, biological endpoints comparable to those used in other sentinel bivalves appear to be suitable as biomarkers of health disturbance; however, more basic research is needed to understand their general biology, ecology and disease. Overall, our study supports that slender marsh clams in the Caribbean, and pustulose arks and ark shell clams in the Pacific coast could be used as biomonitors and sentinels for assessing pollution and ecosystem health disturbance in mangrove-lined coastal systems in Nicaragua. Besides, seasonality has been found to be crucial to properly design and conduct pollution monitoring programmes using the three species investigated herein.

Resume: Esta investigación tiene como objetivo contribuir al uso del guacuco de marjal esbelto (*Polymesoda arctata*), la concha negra (*Anadara tuberculosa*), y el casco de burro (*Larkinia grandis*) como potenciales especies biomonitoras y centinelas para el biomonitoreo de contaminación en los sistemas costeros de manglar en Nicaragua, a partir de la experiencia previa adquirida con ostras de manglar *Crassostrea rhizophorae* (Capitulo 1 y 2). Para ello, se seleccionaron localidades con diferentes fuentes de contaminantes en las costas Nicaragüenses. En el Caribe, se recolectó *P. arctata* in Pigeon Cay (Laguna de Perla) y en Punta San Gabriel (Laguna de Bluefields). En el Pacífico, se recolectó *A. tuberculosa* en la Reserva Padre Ramos y Corinto, y *L. grandis* en la Reserva Padre Ramos. Se recolectaron las muestras en las épocas de lluvia y seca durante 2012-2013. Se determinaron las concentraciones tisulares de metales, PAHs y POPs, y se integraron en índices de contaminación (CPI y PLI). Paralelamente, se determinaron parámetros biológicos a diferentes niveles de complejidad biológica (índices de condición, parámetros de reproducción, histopatología y respuesta de estrés sobre estrés) como biomarcadores de perturbación de la salud del ecosistema. En el Caribe, las concentraciones tisulares de contaminantes en *P. arctata* fueron bajas, en general, con algunas excepciones. Por otro lado, Ag, As, Cd, Hg, Ni y V se registraron principalmente en la época seca, mientras que PAHs y POPs (tales como HCHs, DDTs, AHTN, PCBs y BDE85) se registraron principalmente en la época de lluvia. En general, los valores más altos de CPI y PLI se registraron en Punta San Gabriel, lo que es consistente, porque esta localidad se ubica en la zona de sedimentación de la Laguna de Bluefields. Sin embargo, parece que los metales y PAHs no son motivo de gran preocupación en estas lagunas Caribeñas. Por el contrario, se registraron altos niveles de HCHs y DDTs, y niveles de bajos a medios de almizcles sintéticos. Aunque se encontraron mínimas diferencias entre localidades y entre épocas en los parámetros biológicos estudiados, se registraron valores bajos de LT_{50} en Punta San Gabriel durante la época de lluvia, asociados a valores más altos de CPI y PLI. En la costa del Pacífico, los principales contaminantes registrados en *A. tuberculosa* y *L. grandis* fueron HCHs, DDTs, AHTN y PDBEs en la época de lluvia y Cd en la época seca. En estas dos especies, parámetros biológicos comparables a los utilizados en otros bivalvos centinelas parecen ser adecuados como biomarcadores de perturbación de la salud; sin embargo, se necesita más investigaciones básicas para entender su biología general, ecología y enfermedad. En general, nuestra investigación sustenta que el guacuco de marjal esbelto en el Caribe, y la concha negra y el casco de burro en el Pacífico podrían ser usadas como especies biomonitoras y centinelas para evaluar la contaminación y las perturbaciones de la salud del ecosistema en los sistemas costeros de manglar en Nicaragua. Además, se ha comprobado que la estacionalidad es crucial para diseñar y llevar a cabo adecuadamente programas de monitoreo de contaminación usando las tres especies estudiadas en éste trabajo.

1. Introduction

The mangrove forest from Nicaragua extends throughout 69050 ha and represents one of the main coastal ecosystems, which is approximately equally distributed between the Pacific and the Caribbean coasts (Jiménez, 1999; FAO, 2007). Overall, mangrove ecosystems are threatened by a combination of natural disasters, tourism, aquaculture, deforestation and chemical pollution (Ellison, 2004; Defew et al., 2005; Fernandez et al., 2007; Polidoro et al., 2010; Lewis et al., 2011; Bayen, 2012). Concretely, Nicaraguan mangroves are influenced by 21 watersheds, 13 of them drain in the Caribbean and 8 in the Pacific Coast (González, 1997). Consequently, the Caribbean coastal area is a major sink for contaminants transported along the watersheds as it receives the 90% of the Nicaragua's territory catchment (UNEP, 2002). Nevertheless, maritime traffic and ports, urban settlements, shellfish farming and intensive agriculture are also potential sources of pollutants in the Pacific coast (Spongberg and Davies, 1998; Gener, 2009; Spongberg and Witter, 2008; Vargas et al., 2015).

Thus, there exists a need to carry out pollution monitoring programmes using bivalve molluscs as biomonitors and sentinels, following the Mussel-Watch approach (Goldberg, 1975; Sericano et al., 1995; Monirith et al., 2003; Kimbrough et al., 2008; 2009; Garmendia et al., 2011; Marigómez et al., 2013). However, to our knowledge, such a kind of pollution monitoring activities in Nicaragua are based on sporadic studies carried out by academics and research groups (GEF-REPCar, 2011; Ebanks-Mongalo et al., 2013; Chapters 1 and 2), as well as by international organisations such as the International Mussel Watch and the UNEP Caribbean Environment Programme (Farrington and Tripp, 1995; Siung-Chang, 1997; UNEP, 2006).

The mangrove cupped oysters (*Crassostrea rhizophorae*) have been proposed as biomonitors and sentinels for pollution monitoring programmes in mangrove ecosystems (Nascimento et al., 1998; Wallner-Kersanach et al., 2000; Rebelo et al., 2005; Silva et al., 2003; 2006; da Silva et al., 2005; Zanette et al., 2006; Valdez Domingos et al., 2007; Torres et al., 2012; Chapters 1 and 2). However, their distribution in Nicaragua is restricted to the Caribbean coast and therefore they cannot be used in pollution monitoring programmes in Pacific Coast mangrove-lined coastal systems. Thus, we need additional potential biomonitor and sentinel species, suitable for biomonitoring of pollutants and ecosystem health disturbance in Nicaraguan mangrove–lined coastal systems, both in the Caribbean and in the Pacific Coasts. Using more than one biomonitor/sentinel species in the same pollution monitoring programme is not

exceptional. Pollution monitoring programmes carried out from North to South America (The National Status & Trends NS&T, 1986-1993; and the International Mussel Watch, IMW, 1991-1992) use different bivalve species, such as oysters, clams and cockles, depending on their availability at each particular geographical region (Farrington and Tripp, 1995; Sericano et al., 1995; Kimbrough et al., 2008). Moreover, the sensitivity against pollutants may vary amongst species and therefore usually more than one species are simultaneously used to assess pollution and its biological consequences in marine biomonitoring programmes (Fernández-Tajes et al., 2011; Pereira et al., 2011).

Within this framework, the slender marsh clam, *Polymesoda artacta* from the Caribbean Sea, and mangrove cockles, *Anadara tuberculosa* and *Larkinia grandis* from the Pacific coast were selected as potential biomonitor/sentinel species for Nicaraguan mangrove ecosystems to be used either in parallel or as alternative to mangrove cupped oysters. These three species had been used as biomonitors in previous reports (Farrington and Tripp, 1995; Pérez-Cruz et al., 2013; Leal et al., 2014; Ziarrusta et al., 2015). *P. artacta* (Deshayes, 1854; syn., *P. solida* (Deshayes, 1854)[1], according to Bouchet, 2015) is a benthic mollusc with a biogeographical range extending from Belize to the Orinoco River in Venezuela (Severeyn et al., 1994). It inhabits fine, sandy sediments of the low-to-medium salinity estuarine zone (high organic content, salinity: 3-20 ppt; Severeyn et al., 1994). In Nicaragua, this species has been recorded in the Caribbean coast from Pearl Lagoon (North) to Monkey Point (South), as *P. solida*, *Polymesoda sp* or simply marsh clam (Mackenzie and Stehlik, 2001; MARENA, 2004; GTR-K, 2007; Ziarrusta et al., 2015). Mangrove cockles, *A. tuberculosa* (GB Sowerby I, 1833) and *L. grandis*, (Broderip & GB Sowerby I, 1829; syn., *A. grandis* (Broderip & GB Sowerby I, 1829)[2], according to Huber, 2015) are found along an ample biogeographical range from Baja California in Mexico to Bahia de Tumbes in Perú (Mora Sanchez, 1990; Cruz and Jimenez, 1994). In general, *A. tuberculosa* is more abundant than *L. grandis* (Mackenzie, 2001). *A. tuberculosa* inhabits level mud sediments in mangrove swamps that occur along the main-lands and islands of lagoons. These cockles occur among the aerial prop roots and under the canopies of the mangrove trees; most are about 15 cm deep in the mud (Mackenzie, 2001; Stern-Pirlot and

[1] According to the World Register of Marine Species (http://www.marinespecies.org/index.php), the currently accepted name of *P. solida* is *Polymesoda arctata* (Deshayes, 1854), Bivalvia: Cyrenidae (Bouchet 2015).

[2] On the other hand, taxonomic name of *Anadara grandis* is not accepted according to the World Register of Marine Species (http://www.marinespecies.org/index.php), the currently accepted name is *Larkinia grandis* (Broderip & GB Sowerby I, 1829), Bivalvia: Arcidae (Huber, 2015).

Wolff, 2006). In Nicaragua, the pustulose ark, *A. tuberculosa* (commonly named "*black shell*") has been recorded along the Pacific coast (Pérez et al., 2003; Gener et al., 2009; USAID, 2012). *L. grandis* lives in intertidal mudflats and some subtidal areas beyond the edges of mangrove swamps (Mackenzie, 2001). Unlike in neighbouring countries such as Honduras and El Salvador where it is commercially exploited (Galdámez et al., 2007), in Nicaragua this mangrove cockle (commonly known as "*casco de burro*" -donkey's hoof-) is endangered by overharvest (Gener et al., 2009), except in the Padre Ramos reserve, where it is under community management for recovery (Manzanares L, personal comm.).

Both chemical and biological effects endpoints are included in pollution monitoring programs (ICES, 2011; OSPAR Commission, 2013), because the chemical data alone do not provide any indication of the deleterious effects exerted to biota and ecosystems by cocktails of pollutants in multiple stress scenarios (Cajaraville et al., 2000; Allan et al., 2006). Within this context, in a previous study, both pollutant bioaccumulation and biological effects were investigated in mangrove cupped oysters *C. rhizophorae* (Chapters 1 and 2). However, in slender marsh clams, pustulose arks and *casco de burro* ark shell clams there are only few bioaccumulation studies (Farrington and Tripp, 1995; Pérez-Cruz et al., 2013; Leal et al., 2014; Ziarrusta et al., 2015) and practically no biological effects studies, to our knowledge.

Moreover, as elsewhere discussed (Van Lavieren et al., 2011; Blaise et al., 2016; Chapter 2) although developing countries, such as Nicaragua, may lack the monitoring capacities and facilities required to conduct most-advanced biomarker-based monitoring using bivalves as sentinels, effortless biomarkers can provide basic knowledge on animal health and water quality and are technically achievable (Blaise et al., 2016; Chapter 2). For this purpose, a toolbox of non-sophisticated and reliable biological effects endpoints was selected to assess the potential of the mangrove cupped oyster, *C. rhizophorae*, as sentinel for pollution monitoring in Caribbean mangroves and coastal zones from Nicaragua (Chapter 2). This toolbox included responses easy to measure at population and individual level and histopathological analyses; these latter allow scoring responses at systemic and tissue levels on the basis of cheap, solid and straightforward technology.

This is a preliminary investigation that aims at contributing to the future use of the slender marsh clams (*P. arctata*), pustulose arks (*A. tuberculosa*), and casco de burro ark shell clams (*L. grandis*) as potential biomonitors and sentinels species for pollution biomonitoring in mangrove-lined coastal systems in Nicaragua, after applying the experience and toolbox developed

previously for mangrove cupped oysters (Chapter 1 and 2). For this purpose, a pilot field study was carried out in 4 localities (2 in the Caribbean and 2 in the Pacific coast) subjected to different types and levels of pollution, in two sampling campaigns during 2012-2013. Samples were collected in the rainy and dry seasons and the tissue concentration of metals, PAHs, POPs and biological effects in slender marsh clam and mangrove cockles were recorded.

2. Material and Methods

2.1. Sampling sites and sample collection

Three species of bivalves were collected along one year (2012-2013) in the rainy season (October) and in the dry season (March); seawater surface temperature was in the range of 30-32ºC at both seasons.

Subtidal (<1 m depth) slender marsh clams (*Polymesoda arctata*) were collected from two localities of t*he Caribbean coast* (Fig. 1): Bluefields (sampling site: Punta San Gabriel) and Pearl Lagoon (sampling site: Pigeon Cay). Punta San Gabriel was considered as a prospective reference site (far away from urban settlements, ~6 Km) whilst Pigeon Cay was selected as a potentially polluted site influenced by aquatic transport and urban discharges (GEF-REPCar, 2011; Ebanks-Mongalo et al., 2013). Intertidal pustulose arks (*Anadara tuberculosa*) were studied in two localities (mangrove swamp) in the Pacific coast (Fig. 2): Corinto (sampling site: Isla Machuca) and the Natural Reserve Padre Ramos (sampling site: Estero La Virgen). Isla Machuca was selected as seemingly polluted sites because it is subject to strong anthropogenic influence (UICN / ORMA, 2001) and the Corinto port. Beside, Estero La Virgen was selected a priori as reference site because it is a Natural reserve. Intertidal casco de burro ark shell clams (*Larkinia grandis*) were studied in one locality (mudflat) of the Pacific coast (Fig. 2); the Natural Reserve Padre Ramos (sampling site: Estero Venecia), considered as a reference clean site.

Up to 85 individuals were collected per locality/sample; up to 25 of them were used for the chemical analyses and the remaining 60 were used for biological effect assessment. Upon collection, the bivalves were put in 15 L plastic boxes (2 individuals / L) in seawater at ambient temperature and transported to the laboratory (for 3h) before processing. Due to logistic problems, sampling could not be conducted in Pigeon Cay at the dry season.

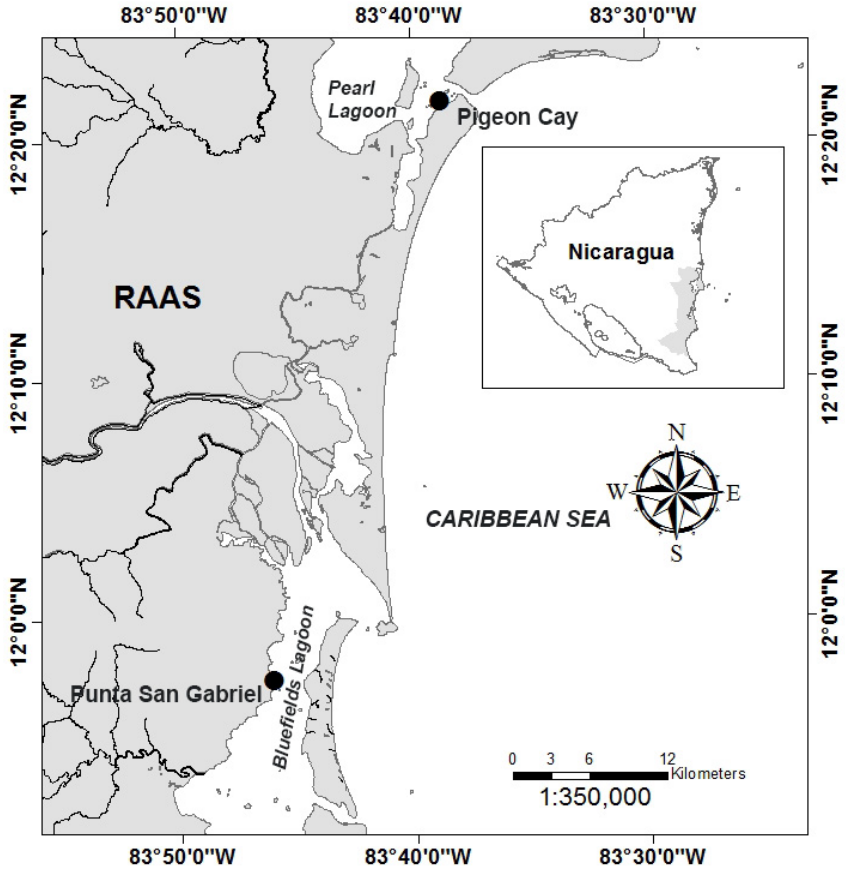


Fig. 1. Caribbean coastal maps from Nicaragua, showing the localities where slender marsh clam *Polymesoda arctata* were collected in 2012-2013. Nicaraguan coast: Pigeon Cay (12°21'42.50"N-83°38'32.11"W) and Punta San Gabriel (11°57'24.40"N-83°46'19.90"W).

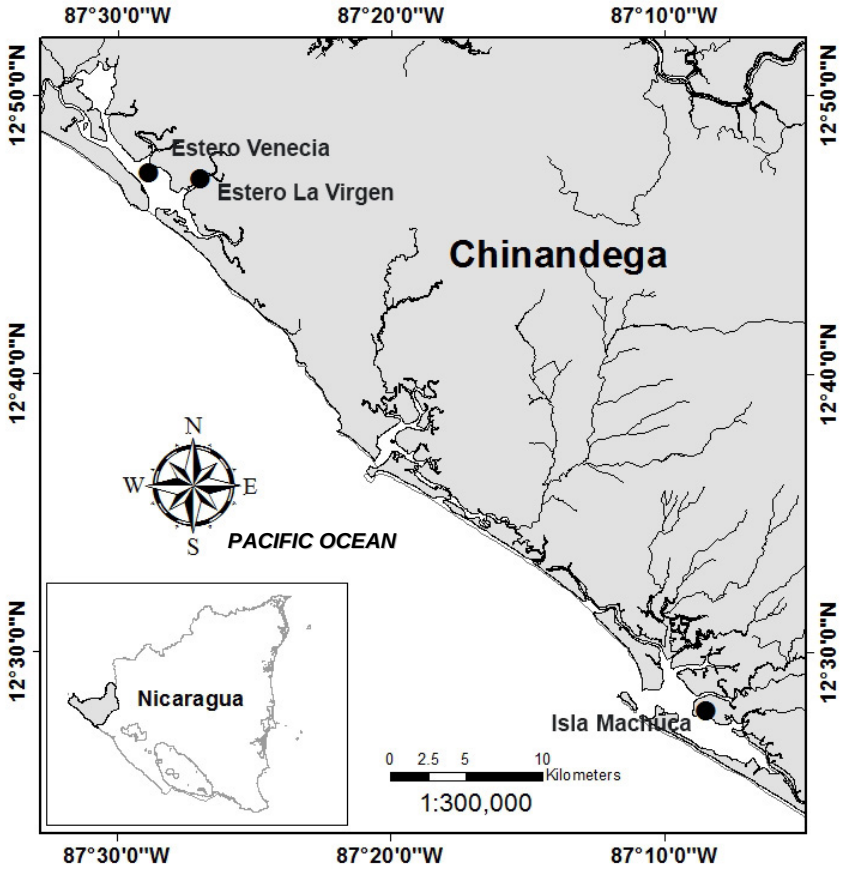


Fig. 2. Pacific coastal maps from Nicaragua, showing the localities where mangrove cockles *Anadara tuberculosa* and *Larkinia grandis* were collected in 2012-2013. Nicaraguan coast: Isla La Virgen (12°46'59.40"N-87°26'59.60"W); Estero Venecia (12°47'10.70"N-87°28'53.20"W) and Isla Machuca (12°27'52.70"N-87°08'27.40"W.

2.2. Tissue concentrations of contaminants

Pools of 25 individuals were homogenised and freeze-dried before being stored (at -20ºC) and analysed. For the analysis of metals (Ag, Al, As, Cd, Cr, Cu, Hg, Ni, Pb, Ti, V and Zn), 100 mg of freeze-dried samples were digested in a microwave oven (Multi-wave 3000, Anton Paar, Austria) and the extracts were first filtered and then measured in an Inductively Coupled Plasma - Mass Spectrometer (ICP-MS; NexION 300 Perkin Elmer, USA) (Bartolomé et al., 2010; Navarro et al., 2010). For the analysis of the organic contaminants, 0.3 g of freeze-dried samples and 0.3 g of Florisil dispersant were manually blended in a glass mortar and were transferred to a 10-mL glass syringe containing 0.6 g of deactivated silica and 4.0 g of activated silica. The target analytes were eluted with 25 mL of dichloromethane, and the eluate was evaporated to dryness using N_2 blowdown and reconstituted to a final volume of 140 L of n-hexane. All these extracts were analysed by Gas Chromatography - Mass Spectrometry (GC–MS) using an Agilent 7890A gas chromatograph coupled to an Agilent 7000 triple quadrupole mass spectrometer and an Agilent 7693 autosampler (Agilent Technologies). The MassHunter WorkStation Acquisition Software (Version B.05.02/Build 5.2.365.0, Agilent Technologies, 2008) was used for data acquisition and automatic integration and quantification of the results. This method was designed to measure up to 40 non-polar or slightly non-polar organic pollutants such as PAHs, PCBs, PBDEs, organochlorine pesticides (OCPs), organophosphorus pesticides and musk fragrances (Ziarrusta et al., 2015).

2.3. Chemical pollution indices

Pollutant concentrations in the tissues of slender marsh clams, pustulose arks and casco de burro ark shell clams were compared with available environmental quality criteria for individual pollutants (OSPAR Commission, 2005; 2009; 2013; Kimbrough et al., 2008; Green et al., 2012): Background/Reference Concentrations (BRCs); Background Assessment Criteria (BACs), Highest Background Concentrations (HBCs) and Highest Low Concentrations (HLC). BRCs are intended to provide baseline or reference concentrations, and to describe environmental conditions under which there is no anthropogenic influence on the concentrations of the target substances in the environment (Davies, 2004; OSPAR Commission, 2005). BACs describe the threshold value for the background level, using data from reference sites (OSPAR Commission, 2005; 2009; 2013). HBCs correspond to the upper limit to Class I in the Norwegian marine pollution monitoring approach, which recognises five classes from Class I, insignificantly

polluted, to Class V, extremely polluted (Green et al., 2012). HLCs correspond to the upper limit of the Low Concentration Range in the NOAA's mussel watch pollution monitoring; this recognises three concentration ranges (low, medium and high) for the tissue concentration of pollutants in oysters (Kimbrough et al., 2008).

The chemical pollution index (CPI; Bellas et al., 2011; 2014; Beiras et al., 2012) was calculated for each site and season. For this purpose, Concentration Factors (CF) were calculated for each pollutant by dividing the tissue pollutant concentrations (C_{tiss}) by the corresponding environmental quality criterion (C_{eqc}): $CF=C_{tiss}/C_{eqc}$. Then, the following formula was applied: $CPI=\Sigma_i[log(CF_i)]$. Likewise, the Pollution Load Index (PLI; Tomlinson et al., 1980) was computed for each site and season by obtaining the n-root from the n-CFs that were obtained for all the pollutants, according to the following equation:

$$PLI = \sqrt[n]{CF_1 \, X \, CF_2 \ldots \ldots CF_n}.$$

2.4. Condition and histopathology

2.4.1. Stress on Stress (SoS) test

LT_{50} was calculated after the "survival-in-air" SoS test (Veldhuizen-Tsoerkan et al., 1991; Viarengo et al., 1995) performed on 30 individuals from each site and sampling period that were placed over wet paper on plastic trays at constant room temperature (34ºC) and 100% humidity. Survival was assessed daily and individuals were considered to be dead when their valves gaped and failed to close when they were physically stimulated, or simply presented a bad smell (Pampanin et al., 2005).

2.4.2. Flesh Condition Index

Soft parts from each individual were dried at 105ºC and weighed to the nearest 0.1 mg on an analytical balance in order to calculate flesh dry-wt (FDW; Lobel and Wright, 1982). Then the flesh condition Index (FCI) was as FDW/SDW, where SDW is the shell dry weight (dried at room temperature) according to Crosby and Gale, (1990). SDW was measured in g and shell length (L) was determined as the length in mm of the largest valve (the long axis measured to the nearest 0.1 mm with Vernier callipers; Dame, 1972). FDW/L was calculated and used to as an indication of bivalves flesh condition suitable for a regional comparison (Hellou et al., 2003; Blaise et al., 2016).

2.4.3. Histology and histopathology

Upon dissection of their soft body (N=20 individuals/sample), central cross-sectioned slices (3–5 mm thick, including digestive gland, gonad (mantle) and additional tissues) were fixed in Davidson's fixative for 48 h and paraffin embedded. Microtome sections (5 µm) were obtained using an automated rotary Leica RM 2255 microtome (Leica Microsystems, Nussloch, Germany), stained with haematoxylin-eosin and examined under the light microscope (Olympus BX61; Tokyo, Japan) for gamete development determination and histopathological diagnosis (Kim et al., 2006).

Gamete development and reproductive disturbance. Gamete developmental stages were examined microscopically. Slides of 20 individuals per sample were examined one-by-one under the light microscope using 10× and 20× objective lenses. The sex of each animal was recorded and the total number of female and male individuals was calculated for each sampling time and locality, and with these data, the sex ratio was calculated for each sampling time and locality (Ortiz-Zarragoitia et al., 2011). Further on, the sex ratio index (SRI) was calculated as the G value of females-to-males ratio (F:M) upon the gross assumption of theoretical F:M ratios of 1:1 in *P. arctata* (no referecne available; arbitrarily decided as a fist approach); *A. tuberculosa* (Gener et al., 2009, Silva-Benavides and Bonilla 2015) and *L. grandis* (Galdámez et al., 2007). Intersex index (IXI) was calculated as the prevalence of intersex individuals at each locality and season. The prevalence of gamete developmental stages was determined upon scoring the maturity of the follicles and gametes, as well as a gonad index (GI) value (Table 1) according to Kim et al., (2006); Ortiz-Zarragoitia and Cajaraville, (2010).

Gamete mass index (GMI) was calculated as the log ratio of the sum of the prevalence of developmental stages 2-4 (gonad tissue growth) to the sum of the prevalence of stages 5-6 (gonad tissue loss), in logarithmic scale. Undifferentiated index (UDI) was estimated as the prevalence of stage 1. Oocyte atresia prevalence was recorded in females and its intensity was scored (0-4) individually according to the scale for abnormal gonad development proposed by Kim et al., (2006) for mussels. Oocyte atresia index (OAI) was calculated by multiplying prevalence × intensity. Reproductive Anomalies Index (RAI) was calculated as weighted average of the deviation from the theoretical maximum anomalies of the specific indices of reproductive disturbance, according to the following formula:

$$RAI = \frac{1}{5} \times \left(\frac{CF \times SRI}{SRI_{50}} + \frac{CF \times IXI}{IXI_{50}} + \frac{CF \times GMI}{GMI_{max}} + \frac{CF \times UDI}{UDI_{max}} + \frac{CF \times OAI}{OAI_{50}} \right)$$

where CF=×100/1.6 (thus RAI changes between 0 and 100); SRI_{50}=27.7 for a 50% deviation from the theoretical sex ratio; IXI_{50}=50, when a 50% prevalence of intersex individuals occurs; (c) where GMI_{max}=2, when 100% of the gametes are mature in absence of any sign of spawning; (d) UDI_{max}=100, when 100% of the individuals do not present differentiated gametes; and OAI_{50}=2, the median value of the scale for atresia scoring after Kim et al., (2006).

Table 1. Gametogenic phases and gonad index applied in slender marsh clam (*Polymesoda arctata*) and mangrove cockles (*Anadara tuberculosa* and *Larkinia grandis*), modified from mytilid and dreissenid development stages (Kim et al., 2006; Ortiz-Zarragoitia and Cajaraville, 2010).

Gonad Index	Gametogenic phase	Description
0	**Resting/spent gonad (I)**	Inactive or undifferentiated
1	**Early development (II)**	Gametogenesis has begun; no ripe gametes visible
2		Ripe gametes present; gonad developed to about one-third of its final size
3	**Advanced development (III)**	Gonad increased in mass to about half the fully ripe condition; each follicle contains, in area, about equal proportions of ripe and developing gametes
4		Gametogenesis still progressing, follicles contain mainly ripe gametes
5	**Mature (IV)**	Gonad fully ripe, early stages of gametogenesis rare; follicles distended with ripe gametes; ova compacted into polygonal configurations; sperm with visible tails
4	**Spawning (V)**	Active emission has begun; sperm density reduced; ova rounded off as pressure within follicles is reduced
3		Gonad about half empty
2	**Post-spawning (VI)**	Gonadal area reduced; follicles about one-third full of ripe gametes
1		Only residual gametes remain; some may be undergoing cytolysis

Digestive gland histopathology. Parasites, tissue lesions and inflammatory responses were examined microscopically. Slides of 20 individuals per sample were examined individually under the light microscope using 10×, 20× or 40× objective lenses. Parasites and histopathological alterations were scored using either quantitative or presence/absence scales (Kim et al., 2006). Intracellular ciliates, unidentified intracellular protists, *Nematopsis* sp., metazoans (trematodes, cestodes) and *Rickettsia/Clamydia*-like organisms (R/CLO) were

recorded quantitatively following procedures previously described (Kim et al., 2006; Kim and Powell, 2007; Garmendia et al., 2010; 2011). Quantitative scores were made by keeping a running count of the incidences as the slide was scanned to avoid re-examination of each slide multiple times for each category. Haemocytic infiltration, without distinction between focal and diffuse and brown cell aggregates were scored as present/absent for each individual bivalve, except to granulocytomas that it was quantified (Zaroogian and Yevich, 1993; Kim and Powell, 2004). Prevalence and intensity of histopahological lesions were determined, according to Kim and Powell, (2007) and Garmendia et al., (2011), as follows:

$$Prevalence = \frac{N.affected\ individuals\ \times 100}{N.examined\ individuals}$$

$$Intensity = \frac{\sum_{i=1}^{n} (N.occurrences\ of\ parasite\ or\ pathology\ per\ individual)}{N.affected\ individuals}$$

The inflammatory response index (IRI) was calculated as the sum of the prevalence values of oedema, granulocytomas and disseminated neoplasia. The parasitic infestation index (PII) was calculated as the sum of the prevalence values calculated individually for each of the 5 groups of parasites aforementioned. The digestive gland atrophy index (DGAI) was estimated according to Kim and Powell (2007).

2.5. Statistical analyses

Statistical analyses were carried out with the aid of SPSS version 22 statistical package (IBM SPSS, Armonk, NY, USA). The normality distribution (Shapiro-Wilk's test) and homogeneity of variance (Levene's test) of data were determined before proceeding with subsequent analyses. Due to the high variability between individuals, even after normal distribution transformation of the data, non-parametric analyses were carried out. Significant differences in the prevalence of inflammatory responses and parasitic lesions and in health condition indices were analysed by the Z score test. Survival values of SoS test were analysed with the non-parametric Kaplan-Meier analysis, followed by the Tarone-Ware post hoc test for comparisons between localities for each season and between seasons for each locality. Differences in L, SDW, FDW/SDW, DGAI and GI were determined by applying Kruskal–Wallis followed by Dunn's post hoc test. Sex ratio bias was studied using the G test of association, comparing total number of female and male mussels and normalizing for theoretical gender bias. In all the tests used herein, significant differences were established at $p<0.05$.

3. Results

Table 2. Metal tissue concentrations in *P. arctata* (µg/g) from Caribbean mangrove lagoons. LOD, limit of detection.

	LOD	RAINY SEASON		DRY SEASON
		Pigeon Cay	Punta San Gabriel	Punta San Gabriel
Ag	0.0015	0.06	1.00	1.46
Al	15.3	285.54	631.98	606.05
As	0.01	10.24	9.69	26.33
Cd	0	0.24	0.25	1.00
Cr	0.22	0.63	0.53	0.68
Cu	0.11	11.48	11.12	8.52
Hg	0.004	0.15	0.16	0.24
Ni	0.54	2.67	0.87	1.25
Pb	0.015	0.89	0.27	0.40
Ti	0.94	11.89	14.73	8.34
V	0	0.78	1.80	2.49
Zn	2.36	91.76	99.45	104.23
∑Metals		**416.35**	**771.86**	**761.00**

3.1. *Polymesoda arctata*

Tissue concentrations of contaminants. Overall, metal tissue concentrations were low, although the tissue concentrations of As and Hg were seemingly higher in Punta San Gabriel in the dry season than in the rainy season, when no differences between localities were apparent (Table 2). The tissue concentrations of PAHs were apparently higher in the rainy season than in the dry season (Table 3). In the rainy season, higher PAHs values were recorded in Punta San Gabriel than in Pigeon Cay (Table 3). The most relevant PAHs found were phenanthrene, fluoranthene, pyrene, and benzo[b]fluoranthene+benzo[k]fluoran-thene in Punta San Gabriel and chrysene in Pigeon Cay, in the rainy season (Table 3). The sum of the 16 USEPA PAHs (except napthalene) was higher in Punta San Gabriel during the rainy season than in Pigeon Cay. Pigeon Cay showed high values of $\sum_{HMW}$PAHs and low $\sum_{LMW}$PAHs and a low $\sum_{LMW}$PAH/$\sum_{HMW}$PAHs ratio, whilst the opposite effect was observed in Punta San Gabriel (Table 3). The tissue concentrations of POPs were apparently higher in the rainy season than in the dry season (Table 4). HCHs and DDTs and their derivatives were recorded especially in the rainy season; the tissue

concentration of HCHs being higher in Pigeon Cay than in Punta San Gabriel and the opposite as regards the DDT tissue concentrations, which was higher in Punta San Gabriel than in Pigeon Cay (Table 4). Musk fragrances were only detected in Punta San Gabriel in the rainy season. PCBs were only detected in Pigeon Cay in the rainy season, CB28 and CB118 showing the highest values (Table 4). Only two of the eight analysed PBDEs (BDE85 and BDE100) were found at measurable levels; BDE85 showing markedly high values in Pigeon Cay in the rainy season and in Punta San Gabriel in the dry season (Table 4).

The highest CPI and PLI values were obtained in the rainy season (Figs. 3a and b), Punta San Gabriel being the locality with positive CPI values (<1) and high PLI values (69.58). Overall, the main pollutants contributing to these CPI and PLI values were Hg, HCHs, DDTs, AHTN, PCBs and BDE85 (SM 1 and 2).

Table 3. PAH tissue concentrations in *Polymesoda arctata* (ng/g) from Caribbean mangrove lagoons. The following compounds were under detection limits in all the samples (superscript numbers refer to the sums and indices given in the table where they are integrated): B[ghi]P (LOD=1); Ind (LOD=2)[(2)]. LOD, limit of detection; udl, under detection limits; n/a, not applicable.

	LOD	RAINY SEASON		DRY SEASON
		Pigeon Cay	Punta San Gabriel	Punta San Gabriel
Acy [(1)]	1	udl	udl	udl
Ace [(1)]	1	17	udl	udl
Flu [(1)]	0.07	udl	17	udl
Phe [(1)]	0.49	udl	173	udl
Ant	1	18	udl	udl
Flr [(1)]	0.34	udl	64	udl
Pyr [(1)]	1	4	33	udl
Benz[a]A [(2)]	1	20	udl	udl
Chr [(2)]	1	40	7	udl
B[b]F +B[k]F [(2)]	1	22	27	3
B[a]P [(2)]	1	udl	6	udl
D[ah]A [(2)]	2	udl	13	udl
∑PAHs (16; except Naph)		**120**	**339**	**3**
$\sum_{HMW}$PAHs $^{\Sigma(2)}$ (carcinogenic)		**60**	**7**	**3**
$\sum_{LMW}$PAHs $^{\Sigma(1)}$ (%∑PAHs)		**21 (17%)**	**287 (85%)**	**n/a**
$\sum_{LMW}$PAHs / $\sum_{HMW}$PAHs		**0.34**	**43.00**	**0**
Ind/(Ind+B[ghi]P		**n/a**	**n/a**	**n/a**

Table 4. POP tissue concentrations in *P. arctata* (ng/g) from Caribbean mangrove lagoons. The following compounds were under detection limits in all the samples (superscript numbers refer to the sums and indices given in the table where they are integrated): 2.4-DDD (LOD=5) [3]; HHCB (LOD=9) [4]; BDE28 (LOD=7.2) [5]; BDE47 (LOD=1.3) [5]; BDE66 (LOD=0.94) [5]; BDE99 (LOD=6.6) [5]; BDE153 (LOD=50) [5]. LOD, limit of detection; udl, under detection limits; n/a, not applicable.

	LOD	RAINY SEASON		DRYSEASON
		Pigeon Cay	Punta San Gabriel	Punta San Gabriel
α-HCH	50	90	29	udl
β-HCH	25	81	udl	udl
γ-HCH	2	15	85	udl
δ-HCH	25	87	udl	udl
∑HCHs		**273**	**114**	**0**
4.4-DDE [3]	2	278	udl	udl
2.4-DDT [3]	25	96	8	udl
4.4-DDT [3]	50	udl	750	udl
∑DDTs [3]		**373**	**758**	**0**
4.4-DDT/4.4-DDE		**0**	**n/a**	**0**
2.4-DDD/4.4-DDE		**0**	**n/a**	**0**
(2.4-DDD+4.4-DDE)/ ∑DDTs		**0.74**	**n/a**	**0**
∑OCPs		**649**	**873**	**0**
Chlorpyrifos	2	7	udl	udl
AHTN [4]	10	udl	115	udl
∑Musks$^{\Sigma}$ [4]		**0**	**115**	**0**
CB28	1	80	udl	udl
CB52	1	7	udl	udl
CB101	1	17	udl	udl
CB118	1	87	udl	udl
CB138	1	18	udl	udl
CB153	1	16	udl	udl
CB180	1	5	udl	udl
∑PCBs∑(PCB_7)		**230**	**0**	**0**
BDE85 [5]	1	227	22	115
BDE100 [5]	1	udl	8	udl
∑PBDEs$^{\Sigma}$ [5]		**227**	**30**	**115**
∑POPs		**1113**	**1018**	**119**

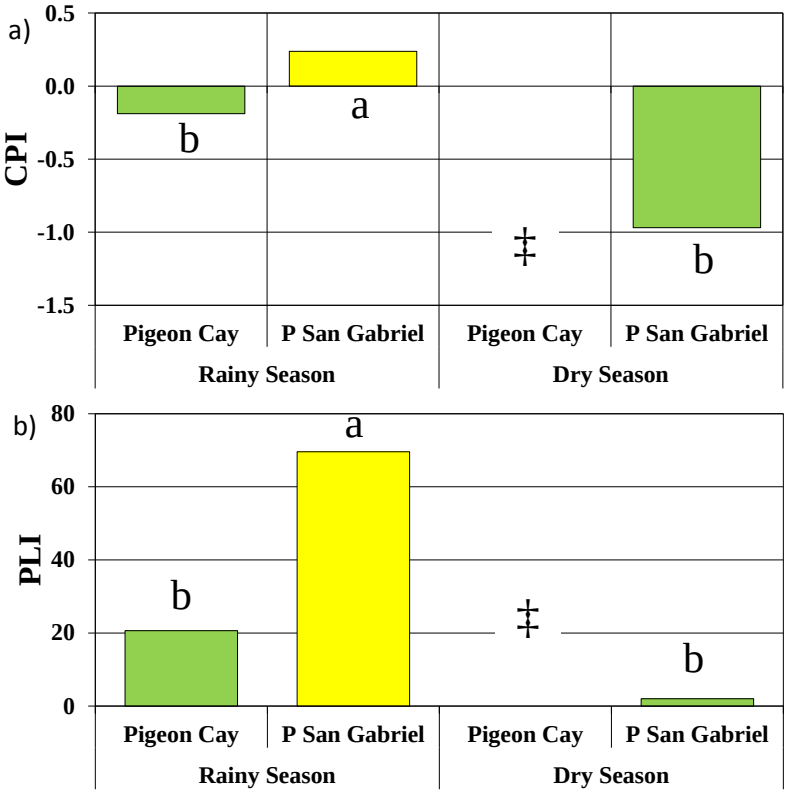


Fig. 3. (a) Chemical Pollution Index (CPI) in *P. arctata* from Caribbean mangrove lagoons. CPI is categorized into three groups: 'low pollution' when CPI ≤ 0, 'moderate pollution' when 0 < CPI ≤ 1 and 'high pollution' when CPI > 1 (according to Bellas et al., 2011; 2014). (b) Pollution Load Index (PLI) derived from the tissue concentrations of pollutants (Tomlinson et al., 1980). A PLI of 100 correspond to very contaminated sites and a PLI > 50 requires a potential risk and moderate contamination (Angulo 1996). ‡, not data.

Health condition. L, SDW, SDW/L and FDW/L showed the highest values in Punta San Gabriel in the dry season, but in the rainy season no statistical differences were recorded between localities in L, FDW and FDW/L (Table 5 and Fig. 4b-c). FDW/SDW in clams from Punta San Gabriel were the highest values recorded (Table 5 and Fig. 4a). Sex ratio values for each sampling locality and time are shown in Table 5. No significant difference was detected in the sex ratio (F:M, 1.2:1; G value=0.82, $p>0.05$) for the investigated slender marsh clams as a whole in comparison with the theoretical sex ratio of 1:1; this population sex ratio resulted from the average between seasonal sex ratios (1.4:1 in the rainy season and 1:1.5 in the dry season). The sex ratio values were similar between localities in rainy season (Table 5). Undifferentiated gonad was only found in Punta San Gabriel in the rainy season with a prevalence value of 2%. No intersex was recorded.

Minor differences in gamete development stages were observed between seasons and localities. The dominant gamete development stages were spawning and post-spawning (>90 %) in both localities (Fig. 5 and 6a-d). Gametogenic development was seemingly more advanced in the dry season than in the rainy season (Fig. 5). Punta San Gabriel showed gamete development stages more advanced than Pigeon Cay in the rainy season (Fig. 5). However, significant differences ($p<0.05$) not were observed in GI between localities and seasons (Table 5).

Likewise, no differences in GMI and UDI were recorded (Table 5). No oocyte atresia were recorded. As a whole, RAI did reflect only a low-to-moderate reproductive disturbance in Punta San Gabriel in the rainy season (Fig. 7a). Only one clam from Pigeon Cay was found to be infested by germinal cells of *Bucephalus sp* in the rainy season (Fig. 6e).

Table 5. Biometry and histopathology of *P. arctata* from Caribbean mangrove lagoons. Shell length, flesh dry weight, shell dry weight and gonad index values are mean ± standard error (n = 20). Sex Ratio Index = SRI G value. The superscript letters (a, b and c) indicate significant differences between groups ($p<0.05$; $p<0.1$, when shown between brackets). Asterisks (*) indicate significant differences between seasons for a given locality ($p<0.05$); ♯, significantly different from the theoretical gender bias (1:1) according to the G test ($p<0.05$). n/a, not applicable.

	RAINY SEASON		DRY SEASON
	Pigeon Cay	P San Gabriel	P San Gabriel
Shell Length (L; mm)	35.2±3.7	35.86±3.12	39.6±6.65*
Flesh dry-wt (FDW; mg)	0.43±0.19	0.52±0.34	0.47±0.13
Shell dry-wt (SDW; g)	18±3.93[a]	13.74±3.03[b]	23.45±5.59*
Sex ratio (Sex Ratio Index)	1.5:1 (1.61)	1.3:1 (0.64)	1:1.5 (0.85)
Intersex Index	0	0	0
Gonad Index	2.88±1	2.95±1.32	3.08±1.23
Gametogenic Mass Index	n/a	1.57	n/a
Undifferentiated Index	0	0.05	0
Oocyte Atresia Prevalence	0	0	0
Oocyte Atresia Intensity	0	0	0
Oocyte Atresia Index	0	0	0
Prevalence (oedema) %	70	80	80
Prevalence (BCA) %	100	100	80
Prevalence (granulocytomas) %	0	0	0
Preval. (dissem. neoplasia) %	0	0	0
Prevalence (ciliates) %	6	0	10
Preval. (Un/aert. Intra. Prot.) %	0	0	10
Prevalence (Total Intr. Prot.) %	6	0	20
Preval. (*Nematopsis* sp) % [(1)]	10	0	0
Prevalence (R/CLO) %	9	13	20

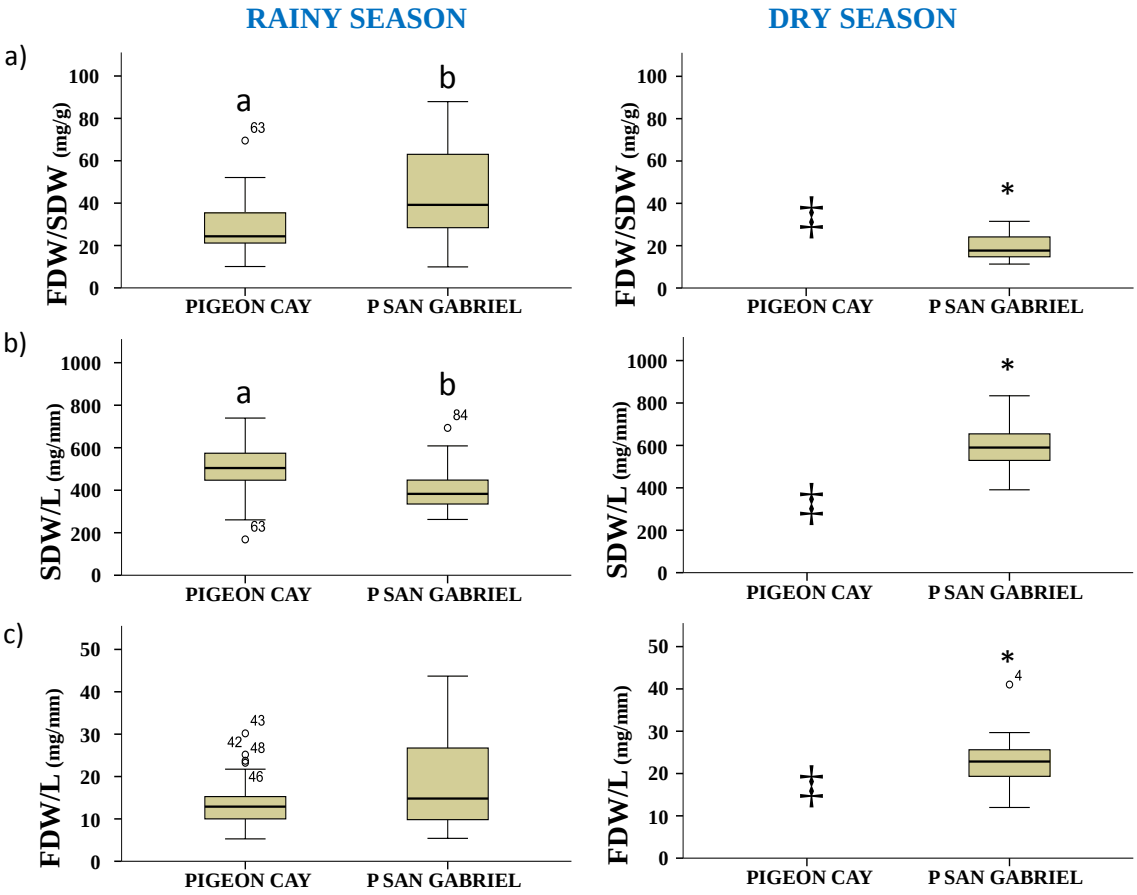


Fig. 4. Biometry and condition of *P. arctata* from Caribbean mangrove lagoons during the rainy and the dry seasons. Different letters denote statistically significant differences between localities and asterisks (*) indicate significant differences between seasons for a given locality (p<0.05). SDW/L, shell growth index; FDW/SDW and FDW/L, condition indices. ‡, not data.

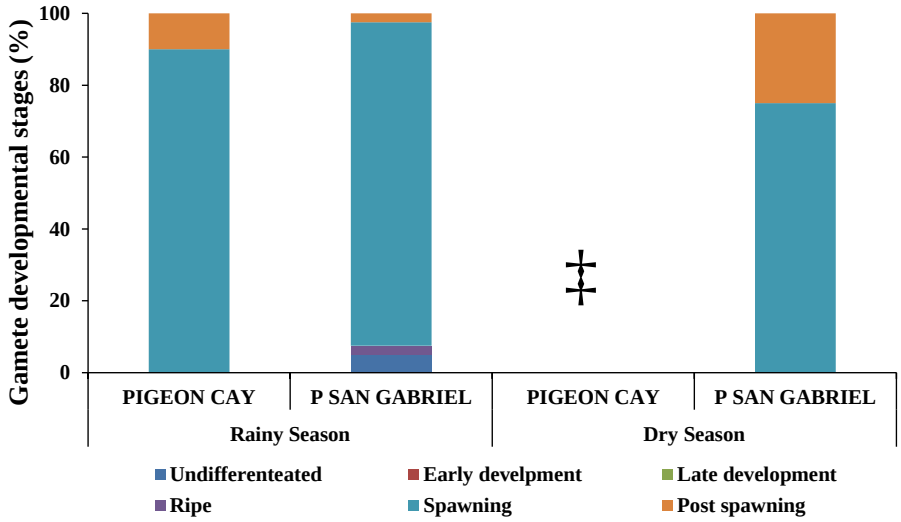


Fig. 5. Gamete development stages of slender marsh clam *P. arctata* from Caribbean mangrove lagoons. ‡, not data.

Histopathological examination in the digestive gland revealed the presence of a variety of parasites such as intracellular ciliates, undetermined intracellular protists, *Nematopsis* sp. and *Rickettsia/Clamydia*-like organisms (R/CLO) (Fig. 8c and d). Some parasites had a local impact such as undetermined intracellular protists in Punta San Gabriel in the dry season and *Nematopsis* sp. in Pigeon Cay in the rainy season (Table 5). Seemingly, parasite prevalence was low in both localities and seasons. PII was higher in dry season than in rainy season (Table 5 and Fig. 7b), which was principally attributed to the incidence of R/CLO. Nevertheless, the intensity of parasitic infestations was generally low (<5 parasites/clam). R/CLO presented the highest intensity values (4-5) all along the study period.

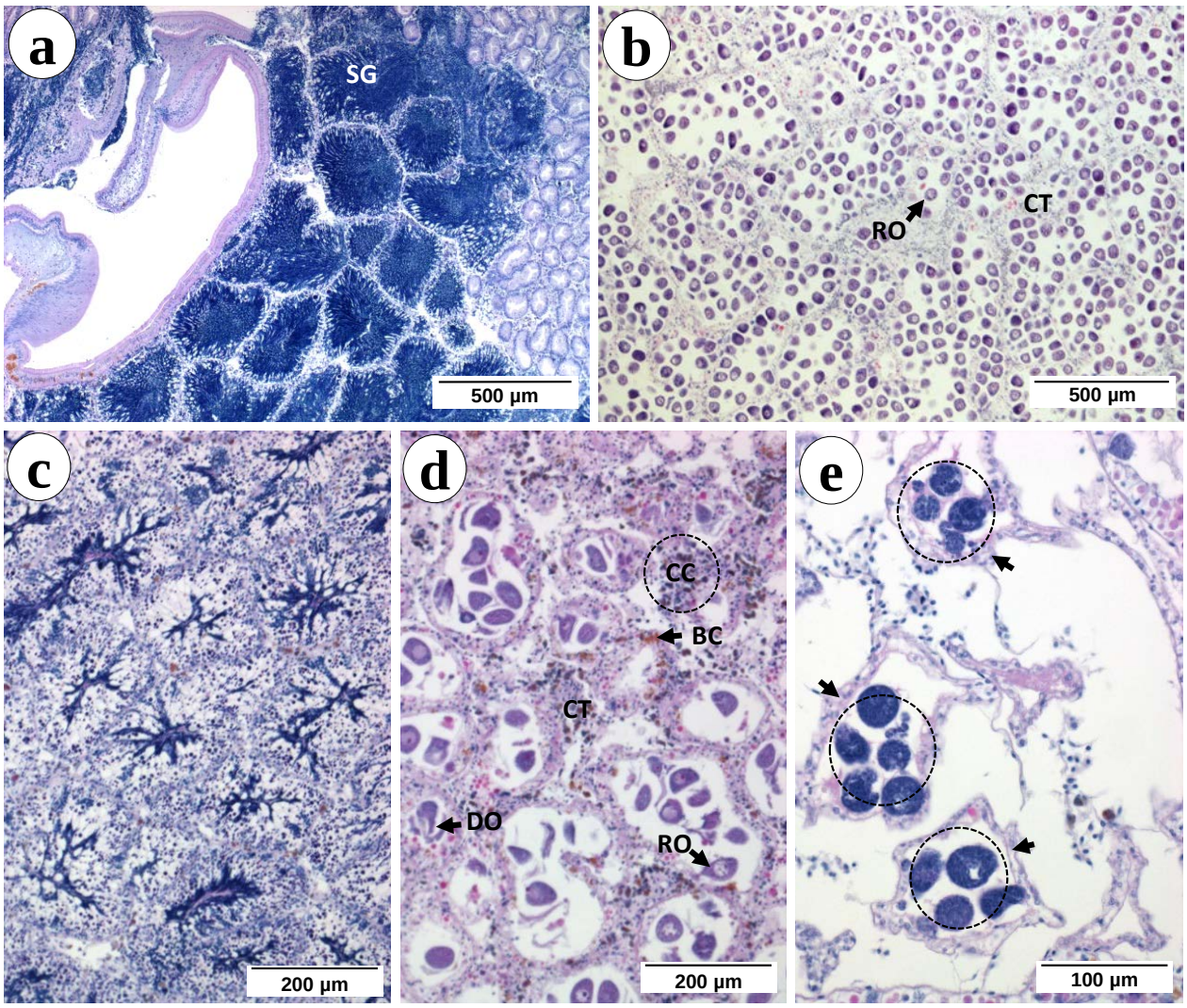


Fig. 6. Light micrographs of gonad sections of *P. arctata* from Caribbean mangrove lagoons. a) spawning gonad of a male; b) spawning gonad of a female; c) post-spawning gonad of a male; d) post-spawning gonad of a female with atretic oocyte; e) germinal cell of *Bucephalus* sp in gonad of a female from Pigeon Cay. Abbrevations: BC, brown cell aggregate; DO, degenerated oocyte; CC, cellular cytolysis; RO, ripe oocyte; SG, spermatogonia; CT, connective tissue. Arrow, change or tissue alteration.

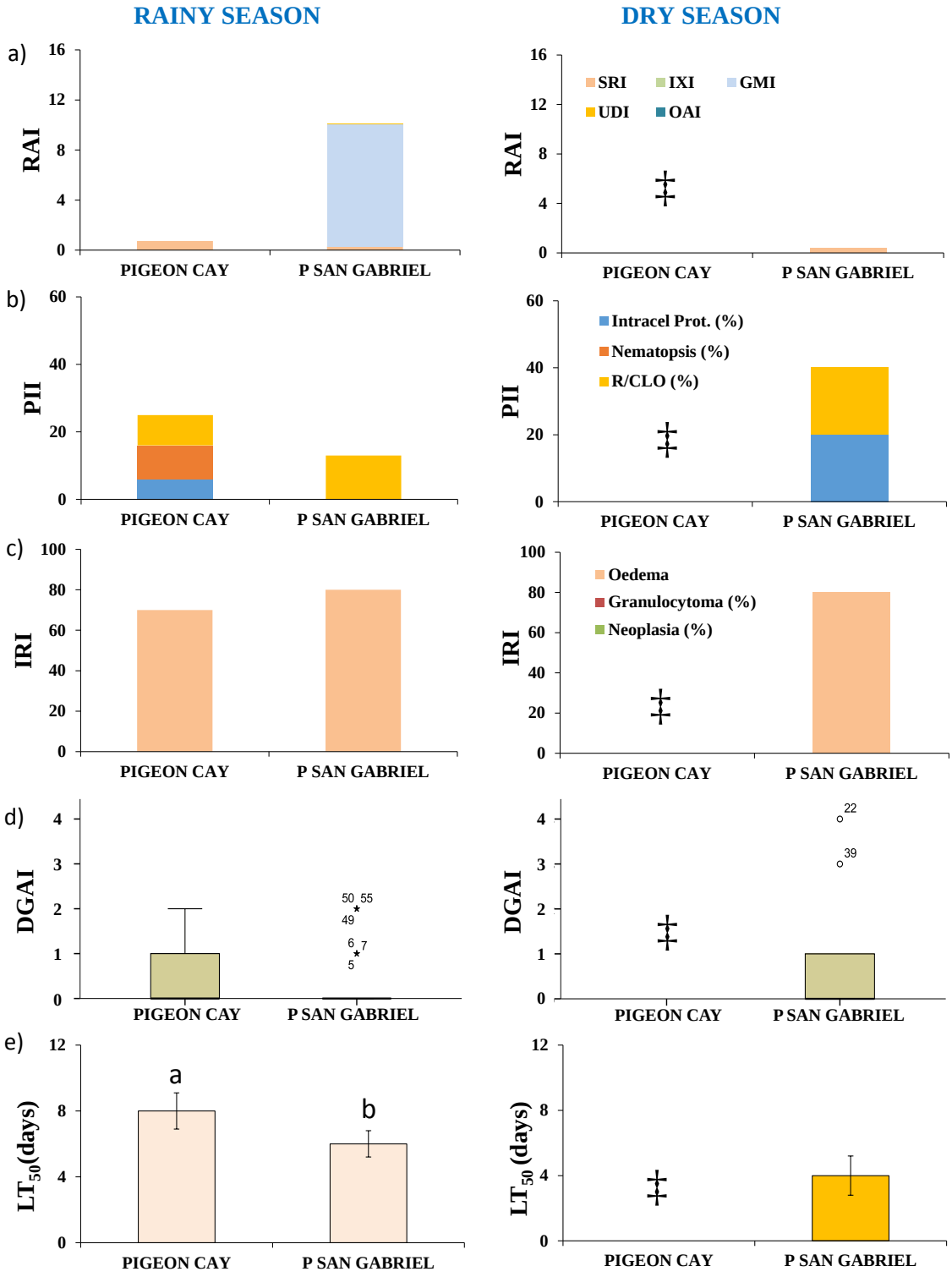


Fig. 7. Biomarkers of anomalies in slender marsh clam health indicative of disturbance in ecosystem health for the rainy and the dry seasons in Caribbean mangrove lagoons. Different letters denote statistically significant differences between localities and asterisk (*) indicate seasonal differences for a locality (p<0.05). RAI, reproductive anomalies index; PII, parasitic infestation index; IRI, inflammatory response index; DGAI, digestive gland atrophy index and LT_{50}, median survival time after SOS test. ‡, not data.

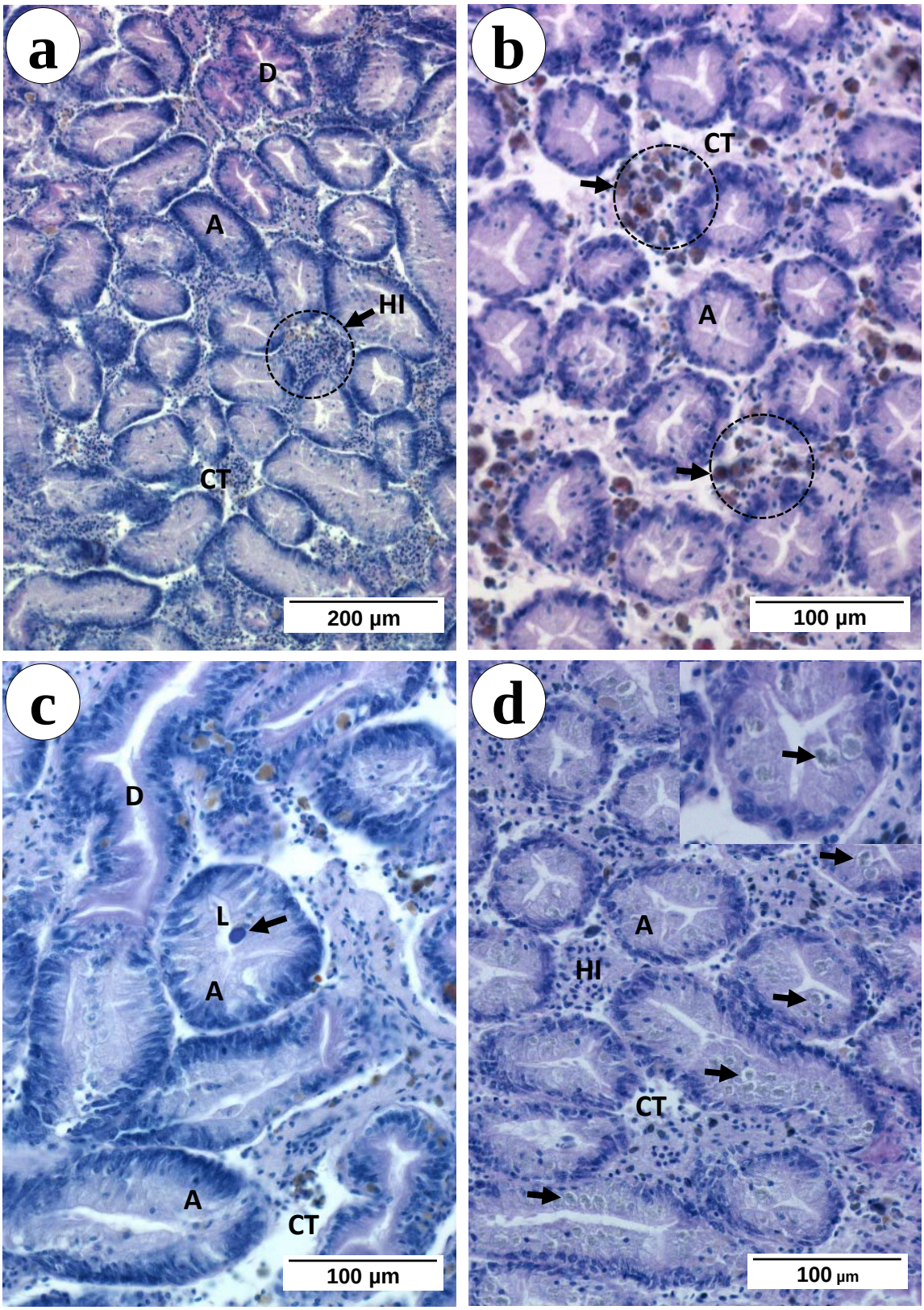


Fig. 8. Paraffin sections of the digestive gland of *P. arctata* stained with haematoxylin-eosin: a) digestive gland with diffuse haemocytic infiltration; b) brown cell aggregates; c) R/CLO; d) undetermined protists in alveoli of digestive gland. Abbreviations: A, alveoli; D, duct; CT, connective tissue; HI, haemocytic infiltration; L, lumen. Arrows, parasites or tissue alterations.

The histological integrity of the digestive gland tissue was seemingly unaltered in all the studied localities in both seasons (Figs. 8a). Inflammatory responses such as massive haemocytic infiltrations of ICT (oedema) and brown cell aggregates were observed (Fig. 8a and b). The prevalence of inflammatory responses and brown cell aggregates was apparently high in both seasons, as the IRI was as well (Table 5 and Fig. 8c). Digestive alveoli presented low degree of atrophy, resulting in low DGAI values; indeed, the lumen was nearly occluded by a high epithelium (Table 5 and Fig. 8a-d).

LT_{50} values were higher in the rainy season than in the dry season (Fig. 5e). In the rainy season, the highest LT_{50} values (Tarone-Ware test, $p<0.05$) were recorded in Pigeon Cay, whilst no statistical differences were observed between seasons (Fig. 5e).

3.2. *Anadara tuberculosa*

Tissue concentrations of contaminants. Metal tissue concentrations were low, except for As and Cd (Table 6). The highest tissue concentration of Cd, and to a lesser in As, was found in Estero La Virgen in the dry season (Table 6). PAH tissue concentration was low and restrained to the rainy season; the most relevant PAHs found being phenanthrene in the both localities (Table 7). The sum of the 16 USEPA PAHs (except naphthalene) and $\sum_{LMW}$PAHs was seemingly equal in Isla Machuca and Estero La Virgen. The $\sum_{LMW}$PAH/$\sum_{HMW}$PAHs ratio, could not be calculated (Table 7). POPs appeared to be higher in rainy season than in the dry season (Table 8). γ-HCH presented higher values in Estero La Virgen than in Isla Machuca in both seasons, with the highest values being recorded in the rainy season (Table 8). The only DDTs metabolite recorded was 4.4-DDE, found in Estero La Virgen in the rainy season and Isla Machuca in the dry season (Table 8). Musk fragrances were recorded only in the rainy season with the highest value in Estero La Virgen (Table 8). PCBs were under detected limit in all localities and seasons and only three of the eight analysed PBDEs (BDE85, BDE100 and BDE154) were found in pustulose ark tissues. BDE154 showed the highest value in the rainy season and BDE85 in the dry season, both in Isla Machuca (Table 8). CPI and PLI values were low in both seasons (CPI<0 and PLI<<50; Figs. 9a and b); the main pollutants contributing to these CPI and PLI values being Cd, Phe, HCHs, DDTs, AHTN and BDE85 (SM 1 and 2).

Table 6. Metal tissue concentration in *A. tuberculosa* (µg/g) from Pacific mangrove swamps. Ni was under detection limits in all the samples (LOD=0.54). LOD, limit of detection.

	LOD	RAINY SEASON		DRY SEASON	
		Estero La Virgen	Isla Machuca	Estero La Virgen	Isla Machuca
Ag	0.0015	0.01	0.05	0.03	0.01
Al	15.3	78.97	188.91	105.67	136.86
As	0.01	9.97	6.42	12.72	7.34
Cd	0	2.94	3.11	13.17	0.52
Cr	0.22	0.78	0.65	1.38	0.57
Cu	0.11	3.34	3.74	3.52	5.22
Hg	0.004	0.09	0.07	0	0
Pb	0.015	0.05	0.06	0	0
Ti	0.94	4.22	9.91	5.74	7.89
V	0	0.84	0.72	0.79	0.61
Zn	2.36	63.77	53.37	68.38	95.91
∑Metals		**165.22**	**267.24**	**211.72**	**255.05**

Table 7. PAH tissue concentration in *A. tuberculosa* (ng/g) from Pacific mangrove swamps. The superscript lyrics (a, b and c) indicate significant differences between groups ($p<0.05$; $p<0.1$, when shown between brackets). The following compounds were under detection limits in all the samples (superscript numbers refer to the sums and indices given in the table where they are integrated): Acy (LOD=1)[(1)]; Ace (LOD=1)[(1)]; Ant (LOD=1); Benz[a]A (LOD=1)[(2)]; Chr (LOD=1)[(2)]; B[b]F+B[k]F (LOD=1)[(2)]; B[a]P (LOD=1)[(2)]; B[ghi]P (LOD=1); Ind (LOD=2)[(2)]; D[ah]A (LOD=2)[(2)]. LOD, limit of detection; udl, under detection limits; n/a, not applicable.

	LOD	RAINY SEASON		DRY SEASON	
		Estero La Virgen	Isla Machuca	Estero La Virgen	Isla Machuca
Flu [(1)]	0.07	3	1	udl	udl
Phe [(1)]	0.49	60	66	udl	udl
Flr [(1)]	0.34	25	udl	udl	udl
Pyr [(1)]	1	3	4	udl	udl
∑PAHs (16; except Naph)		**91**	**70**	**0**	**0**
$\sum_{HMW}$PAHs Σ [(2)] (carcinogenic)		**0**	**0**	**0**	**0**
$\sum_{LMW}$PAHs Σ [(1)] (%∑PAHs)		**91 (100%)**	**70 (100%)**	**0**	**0**
$\sum_{LMW}$PAHs Σ [(1)] (%∑PAHs)		**n/a**	**n/a**	**n/a**	**n/a**
$\sum_{LMW}$PAHs / $\sum_{HMW}$PAHs		**n/a**	**n/a**	**n/a**	**n/a**
Ind/(Ind+B[ghi]P		**n/a**	**n/a**	**n/a**	**n/a**

Table 8. POP tissue concentration in *Anadara tuberculosa* (ng/g) from Pacific mangrove swamps. The superscript lyrics (a, b and c) indicate significant differences between groups ($p<0.05$; $p<0.1$, when shown between brackets). The following compounds were under detection limits in all the samples (superscript numbers refer to the sums and indices given in the table where they are integrated): α-HCH (LOD=50)[3]; β-HCH (LOD=25)[3]; δ-HCH (LOD=25)[3]; 2.4-DDD (LOD=5)[4]; 2.4-DDT (LOD=25)[4]; 4.4-DDT (LOD=50)[4]; Chlorpyrifos (LOD=2); HHCB (LOD=9)[5]; CB28 (LOD=1)[6]; CB52 (LOD=1)[6]; CB101 (LOD=1)[6]; CB118 (LOD=1)[6]; CB138 (LOD=1)[6]; CB153 (LOD=1)[6]; CB180 (LOD=1)[6]; BDE28 (LOD=7.2)[7]; BDE47 (LOD=1.3)[7]; BDE66 (LOD=0.94)[7]; BDE99 (LOD=6.6)[7]; BDE153 (LOD=50)[7].LOD, limit of detection; udl, under detection limits; n/a, not applicable.

	LOD	RAINY SEASON		DRYSEASON	
		Estero La Virgen	Isla Machuca	Estero La Virgen	Isla Machuca
γ-HCH [3]	2	76	7	25	udl
∑HCHs$^{\Sigma}$ [3]		**76**	**7**	**25**	**0**
4.4-DDE [4]	2	28	udl	udl	46
∑DDTs $^{\Sigma}$ [4]		**28**	**0**	**0**	**46**
4.4-DDT/4.4-DDE		**0**	**n/a**	**n/a**	**0**
2.4-DDD/4.4-DDE		**0**	**n/a**	**n/a**	**0**
(2.4-DDD+4.4-DDE)/ ∑DDTs		**1.00**	**n/a**	**n/a**	**1.00**
∑OCPs		**104**	**7**	**25**	**46**
AHTN [5]	10	59	12	udl	udl
∑Musks$^{\Sigma}$ [5]		**59**	**12**	**0**	**0**
∑PCBs∑ [6] (PCB_7)		**0**	**0**	**0**	**0**
BDE85[7]	1	49	23	87	170
BDE100[7]	1	udl	14	udl	udl
BDE154[7]	1	udl	272	udl	udl
∑PBDEs$^{\Sigma}$ [7]		**49**	**309**	**87**	**170**
∑POPs		**212**	**328**	**112**	**216**

Health condition. Overall, L, FDW, SDW, FDW/SDW, SDW/L and FDW/L values showed to be statistically higher in the dry season than in the rainy season in pustulose arks (Figs. 10a-c and Table 9). Low values were observed in Estero La Virgen in the rainy season, except for L (Figs. 10a-c and Table 9). However, in the dry season no significant differences were observed between localities. Sex ratio values for each sampling location at each sampling time are shown in Table 9. Statistically significant bias in the sex ratio towards female condition (F:M, 4.9:1; G value=42.64, $p<0.05$) was found for the whole set of studied pustulose arks in comparison with the theoretical sex ratio of 1:1 (Gener et al., 2009, Silva-Benavides and Bonilla, 2015); this overall sex ratio results from the average between 5.3:1 (rainy season) and 4.3:1 (dry season) sex

ratios. This bias towards female condition was found in at both seasons (Table 9). Undifferentiated gonad was only observed in Estero La Virgen, with a 5.2% of prevalence in the rainy season, and in Isla Machuca, with a 5.9% in the dry season (Table 9). Intersex cases were sporadically found: one female from Estero La Virgen in the rainy season, and one male and female from Estero La Virgen and one female from Isla Machuca in the dry season (Table 9); always with separate male and female gonad follicles (Fig. 11e). Gamete development was more advanced in the rainy season than in the dry season, and in Estero La Virgen than in Isla Machuca in both seasons (Figs. 11a-d and 12); however, significant differences not were observed in GI between localities (Table 9). Oocyte atresia was recorded all along the study period, mainly in Isla Machuca (Fig. 11f and Table 9). RAI reflected only a low-to-moderate (<18) reproductive disturbance in both localities and seasons, mainly featured by deviations in the expected SRI, GMI and OAI values (Fig. 13a). No parasite was observed in the gonad during the study period.

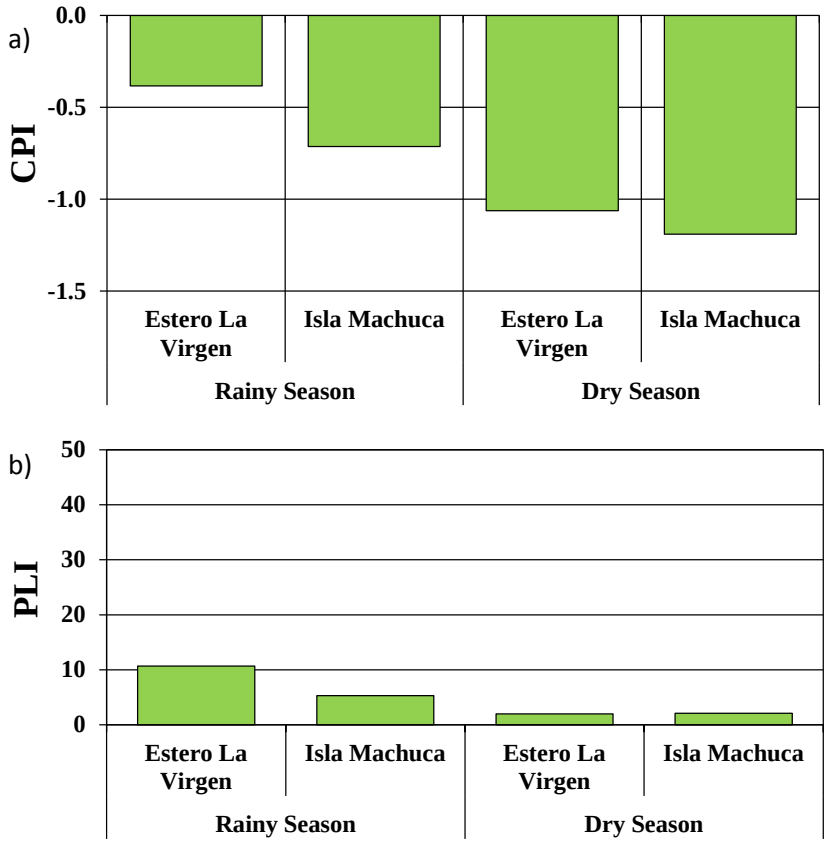


Fig. 9. (a) Chemical Pollution Index (CPI) in *A. tuberculosa* from Pacific mangrove swamps. CPI is categorized into three groups: 'low pollution' when CPI ≤ 0, 'moderate pollution' when 0 < CPI ≤ 1 and 'high pollution' when CPI > 1 (according to Bellas et al., 2011; 2014). (b) Pollution Load Index (PLI) derived from the tissue concentrations of pollutants (Tomlinson et al., 1980). A PLI of 100 correspond to very contaminated sites and a PLI > 50 requires a potential risk and moderate contamination (Angulo 1996).

Table 9. Biometry and histopathology of *A. tuberculosa* from Pacific mangrove swamps. Shell length, flesh dry weight and shell dry weight their values are mean ± standard error (n = 20). Sex Ratio Index = SRI G value. The superscript letters (a, b and c) indicate significant differences between groups ($p<0.05$). Asterisks (*) indicate seasonal difference for a locality ($p<0.05$); ♯, significantly different from the theoretical gender (Gener et al., 2009; Silva-Benavides and Bonilla, 2015) bias (1:1) according to the G test ($p<0.05$).

	RAINY SEASON		DRY SEASON	
	Estero La Virgen	Isla Machuca	Estero La Virgen	Isla Machuca
Shell Length (L; mm)	49.1±3.0	49.1±6.7	51.3±3.0*	50.7±6.7*
Flesh dry-wt (FDW; mg)	0.9±0.4[a]	1.3±0.5[b]	1.3±0.3*	1.3±0.3
Shell dry-wt (SDW; g)	18.2±4.2	20±6.9	21.7±5.2*	21.2±7.0
Sex ratio [1] (Sex Ratio Index)	6:1[♯] (19.8)	4.5:1[♯] (9.7)	7.5:1[♯]*(11.2)	2.8:1* (3.4)
Intersex Index	2.6	0	10.5	5.9
Gonad Index	3.3±1.2	3.7±0.8	3.7±1.6*	3.8±1.3
Gametogenic Mass Index	0.36	0.80	0.73	0.05
Undifferentiated Index	5.2	0	0	5.9
Oocyte Atresia Prevalence	26.7	55.6	73.3	63.6
Oocyte Atresia Intensity	0.5	1.4	1.1	1.2
Oocyte Atresia Index	12.4	80.2	83.1	86.8
Prevalence (oedema) %	10.7	13	0.7	10
Prevalence (BCA) %	93	63	100	80
Prevalence (granulocytomas) %	0	0	0	5
Preval. (dissem. neoplasia) %	0	0	0	0
Preval. (*Nematopsis* sp) %	53.3	27.2	39	85
Preval. (*Bucephalus* sp) %	0	3.1	1.1	0
Preval. (Metacercariae) %	0	3.1	0	5
Prevalence (Copepods) %	3.3	0	0	5

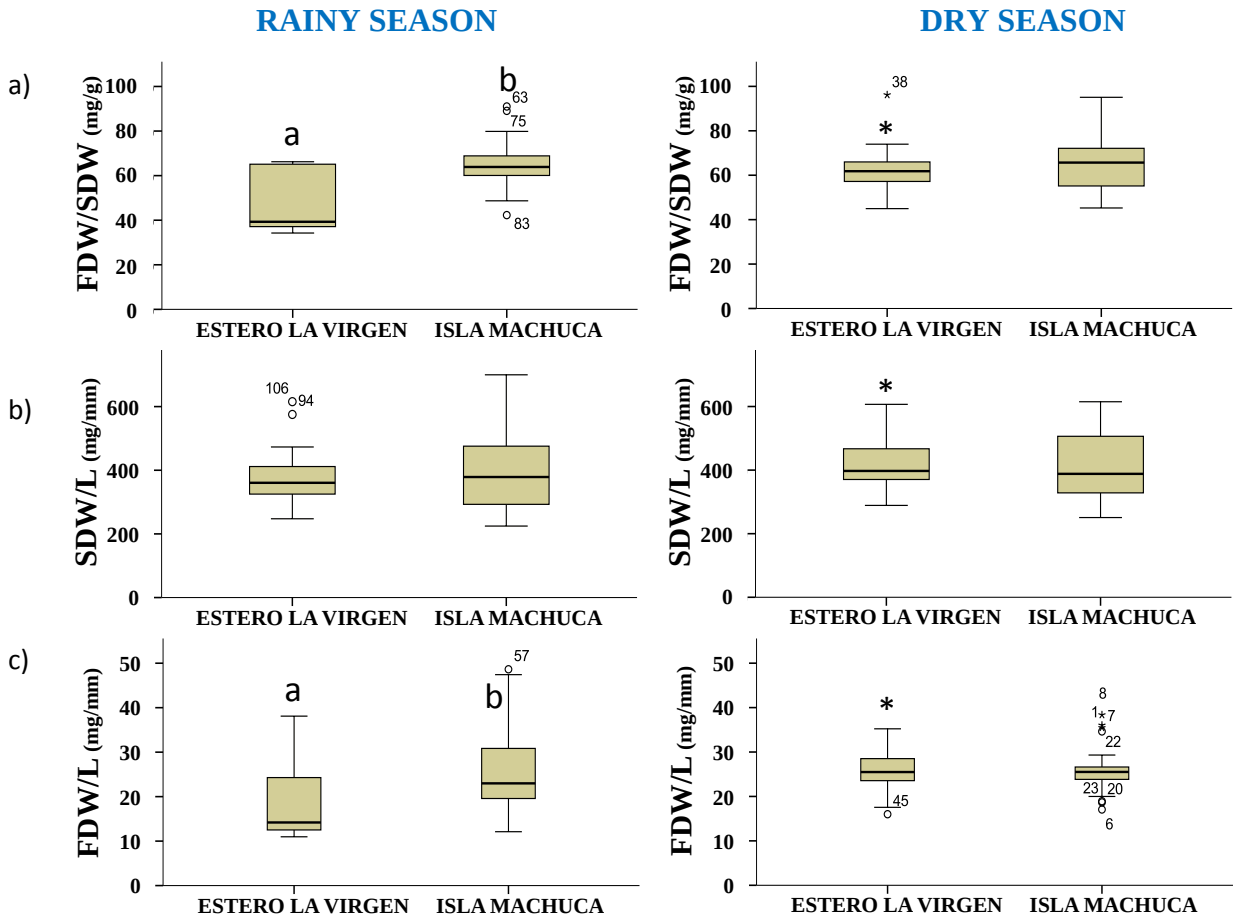


Fig. 10. Biometry and condition of *A. tuberculosa* from Pacific mangrove swamps during the rainy and the dry seasons. Different letters denote statistically significant differences between localities and asterisks (*) indicate significant differences between seasons for a given locality (p<0.05). SDW/L, shell growth index; FDW/SDW and FDW/L, condition indices.

Histopathological examination of the digestive gland revealed the presence of a variety of parasites such as *Nematopsis* sp., *Bucephalus* sp., metacercariae and copepods (Table 9). *Nematopsis* sp. was the parasite more commonly recorded, and with the highest prevalence in all the study period. *Bucephalus* sp., metacercariae and copepods had a local and seasonal impact (Figs. 14e and f and Table 9). Seemingly, parasite prevalence was low in both localities and seasons, except for *Nematopsis* sp. (Table 9). Overall, PII was higher in dry season than in rainy season (Fig. 13b and Table 9), which can be mainly attributed to the incidence of *Nematopsis* sp. Except for the case of *Nematopsis* sp. (intensity<70), the intensity of parasitic infestations was generally low (intensity<2). Inflammatory responses such as oedema, granulocytomas and brown cell aggregates were observed (Figs. 14a-c and Table 9): (a) prevalence of brown cell aggregates was slightly higher in Estero La Virgen than in Isla Machuca; (b) oedema was more relevant in the dry season than in the rainy season; and (c) only one case of granulocytoma was found in Isla Machuca in the dry season. Consequently, IRI was apparently the highest in the dry season (Fig.

13c). DGAI was highly variable amongst individuals and therefore no significant differences were found between seasons and between localities; however, some seasonal trends might be envisaged (Figs. 13 d and 14a-d). Digestive alveoli presented the lowest atrophy and DGAI with a lumen nearly occluded and a high epithelium frequently in the dry season (Table 9, Fig. 13d and Fig. 14a-c).

LT_{50} values tended to be higher in the rainy season than in the dry season; nevertheless, statistical differences were only observed between seasons in Isla Machuca (Fig. 13e).

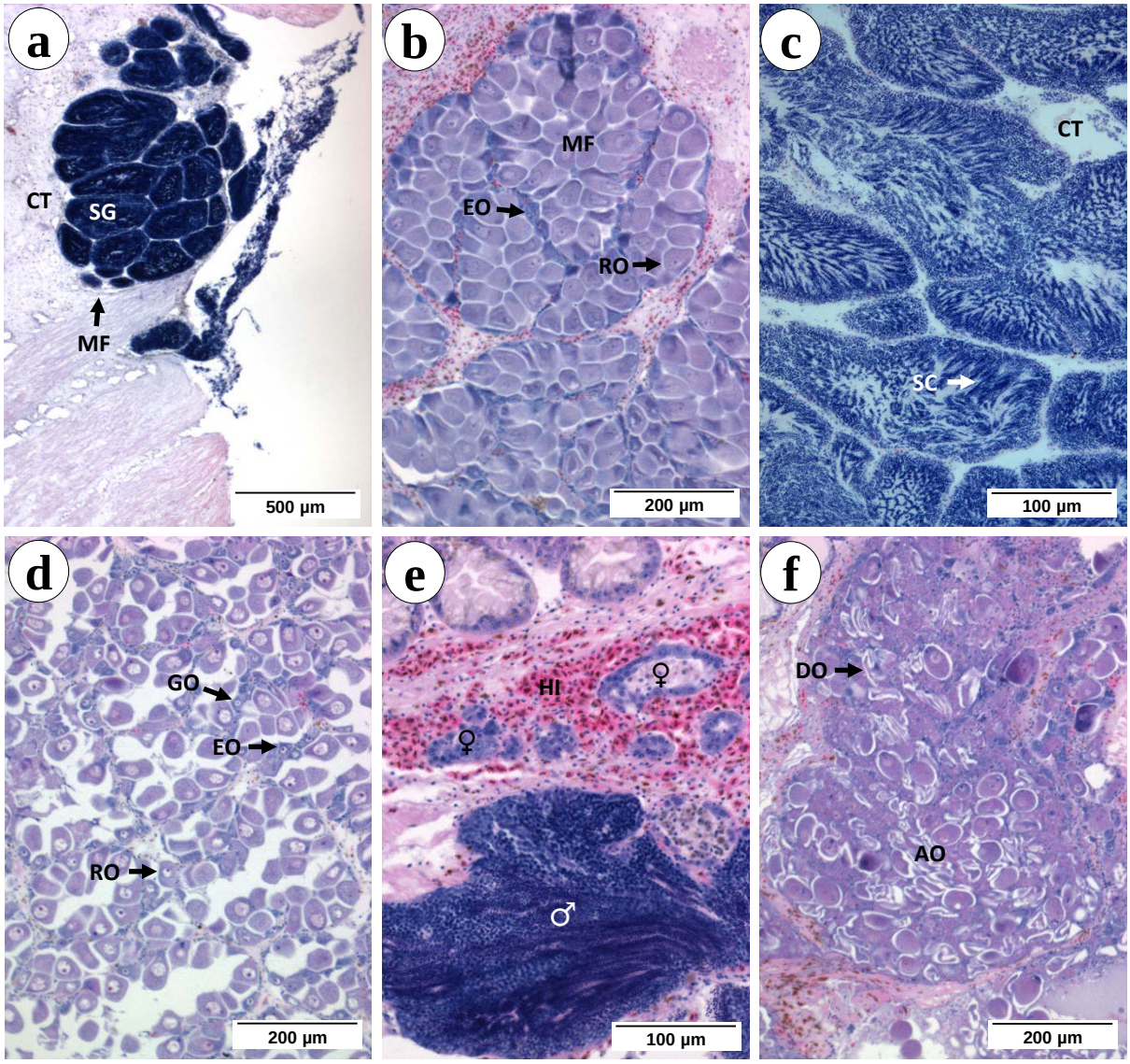


Fig. 11. Light micrographs of gonad sections and intersex individuals of *A. tuberculosa*. a) normal and ripe gonad of a male; b) normal and ripe gonad of a female; c) spawning gonad of a male; d) spawning gonad of a female; e) male from Estero La Virgen with intersex; f) female from Isla Machuca with atretic oocytes. Abbreviations: AO, atretic oocytes; EO, early oocyte; GO, growing oocyte; DO, degenerated ovum; HI, haemocyte infiltration; RO, ripe ovum; SG, spermatogonia; SC, spermatocytes; CT, connective tissue; MF, mature follicles; ♀, female germ cells; ♂, male germ cells. Arrows, change or tissue alterations.

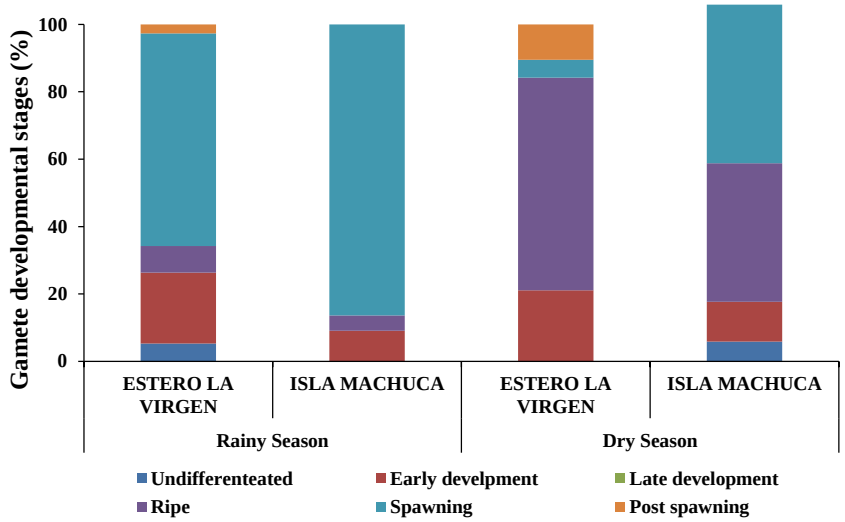


Fig. 12. Gamete development stages of pustulose ark, *Anadara tuberculosa* collected from Pacific mangrove swamps.

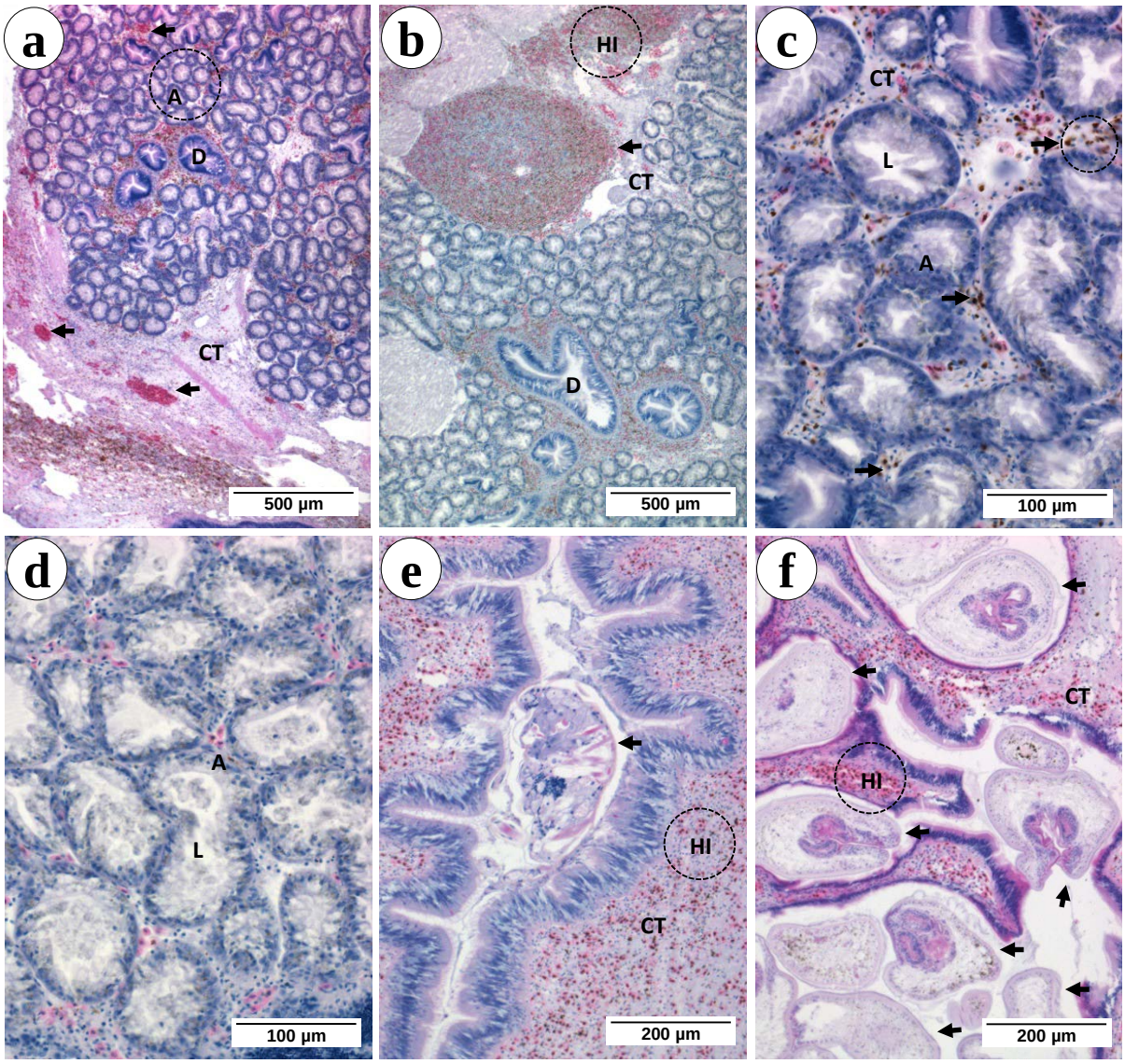


Fig.14. Paraffin sections of the digestive gland of *A. tuberculosa* stained with haematoxylin-eosin: a) normal digestive gland with diffuse infiltration haemocyte b) granulocytoma and diffuse infiltration haemocytic; c) brown cell aggregate in connective tissue; d) atrophied alveoli with a wide lumen; e) copepod in to stomage ; f) trematode in duct of digestive gland. Abbreviations: A, alveoli; D, duct; CT, connective tissue; HI, haemocytic infiltration; L, lumen. Arrows, tissue alterations.

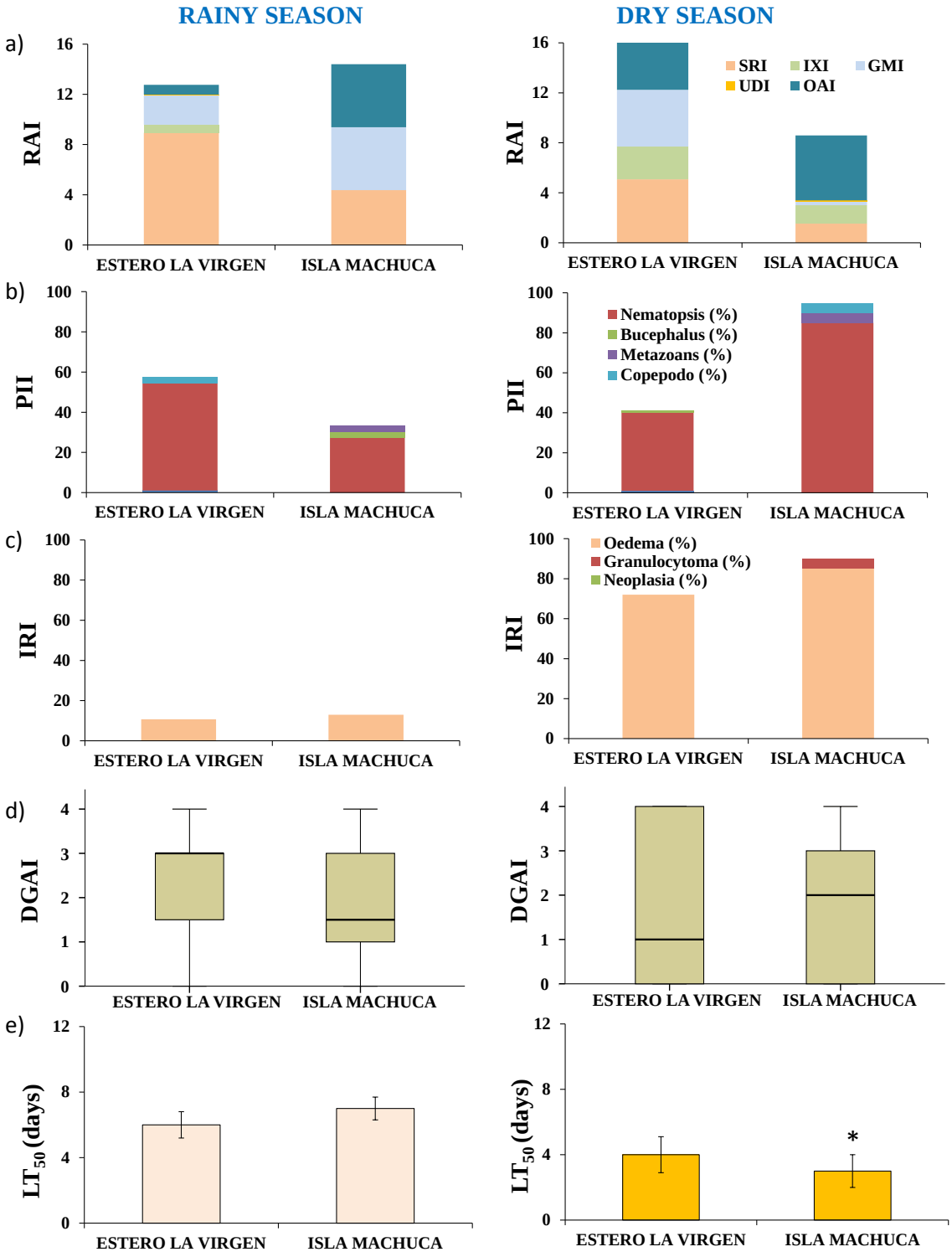


Fig. 13. Biomarkers of anomalies in cockle health indicative of disturbance in ecosystem health for the rainy and the dry seasons in the Pacific mangrove swamps. Asterisk (*) indicate seasonal differences for a locality (p<0.05). RAI, reproductive anomalies index; PII, parasitic infestation index; IRI, inflammatory response index; DGAI, digestive gland atrophy index and LT_{50}, median survival time after SOS test.

Table 10. Metal tissue concentration in *Larkinia grandis* (µg/g) from Pacific mangrove mudflat. The nickel compounds was under detection limits in all the samples (LOD=0.54). LOD, limit of detection.

	LOD	RAINY SEASON	DRY SEASON
		Estero Venecia	Estero Venecia
Ag	0.0015	0.03	0.03
Al	15.3	295.25	535.72
As	0.01	10.48	15.35
Cd	0	3.14	14.22
Cr	0.22	3.00	1.20
Cu	0.11	3.85	4.00
Hg	0.004	0.08	0.10
Pb	0.015	7.87	0.19
Ti	0.94	15.13	27.30
V	0	0.90	1.37
Zn	2.36	43.98	52.55
∑Metals		**383.71**	**652.02**

Table 11. PAH tissue concentration in *Larkinia grandis* (ng/g) from Pacific mangrove mudflat. The following compounds were under detection limits in all the samples (superscript numbers refer to the sums and indices given in the table where they are integrated): Acy (LOD=1)[(1)]; Ace (LOD=1)[(1)]; Ant (LOD=1); Benz[a]A (LOD=1)[(2)]; B[b]F+B[k]F (LOD=1)[(2)]; B[ghi]P (LOD=1); Ind (LOD=2)[(2)]; D[ah]A (LOD=2)[(2)]. LOD, limit of detection; udl, under detection limits; n/a, not applicable.

	LOD	RAINY SEASON	DRY SEASON
		Estero Venecia	Estero Venecia
Flu [(1)]	0.07	14	udl
Phe [(1)]	0.49	74	udl
Flr [(1)]	0.34	37	udl
Pyr [(1)]	1	8	udl
Chr [(2)]	1	4	udl
B[a]P [(2)]	1	2	7
∑PAHs (16; except Naph)		**138**	**7**
$\sum_{HMW}$PAHs $^{\Sigma(2)}$ (carcinogenic)		**4**	**7**
$\sum_{LMW}$PAHs $^{\Sigma(1)}$ (%∑PAHs)		**96 (70%)**	**0 (0%)**
$\sum_{LMW}$PAHs / $\sum_{HMW}$PAHs		24.8	n/a
$\sum_{LMW}$PAHs $^{\Sigma(1)}$ (%∑PAHs)		**n/a**	**n/a**
$\sum_{LMW}$PAHs / $\sum_{HMW}$PAHs		**n/a**	**n/a**
Ind/(Ind+B[ghi]P		**n/a**	**n/a**

3.3. *Larkinia grandis*

Tissue concentrations of contaminants. Metal tissue concentrations were the highest in the dry season, especially for As, Cd and Ti (Table 10). PAH tissue concentration was low and practically restrained to the rainy season; the most relevant PAHs found being phenanthrene and fluoranthene (Table 11). POP tissue levels were low and varied between seasons, with higher tissue concentrations of γ-HCHs and AHTN in the rainy season than in the dry season and higher tissue concentrations of two PBDEs (especially BDE85 but also BDE100) in the dry season than in the rainy season (Table 12). Overall, CPI and PLI were low in both seasons. CPI values were 0.02 in the rainy season and -0.84 in the dry season. PLI values were 11.49 in the rainy season and 4.03 in the dry season. The main pollutants contributing to these CPI and PLI values were Cd, Phe, HCHs, AHTN and BDE85 (SM 1 and 2).

Table 12. POP tissue concentration in *Larkinia grandis* (ng/g) from Pacific mangrove mudflats. The following compounds were under detection limits in all the samples (superscript numbers refer to the sums and indices given in the table where they are integrated): α-HCH (LOD=50)[3]; β-HCH (LOD=25)[3]; δ-HCH (LOD=25)[3]; 4.4-DDE (LOD=5)[4];2.4-DDD (LOD=5)[4]; 2.4-DDT (LOD=25)[4]; 4.4-DDT (LOD=50)[4]; Chlorpyrifos (LOD=2); HHCB (LOD=9)[5]; CB28 (LOD=1)[6]; CB52 (LOD=1)[6]; CB101 (LOD=1)[6]; CB118 (LOD=1)[6]; CB138 (LOD=1)[6]; CB153 (LOD=1)[6]; CB180 (LOD=1)[6]; BDE28 (LOD=7.2)[7]; BDE47 (LOD=1.3)[7]; BDE66 (LOD=0.94)[7]; BDE99 (LOD=6.6)[7]; BDE153 (LOD=50)[7]; BDE154 (LOD=50)[7].LOD, limit of detection; udl, under detection limits; n/a, not applicable.

	LOD	RAINY SEASON	DRY SEASON
		Estero Venecia	Estero Venecia
γ-HCH[3]	2	23	udl
∑HCHs[3]		**23**	**0**
∑DDTs[4]		**0**	**0**
4.4-DDT/4.4-DDE		**n/a**	**n/a**
2.4-DDD/4.4-DDE		**n/a**	**n/a**
(2.4-DDD+4.4-DDE)/ ∑DDTs		**n/a**	**n/a**
∑OCPs		**23**	**0**
AHTN [5]	10	75	udl
∑Musks$^{\Sigma}$ [5]		**75**	**0**
∑PCBs∑ [6] (PCB_7)		**0**	**0**
BDE85[7]	1	19	170
BDE100[7]	8	udl	10
∑PBDEs$^{\Sigma}$ [7]		**19**	**180**
∑POPs		**117**	**180**

Health condition. L, FDW, SDW and SDW/L values were statistically higher in the rainy season than in the dry season; FDW/SDW and FDW/L showed the same trend but this was not significant (Table 13). Sex ratio values for each sampling time are shown in Table 13. The studied set of casco de burro ark shell clams did not deviate from the theoretical sex ratio (after Galdámez et al., 2007), showing a F:M value of 1.2:1 (G value=0.33, $p>0.05$; Table 13). Undifferentiated gonad was not observed in any season (Table 13). Only one female with intersex was recorded in the dry season (Table 13). The gamete development was more advanced in the rainy season than in the dry season, though spawning was always the most prevalent gamete development stage (Figs. 15, 16d and e). Significant differences not were observed in GI between seasons (Table 13). Oocyte atresia was recorded in both seasons (Table 13). RAI reflected only a low-to-moderate (<13) reproductive disturbance in both seasons, mainly featured by the OAI values (Table 13). No parasite was observed in gonad.

Histopathological examination in the digestive gland revealed the presence of a variety of parasites including undetermined intracellular protists, *Nematopsis* sp. and metacercariae (Table 13). *Nematopsis* sp. was the parasite with the highest prevalence, followed by metacercariae and undetermined intracellular protists (Table 13). The prevalence of parasitic infestations was always low (Table 13); likewise, the intensity was as well (intensity<7). The histological integrity of the digestive gland tissue seemingly medium (Figs. 16a-c). Inflammatory responses such as oedema and brown cell aggregates were observed (Figs. 16b and c; Table 13). IRI was the highest in the dry season because the highest prevalence of oedema was found at this season (Table 13). Digestive alveoli presented a large lumen and a thin epithelium in both seasons, which revealed a remarkable atrophy (high DGAI) (Figs. 16b and c; Table 13). LT_{50} values were higher in the rainy season than in the dry season (Table 13).

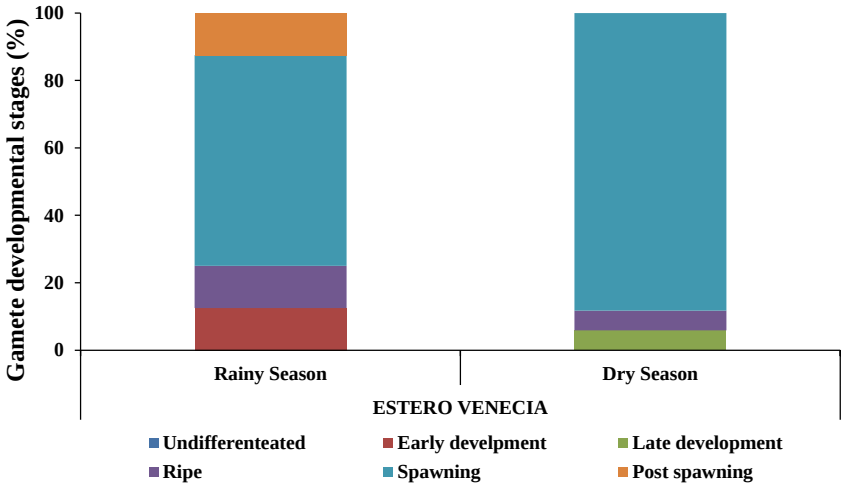


Fig. 15. Gamete development stages of *casco de burro* ark shell clams, *Larkinia grandis* collected from Pacfic mangrove mudflat.

Table 13. Biometry and histopathology of *Larkinia grandis* from Pacific mangrove mudflats. Shell length, flesh dry weight and shell dry weight their values are mean ± standard error (n = 20). Sex Ratio Index = SRI G value. Asterisks (*) indicate seasonal (p<0.05); ♯, significantly different from the theoretical gender (Galdámez et al., 2007) bias (1:1) according to the G test (p<0.05). [1], estimated based on model functions.

	RAINY SEASON	DRY SEASON
	Estero Venecia	Estero Venecia
Shell Length (L; mm)	56.9±6.5	51.3±3.7*
Flesh dry-wt (FDW; mg)	2±1	1.2±0.3*
Shell dry-wt (SDW; g)	78.9±26.1	53.3±13*
FDW/SDW (mg/g)	29.6±4.24	22.9±0.72
SDW/L (mg/mm)	1367.9±92.32	1031.8±36.52*
FDW/L (mg/mm)	3.6±0.44	2.3±0.08
Sex ratio (Sex Ratio Index)	1.5:1 (1.2)	1:1.1 (0.05)
Intersex Index	0	5.9
Reproductive Anomalous Index (RAI)	12.3	10.8
Gonad Index	4±0	3.3±1.3
Gametogenic Mass Index	0.47	0.05
Undifferentiated Index	0	0
Oocyte Atresia Prevalence	0.67	0.75
Oocyte Atresia Intensity	1.9	2.2
Oocyte Atresia Index	1.4	1.44
Prevalence (oedema) %	4	71
Prevalence (BCA) %	96	89
Prevalence (granulocytomas) %	0	0
Preval. (dissem. neoplasia) %	0	0
Inflammatory Response Index (IRI)	4	71
Prevalence (ciliates) %	0	0
Preval. (Undert. Intra. Prot.) %	0	5.5
Prevalence (Total Intr. Prot.) %	0	5.5
Preval. (*Nematopsis* sp) % [(1)]	18.5	0
Prevalence (Metacercariae) %	4	11
Prevalence (R/CLO) %	0	0
Parasitic Infestation Index (PII)	22.5	16.5
DGAI	2.8±1	3.1±1.1
LT^{50}	6±0.9	4±1.1*

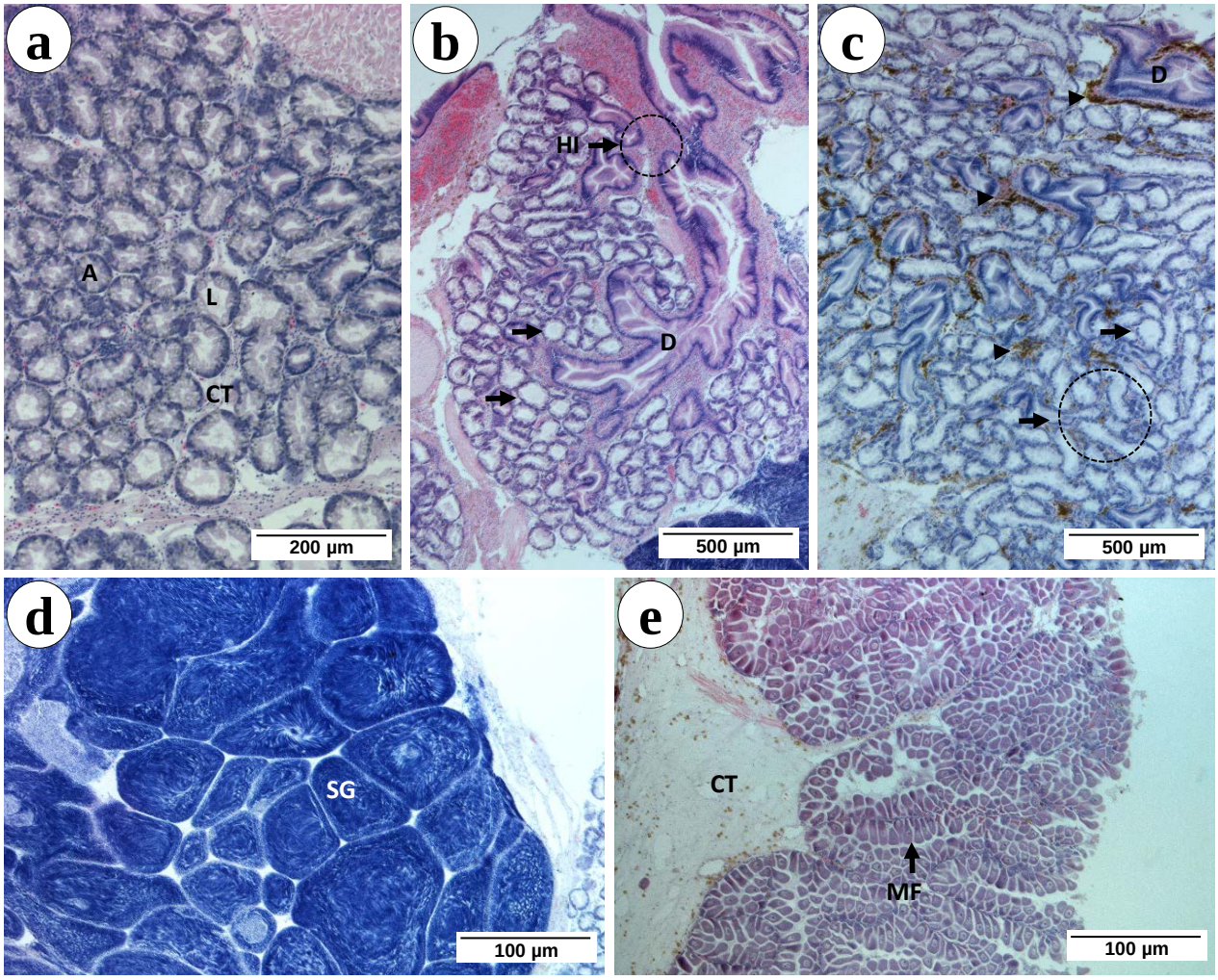


Fig. 16. Paraffin sections of *Larkinia grandis* stained with haematoxylin-eosin: a) normal digestive gland b) digestive gland with diffuse infiltration haemocyte and atrophied alveoli; c) brown cell aggregate and atrophied alveoli; d) gill with diffuse infiltration haemocyte; e) mature gamete of male; f) mature gamete of female. Abbreviations: A, alveoli; D, duct; CT, connective tissue; HI, haemocytic infiltration; L, lumen; MF, mature follicles. Arrows, tissue alterations; arrowheads indicate the presence of brown cell aggregates.

4. Discussion

4.1. Caribbean Coast

Metals. In the Caribbean coast from Nicaragua, metal tissue concentrations in *P. arctata* were low except for As and Hg, according to proposed criteria for oysters and mussels (Kimbrough et al., 2008; Solaun et al., 2013) and existing data in neighbouring regions (Valette-Silver et al., 1999). The sources of As can be discharges of untreated municipal wastes and sediments contaminated by mining activities, industrial processes or the application of pesticides (Smedley and Kinniburg, 2002). The presence of Hg can be attributed to artisanal gold mining

carried out along the Escondido River, which opens into the Bluefields Lagoon (Dumailo, 2003; MARENA, 2010; Cordy et al., 2011). The tissue concentration of Ag, As, Cd, Hg, Ni and V was higher during the dry season than during the rainy season in Punta San Gabriel; and higher for Ag and V but lower for Ni and Pb in Punta San Gabriel in comparison with Pigeon Cay in the rainy season. These findings are consistent because Punta San Gabriel is located at the sediment deposition zone of Bluefields Lagoon, whilst Pigeon Cay is along the outflow of the principal channel in the Pearl Lagoon. In the rainy season in Pigeon Cay, the results were to a large extent comparable to those obtained with sympatric mangrove cupped oysters, in which the metal tissue concentrations were overall low as well (Chapter 1). Nevertheless, *P. arctata* accumulated a little bit less Ag, As, Cd, Ti and V and a little bit more Ni and Pb than *C. rhizophorae*, with equitable tissue levels of Cr and Hg. Doubtless, the most striking difference was the much lower tissue levels of Cu and Zn recorded in *P. arctata* in comparison with *C. rhizophorae*; which is fully justified because unlike other molluscs, oysters (and especially those of the genus *Crassostrea*) are known to be hyperaccumulators of these two metals (Tan et al., 2015). On the other hand, few data are available regarding metal accumulation in slender march clams. The tissue concentrations of Cd, Cu, Pb and V in *P. arctata* from Lake Maracaibo and El Tablazo Bay (Venezuela) were similar to those reported in the present study, whilst those of As and Zn were lower (Sarria-Panasá et al., 2016). In contrast, the tissue concentration of Cd, Pb, Zn and Ni recorded in the rainy and dry seasons in slender marsh clams from Moin River (Costa Rica) and Lake Maracaibo (Venezuela) were higher than those recorded herein (Leal et al., 2014; Vargas et al., 2015). Likewise, the tissue concentrations of Cr, Ni and Pb recorded in *P. arctata* from the "Pantanos de Centla" Reserve of the Biosphere (Tabasco, México) in the dry season were higher than in the present study (Pérez-Cruz et al., 2013). In any case, it seems that metal pollution is not a matter of major concern in these lagoons of the Nicaraguan Caribbean coast.

PAHs. The tissue concentrations of PAHs in *P. arctata* were generally low although they were slightly higher in Punta San Gabriel in the rainy season than in any other case. Similarly, in *C. rhizophorae* from the Pigeon Cay the ∑PAHs values recorded were also low, always according to Kimbrough et al., (2008) who concluded that in *C. virginica,* values of ∑PAHs in the range of 47-828 ng/g must be considered low. Few data are available on the tissue levels of PAHs and related contaminants in *Polymesoda*. For example, total petroleum hydrocarbons were determined in mud clams (*Polymedosa erosa*) from the coastal area of Sabah (Malaysia; Mohd Ali and Yep, 2016). Likewise, mangrove clam *Polymesoda erosa* was found to present

significant tissue concentrations of alkylated and parent PAHs in the mangrove-fringed Segara Anakan Lagoon (Java, Indonesia; Dsikowitzky et al., 2011). In the present study, the most relevant PAHs found in *P. arctata* were phenanthrene, fluoranthene, pyrene, and benzo[b]fluoranthene+benzo[k]fluoranthene in Punta San Gabriel and chrysene in Pigeon Cay, in the rainy season. Likewise, phenanthrene and pyrene where the most relevant PAHs found in sympatric *C. rhizophorae*, though this was observed in the dry season instead (Chapter 1). As a whole, carcinogenic PAHs (PAH_{HMW}; IARC, 1987) were relevant in *P. arctata* (present results) and *C. rhizophorae* (Chapter 1) from Pigeon Cay in the rainy season. A chemical indicator of the petrogenic *vs.* pyrolytic origin of PAHs was calculated based on the $_{LMW}PAHs/_{HMW}PAHs$ ratio (Baumard et al., 1998; Soclo et al., 2000). A $_{LMW}PAHs/_{HMW}PAHs$ ratio of 43 (>>1) was found in slender march clams from Punta San Gabriel, indicating a predominant petrogenic origin of the PAHs, whilst a $_{LMW}PAHs/_{HMW}PAHs$ ratio of 0.34 was found in Pigeon Cay slender marsh clams, revealing a pyrolytic origin of the PAHs in this locality; in agreement with the results obtained on the basis of PAH tissue concentrations recorded in sympatric mangrove cupped oysters from Pigeon Cay ($_{LMW}PAHs/_{HMW}PAHs_{oysters}$=0.58; Chapter 1).

POPs. To our knowledge few reports deal with tissue concentrations of POPs in *Polymesoda* clams. Bayen et al., (2005) carried out an exhaustive analysis of POPs in a variety of mangrove species in Singapore that included the Lokan clams, *Polymesoda expansa*, in which they reported significant tissue concentrations of HCHs, PBDEs (mainly PBDE47 and PBDE100) and most relevantly a dominance of DDTs and certain PCBs (tetra-, penta-, hexa-, and heptachlorinated). In our study, HCHs, DDTs, musk fragrances, PCBs (CB28 and CB118) and PBDEs (BDE85 and BDE100) were found at measurable concentrations in the soft tissues of *P. arctata*. The highest tissue concentrations of POPs were recorded in the rainy season: DDTs, musk fragrances and PCBs in Punta San Gabriel; and HCHs in Pigeon Cay. In agreement with previous results obtained with *C. rhizophorae* from the same locality (Chapter 1), high levels of HCHs and DDTs were recorded in the tissues of slender march clams from Pigeon Cay; however, whilst α-HCH and 4,4,-DDE were dominant in the case of the clams, γ-HCH and 2,4-DDT were more relevant in the case of sympatric oysters. It is conceivable that these two species of bivalves may represent either different environmental compartments or different metabolic strategies. Interestingly, γ-HCH and 4,4-DDT tissue levels in *P. arctata* are higher in Punta San Gabriel than in Pigeon Cay during the rainy season. In any case, HCHs and DDTs are at detectable levels both in *P. arctata* and *C. rhizophorae*, which would clearly indicate the

presence of these POPs in these mangrove-lined coastal systems, most likely associated to agricultural and insect control practices (de Brito et al., 2002; Bodin et al., 2011). Indeed, Punta San Gabriel is the main sink area in Bluefields Lagoon, which receives water load from the Escondido River basin where intensive agriculture has been promoted for the last decades (GEF-REPCar, 2011). In agreement, high concentrations of HCHs and DDTs have been reported in water and sediments in other Nicaraguan Caribbean localities, where these chemicals may constitute a serious environmental hazard (GEF-REPCar, 2011; Ebanks-Mongalo et al., 2013). Detectable tissue levels of chlorpyrifos were determined in the tissues of *P. arctata* from Pigeon Cay, exactly as it had been previously determined in sympatric *C. rhizophorae* (Chapter 1). Chlorpyrifos is a broad spectrum organophosphate insecticide widely used on food crops that has been reported in the tissues of oysters and mussels in the range of 0-53 ng/g dry-wt, with a regional average for the USA coastline of 0.78 ng/g dry-wt (Wade et al., 1998). In Nicaraguan mangrove cupped oysters was reported at a tissue concentration of in the range of 5-11 ng/g dry-wt (Chapter 1; Ebanks-Mongalo et al., 2013), similar to that recorded herein in slender march clams. Although musk fragrances were not detected in mangrove cupped oysters from Nicaraguan mangrove-lined Caribbean coastal systems, in the present study AHTN was revealed in the tissues of slender marsh clams from Punta San Gabriel at concentrations higher than those reported in *C. rhizophorae* from Colombia (Chapter 1), but still lower than those reported in mussels and clams from industrialised areas of Canada (Gatermann et al., 1999). The source of this contaminant would be wastewater discharges from nearby sewage outfalls, since musk fragrances are known to be discharged into estuaries through sewage (Wade et al., 1998). PCBs congeners are non- or poorly metabolised in molluscs and therefore they are bioaccumulated (Kannan et al., 1995; Nhan et al., 2001). ∑PCBs reported in bivalves from Nicaragua within the framework of the International Mussel Watch were <10 ng/g dry-wt (Sericano et al., 1995). BDE85 showed markedly high values in the rainy season in Pigeon Cay, both in slender march clams and mangrove cupped oysters, with values of total PBDEs in the range of 227-325 ng/g dry-wt. These values are much higher than previously reported in bivalves (Moon et al., 2007; Oros et al., 2007) and cannot be associated with a particular source at local scale.

Biological effects assessment. Only minor and less-consistent differences were recorded between localities in biometry and flesh condition of slender march clams, although a higher flesh condition seemed to be envisaged in the rainy season. A similar seasonal trend was reported in *C. rhizophorae* from our study area (Chapter 2) and, as a general rule, seasonal

changes in flesh condition appear to be normal in mangrove bivalves (Nascimento and Pereira 1980; Nascimento et al., 1980; Meyer et al., 1998; Rebelo et al., 2005). Likewise, reproductive disturbance was seemingly minimal and, though a variety of parasites (intracellular ciliates, undetermined intracellular protists, *Nematopsis* sp. and R/CLOs) were identified in *P. arctata*, their prevalence was low in both localities and seasons. In contrast, RAI, PII and IRI reflected a low-to-moderate disturbance in reproduction, disease and immune response in *C. rhizophorae* from Pigeon Cay (Chapter 2) in the rainy season. In parallel, the histological integrity of the digestive gland tissue was similar in both localities and seasons in *P. arctata*, and atrophy of digestive alveoli was stumpy; unlike in *C. rhizophorae* from Pigeon Cay in which disorganised ICT, shrinkage of digestive diverticula, digestive alveoli atrophy and digestive cell vacuolisation were observed and associated to enhanced pollution (Chapter 2; Valdez Domingos et al., 2007). These results might suggest that slender marsh clams are less responsive to environmental insult than mangrove cupped oysters; however, IRI was very high in *P. arctata* from both Pigeon Cay (only rainy season data) and Punta San Gabriel (at both seasons). Thus, it is conceivable that the high prevalence of oedema and brown cell aggregates might be indicating that slender marsh clams, unlike mangrove cupped oysters, possess a highly active immune defence system able to cope with the extreme environmental conditions of the shallow muddy flats (deposition sinks of coastal lagoons) where they inhabit. However, the lower LT_{50} values recorded in *P. arctata* from Punta San Gabriel during the rainy season in comparison with the dry season might be associated to the highest levels of environmental contaminants recorded during that season at that locality, as reflected in the PLI and CPI values.

P. artacta vs C. rhizophorae. Although the tissue concentration of contaminants was, in general terms, slightly lower in *P. arctata* than in sympatric *C. rhizophorae*, the slender march clam appears to be a suitable biomonitor for chemical pollution assessment in the Caribbean that could be used as alternative biomonitor species; e.g., when *C. rhizophorae* is not available. Moreover, although these are preliminary observations that deserve more specific research in order to be confirmed, it seems that for some specific chemicals, such as for instance musk fragrances and PBDEs, *P. arctata* exhibited a greater bioaccumulation capacity than *C. rhizophorae*. Thus, whilst the main contaminants contributing to CPI and PLI in *P. artacta* were Hg, HCHs, DDTs, AHTN, PCBs and BDE85, in *C. rhizophorae* (Chapter 1), these were As, Cd, benzo[a]pyrene, HCHs, DDTs and PCBs for CPI and benzo[a]pyrene HCHs and DDTs for PLI. In agreement with previous results obtained using mangrove cupped oysters, the highest CPI and

PLI values were obtained in the rainy season also in slender marsh clams. CPI and PLI were higher in Punta San Gabriel than in Pigeon Cay, which suggests a higher bioavailability of pollutants in the former locality. Based on the use of oysters as biomonitors Pigeon Cay was determined to be more polluted than Half Way Cay and Punta Lora (Chapter 1) and therefore the present results would indicate that the greatest bioavailability of pollutants in the study area occurs in Punta San Gabriel (where oysters were absent). Altogether, these findings suggest that *P. arctata* would be complementary rather than alternative to *C. rhizophorae* as biomonitor for the mangrove-lined Caribbean coastal systems in Nicaragua. Thus, by using both species as biomonitors in combination we could conclude the following decreasing gradient of contamination in the studied mangrove-lined coastal systems in the Nicaraguan Caribbean area: Punta San Gabriel > Pigeon Cay > Half Way Cay > Punta Lora. Moreover, one could hypothesise that mangrove cupped oysters, absent in Punta San Gabriel, are more sensitive to environmental deterioration than slender marsh clams; however, it rather appears that the sedimentation zone of the lagoon, which would be the natural habitat for *P. arctata* but not for oysters, is also a locally relevant contamination hot spot.

Likewise, it seems that *P. arctata* would be complementary to *C. rhizophorae* as sentinel for biological-effects assessment in mangrove-lined Caribbean coastal systems in Nicaragua. Based on the use of both species as sentinels we could conclude the following decreasing gradient of ecosystem health disturbance in the studied mangrove-lined coastal systems in the Nicaraguan Caribbean area: Punta San Gabriel > Pigeon Cay > Half Way Cay > Punta Lora. Nevertheless, mangrove cupped oysters, appeared to be more responsive to environmental insult than slender marsh clams.

4.2. Pacific Coast

Contaminants. Tissue metal concentrations in *A. tuberculosa* and *L. grandis* were low according to previous records (Tu et al., 2011; Vargas et al., 2015) and to background levels reported for oysters and mussels, except for As and Cd (Kimbrough et al., 2008; Solaun et al., 2013). The values recorded for Cd were higher than in other studies carried out with *A. tuberculosa* as biomonitor (Joiris and Azokwu, 1999; Otchere, 2003; Tu et al., 2011), especially in Estero La Virgen during the dry season. Moreover, the tissue concentration of metals such as As and Cd varied with season, with higher values during the dry season than during the rainy one. Similarly, the tissue concentration of Cd and Hg was higher in the dry season than in the

rainy season in *Anadara (Senilia) senilis* from Ghana (Joiris et al., 1998; Otchere, 2003). Both in *A. tuberculosa* and *L. grandis*, PAH tissue concentration was low and restrained to the rainy season; the most relevant PAHs found being phenanthrene in pustulose arks and phenanthrene and fluoranthene in shell ark clams. POP tissue levels appeared to be higher in rainy season than in the dry season in both species. In *A. tuberculosa*, γ-HCH presented higher values in Estero La Virgen than in Isla Machuca in both seasons, and 4.4-DDE and musk fragrances were found in Estero La Virgen in the rainy season. In *L. grandis*, γ-HCHs and AHTN tissue concentrations were higher in the rainy season than in the dry season. PCBs were under detection limits in all localities and seasons; however, PBDEs (BDE85, BDE100 and BDE154) were found in pustulose ark tissues, especially in Isla Machuca, and in shell ark clams, especially in the dry season. CPI and PLI values were low in both seasons and the main pollutants contributing to these CPI and PLI values were Cd, phenanthrene, HCHs, DDTs, AHTN, PCBs and BDE85 in *A. tuberculosa* and Cd, B[a]P, HCHs, AHTN and BDE85 in *L. grandis*.

Health status. Biometry and flesh condition parameters exhibited somehow opposite seasonal trends in *A. tuberculosa* and *L. grandis*; these parameters were overall higher in the dry season than in the rainy season in pustulose arks and *vice versa* in shell ark clams. In agreement, in shell ark clams, LT_{50} values were higher in the rainy season than in the dry season. Whilst certain bias towards female condition was envisaged in the sex ratio of pustulose arks in comparison with the theoretical sex ratio of 1:1 (Gener et al., 2009, Silva-Benavides and Bonilla, 2015), ark shell clams did not deviate from the theoretical sex ratio (after Galdámez et al., 2007). Intersex cases were sporadically found in both species, always with separate male and female gonad follicles. Gamete development was more advanced in the rainy season than in the dry season, and in Estero La Virgen than in Isla Machuca in both seasons; however, significant differences were not observed in GI between localities. Oocyte atresia was recorded all along the study period, mainly in Isla Machuca. In *A. tuberculosa*, RAI reflected only a low-to-moderate reproductive disturbance in both localities and seasons. A variety of parasites such as *Nematopsis* sp., *Bucephalus* sp., metacercariae and copepods were identified in *A. tuberculosa*, although, parasite prevalence was low, except for *Nematopsis* sp., especially during the dry season. In *L. grandis*, RAI also reflected only a low-to-moderate reproductive disturbance in both localities and seasons. The parasites recorded in *L. grandis* include *Nematopsis* sp. and to a lesser extent undetermined intracellular protists and metacercariae. Inflammatory responses such as oedema and brown cell aggregates were observed in both species (granulocytomas only in *A.*

tuberculosa); however, whereas digestive gland atrophy was negligible in *A. tuberculosa*, remarkable atrophy was recorded in *L. grandis* at both seasons.

A. tuberculosa and L. grandis as biomonitors and sentinels. The present data are preliminary and in absence of previous records and of any comparison with recognised biomonitor and sentinel species from the area are difficult to interpret. However, it seems that the two studied species have the capacity to bioaccumulate metals, PAHs and POPs in their tissues, like regionally recognised biomonitors such as *C. rhizophorae* do (Chapter 1), and therefore they open an opportunity to develop further studies dealing with the use of these species (*A. tuberculosa* and *L. grandis*) as biomonitors in tropical and subropical Pacific mangrove ecosystems (e.g. from Mexico to Peru). Likewise, biological endpoints comparable to those used in sentinel *C. rhizophorae* (Chapter 2) appear to be suitable to be used as biomarkers of health disturbance in these two species from the Nicaraguan Pacific coast. However, more basic research is needed on the general biology, ecology and disease of these species.

Slender marsh clam *P. arctata* (in the Caribbean), and pustulose ark *A. tuberculosa* and ark shell clams *L. grandis* (in the Pacific) are putative biomonitor and sentinel species for assessing chemical pollution and ecosystem health disturbance in the diverse mangrove-lined coastal systems that can be found in Nicaragua. These species bioaccumulate metals, PAHs and POPs in their tissues at measurable levels that are seemingly related with the environmental levels of these contaminants and with the potential sources of contamination. Thus, they can be used as biomonitor species either alternative or complementary to the more widely recognised mangrove cupped oyster *C. rhizophorae* (Chapter 1). On the other hand, a toolbox of low-cost effortless biomarkers and biological endpoints including resilience, reproduction, growth and disease (histopathology), exactly as previously applied in the case of sentinel mangrove cupped oysters (Chapter 2), has been shown be useful for the case of using *P. arctat*a, *A. tuberculosa* and *L. grandis* as sentinel species covering the whole geographical area of Nicaragua. Besides, as also reported in the case of mangrove cupped oysters from the same region (Chapters 1 and 2), seasonality is crucial to properly design and conduct pollution monitoring programmes for mangrove-lined coastal systems in Nicaragua using the three alternative/complementary species investigated herein.

5. References

Allan I, Vrana B, Greenwood R, Mills G, Roig B, Gonzalez C (2006) A “toolbox” for biological and chemical monitoring requirements for the European Union’s Water Framework Directive. Talanta 69: 302–322.

Bartolomé L, Etxebarria N, Martínez-Arkarazo I, Raposo JC, Usobiaga A, Zuloaga O (2010) Distribution of organic microcontaminants, butyltins, and metals in mussels from the Estuary of Bilbao. Arch Environ Contam Toxicol 59: 244–254.

Baumard P, Budzinski H, Michon Q, Garrigues P, Burgeot T, Bellocq J (1998) Origin and bioavailability of PAHs in the Mediterranean Sea from mussel and sediment records. Estuar Coast Shelf Sci 47: 77–90.

Bayen S (2012) Occurrence, bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems: A review. Environ Int 48: 84–101.

Bayen S, Wurl O, Karuppiah S, Sivasothi N, Lee HK, Obbard JP (2005) Persistent organic pollutants in mangrove food webs in Singapore. Chemosphere 61: 303–313.

Beiras R, Durán I, Parra S, Urrutia MB, Besada V, Bellas J, Viñas L, Sánchez-Marín P, González-Quijano A, Franco MA, Nieto O, González JJ (2012) Linking chemical contamination to biological effects in coastal pollution monitoring. Ecotoxicology 21: 9–17

Bellas J, Albentosa M, Vidal-Liñán L, Besada V, Franco MA, Fumega J, González-Quijano A, Viñas L, Beiras R (2014) Combined use of chemical, biochemical and physiological variables in mussels for the assessment of marine pollution along the N-NW Spanish coast. Mar Environ Res 96: 105–117.

Bellas J, Nieto O, Beiras R (2011) Integrative assessment of coastal pollution: development and evaluation of sediment quality criteria from chemical contamination and ecotoxicological data. Cont Shelf Res 31: 448–456.

Blaise C, Gagné F, Burgeot T (2016) Three simple biomarkers useful in conducting water quality assessments with bivalve mollusks. Environ Sci Pollut Res doi:10.1007/s11356-016-6908-6.

Bodin N, N’Gom Ka R, Le Loc’h F, Raffray J, Budzinski H, Peluhet L, de Morais LT (2011) Are exploited mangrove molluscs exposed to persistent organic pollutant contamination in Senegal, West Africa? Chemosphere 84: 318–327.

Bouchet P (2015) *Polymesoda arctata*. In, Mollusca Base. Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=596338.

Cajaraville MP, Bebianno MJ, Blasco J, Porte C, Sarasquete C, Viarengo A (2000) The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. Sci Total Environ 247: 295–311.

Cordy P, Veiga MM, Salih I, Al-Saadi S, Console S, Garcia O, Mesa LA, Velásquez-López PC, Roeser M (2011) Mercury contamination from artisanal gold mining in Antioquia, Colombia: The world's highest per capita mercury pollution. Sci Tot Envir 410–411: 154–160.

Crosby M, Gale L (1990) A review and evaluation of bivalve condition index methodologies with a suggested standard method. J Shellfish Res 9: 233–237.

Cruz DA, Jimenez JA (1994) Moluscos asociados a las áreas de manglar de la Costa Pacífica de América Central: guía. Heredia, C. R. Efuna, p 18–20.

da Silva AZ, Zanette J, Ferreira JF, Guzenski J o, Freire Marques MR, Dias Bainy AC (2005) Effects of salinity on biomarker responses in *Crassostrea rhizophorae* (Mollusca, Bivalvia) exposed to diesel oil. Ecotoxicol Environ Saf 62: 376–382.

Dame RF (1972) Comparison of various allometric relationships in intertidal and subtidal American oysters. Fishery Bulletin 70: 1121–1126.

Davies IM (2004) Background/Reference Concentrations (BRCS) for the UK. Fisheries Research Services Contract Report No 05/04, Aberdeen.

de Brito APX, De Andrade Brüning IMR, Moreira I (2002) Chlorinated pesticides in mussels from Guanabara Bay, Rio de Janeiro, Brazil. Mar Pollut Bull 44: 79–81.

Defew LH, Mair JM, Guzman HM (2005) An assessment of metal contamination in mangrove sediments and leaves from Punta Mala Bay, Pacific Panama. Mar Pollut Bull 50: 547–552.

Dsikowitzky L, Nordhaus I, Jennerjahn TC, Khrycheva P, Sivatharshan Y, Yuwono E, Schwarzbauer J (2011) Anthropogenic organic contaminants in water, sediments and benthic organisms of the mangrove-fringed Segara Anakan Lagoon, Java, Indonesia. Mar Pollut Bull 62: 851–862.

Dumailo S (2003) Evaluación de la problemática ambiental por medio del estudio de algunos aspectos de sedimentación y contaminación en la laguna de Bluefields, RAAS, Nicaragua. MSc Thesis. CIRA-UNAN, 242 pp.

Ebanks-Mongalo BF, Suárez-Sánchez J, Siu-Estrada E, Montoya-Arguello JJ, Mairena-Valdivia DA, Flores-Pacheco A, Van der Wal JC, Valencia-Quintana P (2013) Concentración de plaguicidas en agua, sedimentos y ostiones (*Crassostrea rizophorae*) de la Laguna de Bluefields, RAAS, Nicaragua. WANI: 49-54.

Ellison AM (2004) Wetlands of Central America. Wetl Ecol Manag 12: 3–55.

FAO (2007) The world's mangroves 1980 – 2005. FAO Forestry Paper no. 153.

Farrington JW, Tripp BW (1995) International Mussel Watch Proyect: inicial implementation phase/Final report. NOAA Technical memorandum NOS ORCA 95, pp 188.

Fernandez A, Singh A, Jaffé R (2007) A literature review on trace metals and organic compounds of anthropogenic origin in the Wider Caribbean Region. Mar Pollut Bull 54: 1681–1691.

Fernández-Tajes J, Flórez F, Pereira S, Rábade T, Laffon B, Méndez J (2011) Use of three bivalve species for biomonitoring a polluted estuarine environment. Environ Monit Assess 177(1): 289-300.

Galdámez Castillo AM, Pacheco Reyes SP, Pérez García IM, Kino S (2007) Guía para la producción de *Anadara* spp. 2006-2007: producción artificial de semilla, cultivo intermedio y cultivo de *Anadara tuberculosa* y *A. grandis*. (Guia). JICA/MAGCENDEPESCA, El Salvador, 42pp.

Garmendia L, Soto M, Cajaraville M, Marigómez I (2010) Seasonality in cell and tissue-level biomarkers in *Mytilus galloprovincialis*: relevance for long-term pollution monitoring. Aquat Biol 9: 203–219.

Garmendia L, Soto M, Vicario U, Kim Y, Cajaraville M, Marigómez I (2011) Application of a battery of biomarkers in mussel digestive gland to assess long-term effects of the Prestige oil spill in Galicia and Bay of Biscay: tissue-level biomarkers and histopathology. J Environ Monit 13: 915–932.

Gatermann R, Hellou J, Huhnerfuss H, Rimkus G, Zitko V (1999) Polycyclic and nitro musks in the environment: A comparison between Canadian and European aquatic biota. Chemosphere 38: 3431-3441.

GEF-REPCar (2011) Agricultural pesticide residues in Caribbean coastal waters. Colombia, Costa Rica and Nicaragua 2008-2011. GEF-REPCar Rpt UNEP, 63 pp.

Gener RL, Rivas F, Argüello G (2009) Estudio de mercado de la concha negra (*Anadara similis* y *Anadara tuberculosa*) en Nicaragua: Comercialización con garantía de inocuidad, 55p.

Goldberg ED (1975) The mussel watch — A first step in global marine monitoring. Mar Pollut Bull 6: 111.

González, LI (1997) Diagnostico ecológico de las zonas costeras de Nicaragua: MAIZCo (Programa de manejo integrado de zonas costeras). Ministerio del Ambiente y Recursos Naturales, Dirección General del Ambiente. Managua-Nicaragua, 69 pp.

Green NW, Schøyen M, Øxnevad S, Ruus A, Høgåsen T, Beylich B, Håvardstun J, Rogne ÅKG, Tveiten L (2012) Hazardous Substances in Fjords and Coastal Waters - 2011. Levels, Trends and Effects. Long-term Monitoring of Environmental Quality in Norwegian Coastal Waters. NIVA.

GTR-K (2007) Diagnóstico del territorio Rama y Kriol, pp 290.

Hellou J, Yeats P, Steller S, Gagne F (2003) Chemical contaminants and biological indicators of mussel health during gametogenesis. Envir Toxicol Chem 22: 2080–2087.

Huber M (2015) Compendium of bivalves 2. Harxheim: ConchBooks, 907 pp.

IARC (1987) Monographs on the evaluation of the carcinogenic risk of chemicals to humans. In: Overall Evaluation of Carcinogenicity: an Updating of IAPC Monographs Vols 1–42. International Agency for Research on Cancer, Lyon, France (Suppl. 7).

ICES (2011) Report of the Study Group on Integrated Monitoring of Contaminants and Biological Effects (SGIMC), 14-18 March 2011. Copenhagen, Denmark. ICES CM 2011/ACOM:30, 265 pp.

Jiménez JA (1999) Ambiente, distribución y características estructurales en los Manglares del Pacífico de Centro América: Contrastes climáticos. In: Yáñez–Arancibia A, Lara–Domínguez AL (eds). Ecosistemas de Manglar en América Tropical. Instituto de Ecología A.C. México, UICN/ORMA, Costa Rica, NOAA/NMFS, pp 51-70.

Joiris CR, Azokwu MI (1999) Heavy metals in the bivalve *Anadara (Senilia) senilis* from Nigeria. Mar Pollut Bull 38(7): 618-622.

Joiris CR, Azokwu MI, Otchere FA, Ali IB (1998) Mercury in the bivalve *Anadara (Senilia) senilis* from Ghana and Nigeria. Sci Total Environ 224: 181-188.

Kannan N, Reusch TBH, Schulz-Bull DE, Petrick G, Duinker JC (1995) Chlorobyphenyls: model compounds for metabolism in food chain organisms and their potential use as ecotoxicological stress indicators by application of the metabolic slope concept. Environ Sci Technol 29: 1851–1859.

Kim Y, Ashton-Alcox KA, Powell EN (2006) Histological techniques for marine bivalve molluscs : update. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 27.

Kim Y, Powell EN (2004) Surfclam histopathology survey along the Delmarva mortality line. J Shellfish Res 23: 429–441.

Kim Y, Powell EN (2007) Distribution of parasites and pathologies in sentinel bivalves: NOAA status and trends "Mussel Watch" program. J Shellfish Res 26: 1115–1151.

Kimbrough KL, Johnson WE, Lauenstein GG, Christensen JD, Apeti DA (2008) An assessment of two decades of contaminant monitoring in the nation's coastal zone. NOAA Technical Memorandum NOS NCCOS 74, 118 pp.

Kimbrough KL, Johnson WE, Lauenstein GG, Christensen JD, Apeti DA (2009) An Assessment of Polybrominated Diphenyl Ethers (PBDEs) in Sediments and Bivalves of the U.S. Coastal Zone. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 94, 87 pp.

Leal JCM, Polo C, Behling E, Colina G, Rincón N, Carrasquero S (2014) Distribución espacial de Cd y Pb en *Polymesoda solida* y sedimentos costeros del Lago de Maracaibo. Multiciencias 14(1): 7-15.

Lewis M, Pryor R, Wilking L (2011) Fate and effect of anthropogenic chemicals in mangrove ecosystems: a review. Envir Pollut 159: 2328–2346.

Lobel PB, Wright DA (1982) Relationship between body zinc concentration and allometric growth measurements in the mussel *Mytilus edulis*. Mar Biol 66: 145–150.

Mackenzie CL (2001) The Fisheries for Mangrove Cockles, *Anadara* spp., from Mexico to Peru, With Descriptions of Their Habitats and Biology, the Fishermen's Lives, and the Effects of Shrimp Farming. Mar Fish Rev 63(1): 1-39.

Mackenzie CL, Stehlik LL (2001) Las pesquerías de moluscos en la costa Caribeña de Honduras y Nicaragua. Rev Invest Mar 22 (2): 133-141.

MARENA (2004) plan de manejo de la Reserva natural Cerro Silva. Proyecto MARENA-CBA/BRLi/VEGA/WCS, Elaboración de Planes de Manejo de las Áreas Protegidas de Cerro Silva, Cerro Silva y Cayos Miskitos, y Ordenamiento Territorial del Municipio de Waspam, pp 143.

MARENA (2010) Caracterización de la cuenca No. 61. Río Escondido. Ministerio del Ambiente y los Recursos Naturales de Nicaragua, DGPN & DRHCH, pp 38.

Marigómez I, Garmendia L, Soto M, Orbea A, Izagirre U, Cajaraville MP (2013) Marine ecosystem health status assessment through integrative biomarker indices: a comparative study after the Prestige oil spill "Mussel Watch". Ecotoxicology 22: 486–505.

Meyer U, Hagen W, Medeiros C (1998) Mercury in a northeastern Brazilian mangrove area, a case study: potential of the mangrove oyster *Crassostrea rhizophorae* as bioindicator for mercury. Mar Biol 131: 113–121.

Mohd Ali, SA and Yep, YM (2016) Petroleum hydrocarbon in mud clam (*Polymesoda erosa*) and its ambient medium in the coastal area of Putatan and Tuaran, Sabah. Trans Sci Technol 3: 218 - 226.

Monirith I, Ueno D, Takahashi S, Nakata H, Sudaryanto A, Subramanian A, Karuppiah S, Ismail A, Muchtar M, Zheng J, Richardson B, Puidente M, Hue ND, Tana TS, Tkalin AV, Tanabe S (2003) Asia–Pacific mussel watch: monitoring contamination of persistent organochlorine compounds in coastal waters of Asian countries. Mar Pollut Bull 46: 281–300.

Moon H-B, Kannan K, Lee S-J, Choi M (2007) Polybrominated diphenyl ethers (PBDEs) in sediment and bivalves from Korean coastal waters. Chemosphere 66: 243–251.

Mora Sanchez EM (1990) Catologo de bivalvos marinos del Ecuador. Bol Cient Tec 10(1): 1–136.

Nascimento IA, Pereira AS (1980) Changes in the condition index for mangrove oysters (*Crassostrea rhizophorae*) from Todos os Santos Bay, Salvador, Brazil. Aqualculture 20: 9-15.

Nascimento IA, Pereira SA, Souza RCE (1980) Determination of the optimum commercial size for the mangrove oyster (*Crassostrea rhizophorae*) in Todos os Santos Bay, Brazil. Aquaculture 20: 1–8.

Nascimento, IA, Leite, MBN, Sansone, G, Pereira, SA, Smith, DH (1998) Stress protein accumulation as an indicator of impact by the petroleum industry in Todos os Santos Bay, Brazil. J Aquat Ecosyst Hlth Manag 1: 101–108.

Navarro P, Bartolomé L, Raposo JC, Zuloaga O, Arana G, Etxebarria N (2010) Preparation of a reference mussel tissue material for polycyclic aromatic hydrocarbons and trace metals determination. Anal Chim Acta 675: 91–96.

Nhan DD, Carvalho FP, Am NM, Tuan NQ, Yen NTH, Villeneuve J-P, Cattini C (2001) Chlorinated pesticides and PCBs in sediments and molluscs from freshwater canals in the Hanoi region. Environ Pollut 112: 311–320.

Oros DR, Ross JRM, Spies RB, Mumley T (2007) Polycyclic aromatic hydrocarbon (PAH) contamination in San Francisco Bay: A 10-year retrospective of monitoring in an urbanized estuary Environ Res 105: 101–118.

Ortiz-Zarragoitia M, Cajaraville MP (2010) Intersex and oocyte atresia in a mussel population from the Biosphere's Reserve of Urdaibai (Bay of Biscay). Ecotoxicol Environ Safety 73: 693–701.

Ortiz-Zarragoitia M, Garmendia L, Barbero MC, Serrano T, Marigómez I, Cajaraville MP (2011) Effects of the fuel oil spilled by the Prestige tanker on reproduction parameters of wild mussel populations. J Environ Monit 13: 84–94.

OSPAR Commission (2005) Agreement on Background Concentrations for Contaminants in Seawater, Biota and Sediment. OSPAR Agreement 2005-6, London, 4 pp.

OSPAR Commission (2009) Background Document on CEMP Assessment Criteria for QSR 2010. In: Monitoring and Assessment Series. OSPAR Commission, London.

OSPAR Commission (2013) Levels and trends in marine contaminants and their biological effects – CEMP Assessment report 2012.

Otchere FA (2003) Heavy metals concentrations and burden in the bivalves (*Anadara (Senilia) senilis*, *Crassostrea tulipa* and *Perna perna*) from lagoons in Ghana: Model to describe mechanism of accumulation/excretion. African J Biotechnol 2(9): 280-287.

Pampanin DM, Volpato E, Marangon I, Nasci C (2005) Physiological measurements from native and transplanted mussel (*Mytilus galloprovincialis*) in the canals of Venice: survival in air and condition index. Comp Biochem Physiol 140A: 41–52.

Pereira SM, Fernández-Tajes J, Rábade T, Flórez-Barrós F, Laffon B, Méndez J (2011) Comparison between two bivalve species as tools for the assessment of pollution levels in an estuarian environment. J Toxicol Environ Health. Part A: Current Issues 74: 15-16.

Pérez AM, Siria I, Sotelo M, Vargas E (2003) Informe final y diagnóstico de la elaboración de la norma técnica para el "Aprovechamiento del recurso ostras en el Pacífico de Nicaragua", 57 pp.

Pérez-Cruz YG, Rangel-Ruiz LJ, Gamboa-Aguilar J (2013) Metales en almejas y sedimentos en la Reserva de la Biósfera "Pantanos de Centla" Tabasco, México. Hidrobiológica 23 (1): 1-8.

Polidoro B a., Carpenter KE, Collins L, Duke NC, Ellison AM, Ellison JC, Farnsworth EJ, Fernando ES, Kathiresan K, Koedam NE, Livingstone SR, Miyagi T, Moore GE, Nam VN, Ong JE, Primavera JH, Salmo SG, Sanciangco JC, Sukardjo S, Wang Y, Yong JWH (2010) The loss of species: Mangrove extinction risk and geographic areas of global concern. PLoS One 5: e10095.

Rebelo MF, Amaral MCR, Pfeiffer WC (2005) Oyster condition index in *Crassostrea rhizophorae* (Guilding 1828) from a heavy-metal polluted coastal lagoon. Braz J Biol 65: 345–51.

Sarria-Panasá J, Mansilla-Rivera I, Colina M, Rodríguez-Sierra CJ (2016) Levels of Trace Metals in the Clam, *Polymesoda solida* (Philippi, 1846) (Bivalvia: Cyrenidae), from the Strait of Lake Maracaibo and Bahía El Tablazo, Venezuela. Life: The Excitement of Biology 4(1): 11-26

Sericano JL, Wade TL, Jackson TJ, Brooks JM, Tripp BW, Farrington JW, Mee LD, Readmann JW, Villeneuve JP, Goldberg ED (1995) Trace organic contamination in the

Americas: An overview of the US National Status & Trends and the International "Mussel Watch" programmes. Mar Pollut Bull 31: 214–225.

Severeyn HJ, García de Severeyn Y, Ewald JJ (1994) Taxonomic revision of *Polymesoda solida* (Philippi, 1846) (Bivalvia: Corbiculidae), a new name for *Polymesoda arctata*, the estuarine clam of Lake Maracaibo and other estuaries of the tropical Atlantic coasts of America. Ciencia 2: 53-65.

Silva CAR, Rainbow PS, Smith BD (2003) Biomonitoring of trace metal contamination in mangrove-lined Brazilian coastal systems using the oyster *Crassostrea rhizophorae*: comparative study of regions. Hydrobiologia 501: 199–206.

Silva-Benavides AM, Bonilla R (2015). Estructura de la población y distribución de *Anadara tuberculosa* Sowerby (1833) (Mollusca: Bivalvia) en los manglares de Golfito y Playa Blanca de Puerto Jiménez, Golfo Dulce, Costa Rica. Rev Biol Trop 63(1): 287-298.

Siung-Chang A (1997) A review of marine pollution issues in the Caribbean. Environ Geochem Health 19: 45–55.

Smedley PL, Kinniburgh DG (2002) A review of the source, behaviour and distribution of arsenic in natural waters. Appl Geochem 17: 517–568.

Solaun O, Rodríguez JG, Borja A, González M, Saiz-Salinas JI (2013) Biomonitoring of metals under the water framework directive: Detecting temporal trends and abrupt changes, in relation to the removal of pollution sources. Mar Pollut Bull 67: 26–35.

Spongberg AI, Davies P (1998) Organochlorinated pesticide contaminants is Golfo Dulce, Costa Rica. Rev Biol Trop 46 (Suppl 4): 111-124.

Spongberg AL, Witter JD (2008) A review of PCB concentrations in tropical media, 1996-2007. Rev Biol Trop 56 (Suppl 4): 1-9.

Stern-Pirlot A, Wolff M (2006) Population dynamics and fisheries potential of *Anadara tuberculosa* (Bivalvia: Arcidae) along the Pacific coast of Costa Rica. Rev Biol Trop 54(1): 87-99.

Tan QG, Wang, Y, Wang, WX (2015) Speciation of Cu and Zn in two colored oyster species determined by X-ray absorption spectroscopy. Environ Sci Technol 49: 6919–6925.

Tomlinson DL, Wilson JG, Harris CR, Jeffrey DW (1980) Problems in assessment of heavy metals in estuaries and the formation of Pollution Index. Helgol Meeresunters 33: 566–575.

Torres RJ, Cesar A, Pereira CDS, Choueri RB, Abessa DMS, do Nascimento MRL, Fadini PS, Mozeto AA (2012) Bioaccumulation of polycyclic aromatic hydrocarbons and mercury in oysters (*Crassostrea rhizophorae*) from Two Brazilian estuarine zones. Int J Oceanogr 2012: 1–8.

Tu NPC, Ha NN, Agusa T, Ikemoto T, Tuyen BC, Tanabe S, Takeuchi I (2011) Trace elements in *Anadara* spp.(Mollusca: Bivalva) collected along the coast of Vietnam, with emphasis on regional differences and human health risk assessment. Fish Sci 77(6): 1033-1043.

UICN / ORMA (2001) Humedales de Centroamérica: síntesis de veintisiete estudios e iniciativas sobre educación, investigación, manejo y conservación de humedales y zonas costeras / (eds): Pizarro F, Gómez C, Córdoba R, (1 ed) – San José, C.R.

UNEP (2002) Central America and the Caribbean Regional Report. Regionally Based assessment of Persistant Toxic Substances. UNEP, CH-1219, Geneva-Switzerland, pp 133.

UNEP (2006) Isaza CFA, Sierra-Correa PC, Bernal-Velasquez M, Londono LM, Troncoso W Caribbean Sea/Colombia & Venezuela, Caribbean Sea/Central America & Mexico. GIWA Regional Assessment 3b, 3c. University of Kalmar, Sweden.

USAID (2012) Co-manejo de Bivaldo "Concha Negra" (*Anadara* ssp) en Aserradores, Nicaragua 2011 Programa regional de USAID para el manejo de recursos acuáticos y alternativas económicas, 22 p.

Valdez Domingos FX, Azevedo M, Silva MD, Randi MAF, Freire CA, Silva de Assis HC, Oliveira Ribeiro CA (2007) Multibiomarker assessment of three Brazilian estuaries using oysters as bioindicators. Environ Res 105: 350–363.

Valette-Silver NJ, Riedel GF, Crecelius EA, Windom H, Smith RG, Dolvin SS (1999) Elevated arsenic concentrations in bivalves from the southeast coasts of the USA. Mar Environ Res 48(4): 311-333.

Van Lavieren H, Metcalfe CD, Drouillard K, Sale PF, Bouchot GG, Reid R, Vermeulen L (2011) Strengthening coastal pollution management in the wider Caribbean region. Proc 64th Gulf Caribb Fish Inst 5: 1–11.

Vargas JA, Acuña-González J, Gómez E, Molina J (2015) Metals in coastal mollusks of Costa Rica. Rev Biol Trop 63: 1007–1019.

Veldhuizen-Tsoerkan MB, Holwerda DA, Zandee DI (1991) Anoxic survival time and metabolic parameters as stress indices in sea mussels exposed to cadmium or polychlorinated biphenyls. Arch Environ Contam Toxicol 20: 259-265.

Viarengo A, Canesi L, Pertica M, Mancinelli G, Accomando R, Smaal AC, Orunesu M (1995) Stress on stress response: a simple monitoring tool in the assessment of general stress syndrome in mussels. Mar Environ Res 39: 245–248.

Wade TL, Sericano JL, Gardinali PR, Wolff G, and Chambers L (1998) NOAA's 'Mussel Watch' Project: Current use organic compounds in bivalves. Mar Pollut Bull 37: 20-26.

Wallner-Kersanach M, Theede H, Eversberg U, Lobo S (2000) Accumulation and elimination of trace metals in a transplantation experiment with *Crassostrea rhizophorae*. Arch Environ Contam Toxicol 38: 40–45.

Zanette J, Monserrat JM, Bianchini A (2006) Biochemical biomarkers in gills of mangrove oyster *Crassostrea rhizophorae* from three Brazilian estuaries. Comp Biochem Physiol 143C: 187–95.

Zaroogian G, Yevich P (1993) Cytology and biochemistry of brown cells in *Crassostrea virginica* collected at clean and contaminated stations. Environ Pollut 79: 191–197.

Ziarrusta H, Olivares M, Delgado A, Posada-Ureta O, Zuloaga O, Etxebarria N (2015) Multiscreening determination of organic pollutants in molluscs using matrix solid phase dispersion. J Chromat 1391A: 18–30.

SM 1. CPI calculations for tissue concentrations of pollutants in slender marsh clam (*P. arctata*), pustulose Arks (*A. tuberculosa*) and ark shell clams (*L. grandis*) from Nicaraguan mangrove lagoons and intertidal roots/docks of Colombian mangrove swamps, collected at both rainy (RS) and dry seasons (DS). Background tissue concentration values (BTC) were selected for calculations according to the following references (Ref.): BRC (Davies 2004; OSPAR 2005); BAC (OSPAR Commission, 2013); LOD: limit of detection.

Ref.	BTC	Chemical Pollutant	*P. arctata*			*A. tuberculosa*				*L. grandis*	
			Pigeon Cay (RS)	P San Gabriel (RS)	P San Gabriel (DS)	Estero La Virgen (RS)	Isla Machuca (RS)	Estero La Virgen (DS)	Isla Machuca (DS)	Estero Venecia (RS)	Estero Venecia (DS)
BRC	0.78	**Ag**	-1.09	0.11	0.27	-1.82	-1.17	-1.35	-1.75	-1.36	-1.36
BRC	21.00	**As**	-0.31	-0.34	0.10	-0.32	-0.51	-0.22	-0.46	-0.30	-0.14
BAC	0.66	**Cd**	-0.44	-0.41	0.18	0.65	0.67	1.29	-0.11	0.68	1.33
BRC	1.80	**Cr**	-0.46	-0.53	-0.42	-0.36	-0.44	-0.07	-0.50	0.22	-0.18
BAC	0.06	**Hg**	0.41	0.43	0.60	0.20	0.04	-0.30	-0.30	0.15	0.20
BRC	2.40	**Ni**	0.05	-0.44	-0.28	-0.99	-1.02	-0.85	-1.31	-0.90	-0.66
BAC	1.2	**Pb**	-0.13	-0.65	-0.47	-1.37	-1.28	-2.08	-2.08	0.82	-0.80
BAC	11.00	**Phe**	-3.04	1.20	-3.04	0.74	0.77	-3.04	-3.04	0.83	-3.04
BAC	12.20	**Flr**	-3.09	0.72	-3.09	0.31	-3.09	-3.09	-3.09	0.48	-3.09
BAC	9.00	**Pyr**	-0.38	0.56	-2.95	-0.47	-0.34	-2.95	-2.95	-0.05	-2.95
BAC	2.50	**Benz[a]A**	0.90	-2.40	-2.40	-2.40	-2.40	-2.40	-2.40	-2.40	-2.40
BAC	8.10	**Chr**	0.69	-0.08	-2.91	-2.91	-2.91	-2.91	-2.91	-0.32	-2.91
BAC	1.40	**B[a]P**	-2.15	0.63	-2.15	-2.15	-2.15	-2.15	-2.15	0.07	0.70
LOD	3.50	**HCHs**	1.89	1.51	-0.24	1.34	0.34	0.87	-0.01	1.10	-0.07
BCR	6.90	**DDTs**	1.73	2.04	-0.04	0.64	-0.36	-0.36	0.84	-0.36	-0.50
LOD	1.00	**AHTN**	0.00	2.06	0.00	1.77	1.09	0.00	0.00	1.88	0.00
BAC	10.20	**PCBS**	1.35								
LOD	50.00	**BDE85**	0.66	-0.36	0.36	-0.01	-0.34	0.24	0.53	-0.42	0.53

SM 2. PLI calculations for tissue concentrations of pollutants in slender marsh clam (*P. arctata*), pustulose Arks (*A. tuberculosa*) and ark shell clams (*L. grandis*) from Nicaraguan mangrove lagoons and intertidal roots/docks of Colombian mangrove swamps, collected at both rainy (RS) and dry seasons (DS). Background tissue concentration values (BTC) were selected for calculations according to the following references (Ref.): BRC (Davies 2004; OSPAR 2005); BAC (OSPAR Commission, 2013); LOD: limit of detection.

Ref.	BTC	Chemical Pollutant	*P. arctata*			*A. tuberculosa*				*L. grandis*	
			Pigeon C (RS)	P San Gabriel (RS)	P San Gabriel (DS)	Estero La Virgen (RS)	Isla Machuca (RS)	Estero La Virgen (DS)	Isla Machuca (DS)	Estero Venecia (RS)	Estero Venecia (DS)
BRC	0.78	**Ag**	0.08	1.28	1.87	0.02	0.07	0.04	0.02	0.04	0.04
BRC	21.00	**As**	0.49	0.46	1.25	0.47	0.31	0.60	0.35	0.50	0.73
BAC	0.66	**Cd**	0.36	0.39	1.51	4.45	4.72	19.31	0.78	4.76	21.54
BRC	1.80	**Cr**	0.35	0.29	0.38	0.43	0.36	0.85	0.32	1.67	0.66
BAC	0.06	**Hg**	2.58	2.72	4.03	1.58	1.09	0.50	0.50	1.41	1.60
BRC	2.40	**Ni**	1.11	0.36	0.52	0.10	0.09	0.14	0.05	0.13	0.22
BAC	1.20	**Pb**	0.74	0.22	0.34	0.04	0.05	0.01	0.01	6.56	0.16
BAC	11.00	**Phe**		15.71		5.48	5.96			6.77	
BAC	12.20	**Flr**		5.25		2.06				3.01	
BAC	9.00	**Pyr**	0.42	3.62		0.34	0.46			0.90	
BAC	2.50	**Benz[a]A**	7.91								
BAC	8.10	**Chr**	4.92	0.82						0.48	
BAC	1.40	**B[a]P**	0.01	4.27	0.01	0.01	0.01	0.01	0.01	1.18	5.05
LOD	3.50	**HCHs**	78.14	32.65	0.58	22.02	2.19	7.40	0.98	12.64	0.86
BCR	6.90	**DDTs**	54.25	110.04	0.91	4.39	0.43	0.43	6.94	0.43	0.32
LOD	1.00	**AHTN**	1.00	115.47	1.00	59.26	12.33	1.00	1.00	75.12	1.00
BAC	10.20	**PCBS**	22.57								
LOD	50.00	**BDE85**	4.55	0.44	2.30	0.97	0.46	1.74	3.40	0.38	3.41
LOD	50.00	**BDE154**					5.44				

Chapter 4. Needs analysis and recommendations for biomonitoring chemical pollution and ecosystem health disturbance in mangrove-lined coastal systems using bivalves as biomonitors and sentinels

Amongst others, chemical pollution is a major problem in mangrove-lined coastal ecosystems in the Wider Caribbean Region (WCR) and other neighbouring tropical and subtropical regions. Whilst pollution monitoring programmes carried out in the Caribbean are mainly based on the chemical analysis of water, sediment and biota (these latter as biomonitors), pollution biomonitoring programs worldwide are increasingly including biological effects as endpoints; for which they employ early warning signals of ecosystem health disturbance recorded in sentinel species, mainly bivalves. Within this context, a novel initiative emerged from a consortium comprised of (a) the Cell Biology & Environmental Toxicology Consolidated Res Grp and (b) the Research Centre for Experimental Marine Biology & Biotechnology (Plentzia Marine Station) from the University of the Basque Country (UPV/EHU), (c) the Infectious Disease Centre of the National Autonomous University of Nicaragua-León (UNAN- León) and (d) the Department of Biological & Environmental Sciences of the University Jorge Tadeo Lozano (UJTL; Bogotá). As a result, a pilot biomonitoring project known as Caribbean Biomonitoring of Pollution (CARIBIOPOL) was carried out along 2012 and 2013, aimed at implementing a science-based, non-sophisticated and low-cost methodology (details in Annexes 1-5) suitable for biomonitoring of chemical pollution and associated ecosystem health disturbance in mangrove-lined Caribbean coastal systems from Nicaragua and Colombia, using bivalves as biomonitor and sentinel species.

Two different working scenarios were conceived: (a) the first one at regional level, using *Crassostrea rhizophorae* as biomonitor and sentinel species in the Caribbean coast from Nicaragua and Colombia in an attempt to contribute to harmonised biomonitoring approaches at the WCR scale; and (b) a second one at national level in Nicaragua, using four species of bivalves (*C. rhizophorae*, *Polymesoda arctata*, *Anadara tuberculosa* and *Larkinia grandis*) as biomonitor and sentinel species in the entire Nicaraguan coastline, both in the Caribbean and the Pacific coasts.

1. *C. rhizophorae* as biomonitor and sentinel in WCR

The first challenge was to determine the suitability of mangrove cupped oysters, *C. rhizophorae*, as biomonitor and sentinel species for pollution biomonitoring in different types of mangrove-lined coastal systems in the Caribbean. For this purpose, an integrated approach was applied, including both contaminant tissue concentrations (Chapter 1) and low-cost and effortless biomarkers of health condition in oysters (Chapter 2). The biomonitoring programme was carried out in 8 localities (3 in Nicaragua and 5 in Colombia) subjected to different types and levels of pollution. Oysters were collected during the rainy and the dry seasons in 2012-2013. The tissue

concentration of chemical pollutants such as metals, PAHs and POPs were measured in parallel with biological effects at different levels of biological complexity (condition index, reproduction, tissue-level biomarkers, histopathological condition and stress-on-stress response). The present study revealed that the pollution profile was different between the two studied geographical areas, Nicaragua and Colombia; as shown by radar plot pollution profiles. Likewise, the levels of pollutants were different, which was clearly reflected in the CPI and PLI values, which were significantly correlated to each other following a linear regression model. Therefore, CPI and PLI seem to be fully comparable integrative estimates of pollutants' levels.

In Nicaraguan oysters, low tissue concentrations of metals (except Hg) and PAHs, moderate-to-high tissue concentrations of Hg, HCHs, DDTs, detectable levels of chlorpyrifos, PCBs and BDE85 and negligible levels of musk fragrances were recorded. The values recorded for HCHs, DDTs and PCBs greatly exceeded the reference values in tissues of *C. rhizophorae* from the WCR. In Colombia, a distinct profile of POPs was identified; thus, the tissue concentrations of HCHs and DDTs were practically negligible, chlorpyrifos and PBDEs were below detection limits, the tissue levels of PCBs were low, the tissue concentrations of synthetic musk fragrances were remarkable in the dry season, and the tissue concentrations of Ag, As, Cd, Pb and PAHs ranged from moderate to extremely high. The values recorded for PCBs occasionally surpassed the average values recorded in tissues of *C. rhizophorae* from the WCR. The different contaminant profiles for oysters from Nicaragua and Colombia were evidenced in radar plots and principal component analysis, as well as in integrated pollution indices. Oyster tissue contaminant profiles varied with season. However this seasonality seemingly did not depend on the oysters' reproductive cycle but on the seasonal variability in physicochemical properties of mangrove ecosystems as well as on the diversification of human activities and practices between the rainy and the dry season (e.g., pest control, sanitation and tourism).

Considering the geographical and environmental diversity of the studied scenarios (which included diverse habitats such as lagoons and swamps and over a wide geographical coastal region from Nicaragua to Colombia), the present results confirm the suitability of *C. rhizophorae* as biomonitor species at Caribbean regional scale for pollution monitoring programmes in mangrove-lined coastal systems, where seasonal variability is a major factor controlling pollutant mobility and bioavailability. National and regional monitoring programmes in the WCR would be greatly improved by including the measurement of tissue concentrations of chemical pollutants in

mangrove cupped oysters in addition to measurements of the levels of pollutants in water and sediments, as it is regularly done in other regions of the world such as Pacific Asia, Europe, Canada and the USA.

These tissue concentrations of contaminants are useful to identify potential pollution problems and pollutant sources in the studied regions but they do not provide reliable indication of the deleterious effects that these pollutants may exert to biota and ecosystems, as recommended for pollution monitoring programs. For this reason, biological effects assessment was carried out in a parallel investigation aimed at relating oyster health condition to pollutant tissue levels (Chapter 2). In order to contribute to develop a suitable multi-biomarker approach for pollution monitoring using *C. rhizophorae* as sentinel species, biological effects were determined at different levels of biological complexity as indicators of health disturbance. These biological endpoints were integrated in IBR/n indices and compared with the tissue burden of chemical pollutants (recorded in Chapter 1).

Our results indicated that oysters were not equally healthy in the different localities and seasons investigated, which was directly evidenced by the IBR/n index, which provided an integrated view on the health status of the mangrove cupped oysters and a general indication of the ecosystem health disturbance. Different indicators of health disturbance alone and in combination were related to the presence of different profiles and levels of chemical pollutants present at low-to-moderate levels. As a result, IBR/n was correlated with pollution indices such as PLI and CPI, even though the levels of biological anomalies used as indicators of health disturbance and the levels of pollutants were in general terms low-to-moderate. Moreover, it is worth noting that it was a real field situation influenced and modulated by biological variables and environmental factors that varied seasonally.

Consequently, the simple methodological approach used herein is useful for the assessment of health disturbance in a variety Caribbean coastal ecosystems using mangrove cupped oysters as sentinel species, which would be a suitable sentinel for biological effects assessment in pollution biomonitoring along mangrove-lined coastal systems in the WCR.

2. A national approach for Nicaragua

The second challenge was to determine the suitability of three potential biomonitor and sentinel species as either alternative or complementary options to mangrove cupped oysters in

order to apply the same approach for biomonitoring chemical pollution and ecosystem health disturbance in mangrove–lined coastal systems at national scale in Nicaragua. *C. rhizophorae* distribution is restricted to the Caribbean coast and this species is not present in muddy areas of coastal lagoons. Therefore, we investigated the suitability of slender marsh clams, *Polymesoda arctata*, as biomonitor and sentinel for muddy areas of Caribbean coastal lagoons; and the suitability of pustulose arks, *Anadara tuberculosa*, and ark shell clams, *Larkinia grandis*, to be used in Pacific Coast mangrove-lined coastal systems. Based on the acquired experience with *C. rhizophorae* and applying the same analytical chemical approach and the same biological effects toolbox, biomonitoring was carried out in 5 localities with different contaminant sources, which represented diverse scenarios of the mangrove-lined coastal systems at national scale in Nicaragua.

In the Caribbean, *P. arctata* was collected in Pigeon Cay (Pearl Lagoon) and Punta San Gabriel (Bluefields Lagoon). In the Pacific, *A. tuberculosa* was collected in Padre Ramos Reserve and Isla Corinto Port, and *L. grandis* in Padre Ramos Reserve. Samples were collected in the rainy and dry seasons during 2012-2013. The tissue concentration of metals, PAHs and POPs were determined and integrated into pollution indices (CPI and PLI). In parallel, biological endpoints at different levels of biological complexity (condition indices, reproduction parameters, histopathology, and stress-on-stress response) were determined as biomarkers of ecosystem health disturbance.

In the Caribbean, the tissue concentration of contaminants in *P. arctata* was, in general terms, low with some exceptions: metals such as Ag, As, Cd, Hg, Ni and V were mainly recorded during the dry season, and PAHs, HCHs, DDTs, musk fragrances, PCBs and PBDEs during the rainy season. The highest levels of contaminants (high CPI and PLI) were recorded in Punta San Gabriel, located at the sediment deposition zone of Bluefields Lagoon. In the rainy season in Pigeon Cay, the profile of the tissue concentrations of contaminants were comparable to those obtained with sympatric mangrove cupped oysters (Chapter 1); although *P. arctata* accumulated much less Cu and Zn, a little bit less Ag, As and Cd, and a little bit more Pb than *C. rhizophorae*. The tissue concentrations of PAHs in *P. arctata* were generally low, as they were in *C. rhizophorae* from the Pigeon Cay. The LMWPAHs/HMWPAHs ratio recorded in slender march clams suggested a predominant petrogenic origin of the PAHs in Punta San Gabriel and a pyrolytic origin in Pigeon Cay (in agreement with the results obtained in this latter locality with sympatric mangrove cupped oysters). Thus, in agreement with the results obtained using mangrove cupped oysters as

biomonitors (Chapter 1), slender marsh clams also revealed that metals and PAHs were less relevant contaminants in these Caribbean lagoons, whilst HCHs, DDTs, musk fragrances and PBDEs could be of concern.

On the other hand, our results might suggest that slender marsh clams are less responsive to environmental insult than mangrove cupped oysters. In any case, the high prevalence of oedema and brown cell aggregates might be indicating that slender marsh clams, unlike mangrove cupped oysters, possess a highly active immune defence system able to cope with the extreme environmental conditions of the shallow muddy flats (deposition sinks of coastal lagoons) where they inhabit. More research is needed to better understand *P. arctata* biomarkers of health status and its responsiveness to environmental insult; which would help in getting an adapted toolbox to measure biological effects in this species. Meanwhile, some biomarkers such as reduction in LT50 were shown to respond and were associated to low-to-moderately high PLI and CPI values in Punta San Gabriel during the rainy season. Our findings suggest that *P. arctata* would be complementary rather than alternative to *C. rhizophorae* as biomonitor and sentinel for the mangrove-lined Caribbean coastal systems in Nicaragua. Thus, if we consider altogether both species as biomonitors and sentinels we could conclude a decreasing gradient of contamination and ecosystem health disturbance in the studied mangrove-lined coastal systems in the Nicaraguan Caribbean area, say: Punta San Gabriel > Pigeon Cay / Half Way Cay > Punta Lora.

In the Pacific coast, significant tissue levels of HCHs, DDTs, musk fragrances and PDBEs were recorded in *A. tuberculosa* and *L. grandis*; which could not be related to the analysed biological endpoints because we had few localities to compare and, mainly, because the general biology, ecology and disease of these species is hitherto unknown and, therefore, we lacked reference data to compare. Nevertheless, our study supports that pustulose arks and ark shell clams in the Pacific coast could be used as biomonitors and sentinels for assessing pollution and ecosystem health disturbance in mangrove-lined coastal systems in Nicaragua. As in the case of mangrove cupped oysters, seasonality was also found to be crucial to properly design and conduct pollution monitoring programmes using these two species. Moreover, the present results constitute a pioneering study to establish baseline of tissue concentrations of contaminants in these species, as well as to characterise their general and reproductive biology, their growth and physiological/nutritional condition, and their health status (disease; including the identification of parasites and pathologies); all these parameters being susceptible to be used as low-cost, effortless

and reliable toolbox of biomarkers, indicative of ecosystem health disturbance in tropical and subtropical regions. The present data are preliminary; however, the two studied species bioaccumulate metals, PAHs and POPs in their tissues and therefore they open an opportunity to develop further studies dealing with the use of these species as biomonitors in tropical and subtropical Pacific mangrove ecosystems from Mexico to Peru. Finally, biological endpoints comparable to those used in sentinel *C. rhizophorae* (Chapter 2) appear to be suitable biomarkers of health disturbance but this cannot be corroborated until more basic research carried out to improve our knowledge of the general biology, ecology and disease of these species.

As a whole, slender marsh clams, *P. arctata*, in the Caribbean, and pustulose arks *A. tuberculosa*, and ark shell clams, *L. grandis*, in the Pacific are putative biomonitor and sentinel species for assessing chemical pollution and ecosystem health disturbance in the diverse mangrove-lined coastal systems that can be found in Nicaragua.

3. Needs analysis and recommendations

Mangrove cupped oysters, *C. rhizophorae*, are suitable biomonitors for pollution monitoring and assessment in Caribbean mangroves, including diverse habitats such as lagoons and swamps and over a wide geographical coastal region from Nicaragua to Colombia. The biomonitoring can be improved by using complementary species such as slender marsh clams, *P. arctata*, in combination; thus covering other neighbouring habitats of interest such as e.g. muddy flats. Likewise, the same conceptual and methodological approaches can be applied to neighbouring coastal areas of the tropical and subtropical regions, such as for instance the Pacific coast. It is worth noting that integrative approaches for marine and coastal pollution monitoring require the application of methodological standards in sampling and analysis of samples, as well as in the interpretation of results.

Regarding the WCR, in view of the present results it is highly recommended that national and regional monitoring programmes such as IMA in Trinidad and Tobago, GIWA in the whole WCR and REDCAM in Colombia would be greatly improved by including measurement of tissue concentrations of chemical pollutants in mangrove cupped oysters (or other biomonitor species) in addition to measurements of the levels of pollutants in water and sediments, as it is regularly done in other regions of the world such as Pacific Asia, Europe, Canada and the USA. Also, it is

relevant to emphasise that seasonality is crucial to properly design and conduct pollution monitoring programmes.

The determination of biological effect biomarkers for the assessment of ecosystem health disturbance often requires delicate equipment and instrumentation, special wares and expensive reagents, not always easy to afford by developing countries. Our study supports that simple methodological approaches (low-cost and effortless biomarkers) can be used to diagnose anomalies in the health status of mangrove cupped oysters (and slender marsh clams, pustulose arks, and ark shell clams), which would be indicative of disturbances in ecosystem health associated to chemical pollution, eutrophication (e.g. from domestic sewage) and other sources of environmental distress. Even if this cost-effective methodological approach is used, the use of biomarkers in tropical and subtropical coastal regions could be constrained by: (a) the difficulties for systematic routine sampling because accessibility to sampling sites is expensive and complicated, and because it is often hampered by natural phenomena such as hurricanes, cyclones and earthquakes (systematic biomonitoring or time-series are a difficult challenge); (b) the limited knowledge on the biology and ecology of target species of local or regional interest, as presently discussed for the case of *P. arctata* and more markedly for *A. tuberculosa* and *L. grandis*; (c) the difficulties to achieve adequate transportation of samples to the laboratory (transportation may take long time under non-optimal conditions for sample quality preservation); and (d) some technological hurdles (e.g. for in situ cryo-processing and further cryo-sample transportation) that hamper the application (at least occasionally for comparison and calibration purposes) of core biomarkers elsewhere applied in coastal biomonitoring such as e.g. lysosomal biomarkers (not applied in the present study).

After the experience acquired during the sampling campaigns and sample processing and in view of the present and other complementary results, the CARIBIOPOL consortium agreed the following recommendations in order overcome the aforementioned constrains: (a) research efforts must be addressed to extend and deepen the knowledge about the biology, ecology and disease of target species (especially *C. rhizophorae*, but also *P. arctata, A. tuberculosa* and *L. gandris*, amongst others of local/regional interest) in order facilitate a better interpretation of the biological results and their relationship with chemical pollution; (b) ad-hoc means, local assistance (this implies also than environmental awareness and education are a must) and networking are crucial to overcome existing logistic difficulties for routine or systematic sampling (e.g., thus facilitating

accessibility to sampling sites, and timely forecasting natural phenomena such as hurricanes, cyclones and earthquakes); (c) realistic consensus procedures (or alternative options) and contact-points must be agreed in order to secure the adequate transportation of samples to the laboratory; and (d) research policies (e.g. special permits for cold-ice transportation for research purposes), reference labs or institutional agreements (e.g. for researchers' mobility and exchanges or for using neighbouring hospital facilities and services) should be developed in order to overcome technological hurdles for applying more sensitive and sophisticated biomarkers.

Aimed at contributing to stimulate these actions in Nicaragua and Colombia, we identified partners/headquarters in the Caribbean Coast, their technical capabilities and their capacity to support accessibility to sampling localities. These were the Dept. of Biology of the Bluefields Indian Caribbean University (BICU) in the Nicaraguan Caribbean coast, the Infectious Disease Center from UNAN-León in the Nicaraguan Pacific coast and the Dept. of Biological and Environmental Sciences from UJTL, with headquarters in Bogotá and Santa Marta.

IV. Conclusions and Thesis

1. Conclusions

1) Mangrove cupped oyster, *Crassostrea rhizophorae*, is a suitable biomonitor for assessing pollution in mangrove-lined coastal systems in the Caribbean, including diverse habitats such as lagoons and swamps and a geographically wide coastal region from Nicaragua to Colombia. Based on the determination of pollutant tissue levels in this species, the present study revealed that the pollution profile was different between Nicaragua and Colombia; as evidenced in both radar plot pollution profiles and PCA. Likewise, the levels of pollutants were different, which was clearly reflected in the CPI and PLI values. National and regional monitoring programmes in the WCR would be greatly improved by including measurements of tissue concentrations of chemical pollutants in mangrove cupped oysters in addition to measurements of the levels of pollutants in water and sediments.

2) The profiles and levels of pollutants recorded in mangrove cupped oyster tissues were useful to identify potential pollution problems and pollutant sources in the studied regions. Overall, low tissue concentrations of metals (except Hg) and PAHs, moderate-to-high tissue concentrations of Hg, HCHs, DDTs, and low levels of chlorpyrifos, PCBs and BDE85 were recorded in Nicaraguan oysters. A distinct profile of POPs was identified in Colombia, where the tissue concentrations of HCHs, DDTs and PCBs were negligible-to-low, noticeable tissue concentrations of synthetic musk fragrances were recorded and the levels of Ag, As, Cd, Pb, and PAHs ranged from moderate to extremely high.

3) Seasonality was a crucial factor affecting contamination profiles and concentrations and was seemingly related to seasonal variability in physicochemical properties of mangrove ecosystems as well as on diversification of human activities and practices (e.g., pest control, sanitation, tourism) between the rainy and the dry season, rather than the oysters' reproductive cycle.

4) Mangrove cupped oysters were not equally healthy in the different localities and seasons investigated herein, as evidenced by the IBR/n index. Complex multi-factorial sources of health disturbance seemed to co-occur over a regional background of low-to-moderate pollution (e.g. by pesticides and domestic sewage) in the Nicaraguan Caribbean coast and therefore correlation between IBR/n and pollution indices (PLI and CPI) was not significant. In contrast, different indicators of health disturbance were related to the presence of different

profiles and levels of chemical pollutants present at low-to-moderate levels, and IBR/n was correlated with pollution indices (PLI and CPI) in a real field situation influenced and modulated by biological variables and environmental factors that varied seasonally.

5) The assessment of ecosystem health disturbance by means of biomarkers in tropical and subtropical coastal regions may be constrained by difficulties for systematic routine sampling, the limited knowledge on the biology and ecology of local/regional target species, and the lack of adequate sample transportation to the laboratory; as well as by technological hurdles for applying core biomarkers elsewhere applied in coastal biomonitoring. However, the present study supports that simple methodological approaches can be used to diagnose anomalies in the health status of mangrove cupped oysters and to identify potential causing agents; these anomalies reflecting disturbances in ecosystem health associated to chemical pollutants, eutrophication (e.g. from domestic sewage) and other environmental stressors. Consequently, based on the low-cost, effortless and non-sophisticated biomarker toolbox used herein, mangrove cupped oysters can be used as sentinel species for the assessment of health disturbance in a variety Caribbean coastal ecosystems.

6) Although the tissue concentration of contaminants was, in general terms, slightly lower in *Polymesoda arctata* than in sympatric *C. rhizophorae*, the slender marsh clam appears to be a suitable biomonitor for chemical pollution assessment in the Caribbean. For synthetic musk fragrances and PBDEs, *P. arctata* exhibited a greater bioaccumulation capacity than *C. rhizophorae*, whilst this latter species exhibited a greater bioaccumulation for As, Cd, benzo[a]pyrene, and PCBs. These findings suggest that *P. arctata* would be complementary rather than alternative to *C. rhizophorae* as biomonitor for the mangrove-lined Caribbean coastal systems in Nicaragua. Likewise, *P. arctata* would be complementary to *C. rhizophorae* as sentinel organism for biological effects assessment.

7) Regarding the suitability of Pacific species, *Anadara tuberculosa* and *Larkinia grandis* as biomonitors and sentinels, our data are preliminary and in absence of previous records and of any comparison with recognised biomonitor and sentinel species from the area are difficult to interpret. However, it seems that the two studied species have the capacity to bioaccumulate metals, PAHs and POPs in their tissues and therefore they open an opportunity to develop further studies dealing with the use of these species as biomonitors in tropical and subtropical Pacific mangrove ecosystems from Mexico to Peru. Likewise, biological endpoints

comparable to those used in sentinel *C. rhizophorae* appear to be suitable to be used as biomarkers of health disturbance in these two species from the Nicaraguan Pacific coast. However, more basic research is needed on the general biology, ecology and disease of these species.

8) A toolbox of low-cost effortless biomarkers and biological endpoints including resilience, reproduction, growth and disease (histopathology), exactly as previously applied in the case of sentinel mangrove cupped oysters, has been shown to be useful for the case of using *P. arctata, A. tuberculosa* and *L. grandis* as sentinel species covering the whole geographical area of Nicaragua. Besides, as in the case of *C. rhizophorae*, seasonality is crucial to properly design and conduct pollution monitoring programmes using *P. arctata; A. tuberculosa* and *L. grandis* as biomonitors and sentinels.

2. Thesis

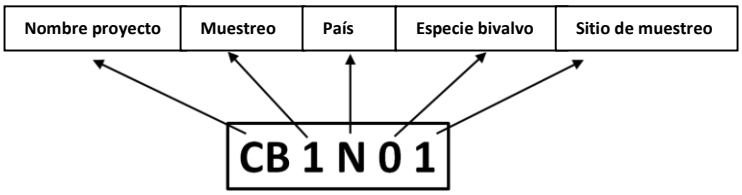
Assessment of chemical pollution and ecosystem health disturbance in mangrove-lined coastal systems in the Pacific region of Nicaragua and in the Caribbean regions of Nicaragua and Colombia, are successfully achieved by using mangrove cupped oysters, *Crassostrea rhizophorae*, and other alternative and locally relevant bivalve species, such as *Polymesoda arctata*, *Anadara tuberculosa* and *Larkinia grandis*, as biomonitors and sentinels. For this purpose, the combination of pollutant tissue burdens together with low-cost and effortless biomarkers in an integrated approach must take into account seasonality and other sources of natural variability and confounding factors, as well as the occurrence of multiple stressors.

V. Annexes

Anexo 1. Protocolo de muestreo

CARIBIOPOL 2012-2013

Planificación Muestreo: / / / /



PUNTOS DE MUESTREO

Localidad	Sitio de muestreo	Etiqueta por especie			

MAREAS

Día	Hora tabla	Altura (mts)

TÉCNICAS Y ANIMALES A USAR

Técnicas a usar	Nº animales	Etiqueta	Envase-Procesado
Histología	20 5	H	20 corte transversal EN Casetes, **DAVIDSON** Órganos separados: 5 Manto (M), 5 Glándula Digestiva (GD) 5 Branquia (Br). Casetes, **FORMALDEHIDO**
BIOLOGIA MOLECULAR	5	BM	5 Manto (M) 5 Glándula Digestiva (GD) 5 Branquia(Br), todos los crioviales deben de llevar 0.5 ml de rNA Later
Stress on stress	30	SOS	Enteros, vivos en agua o lodo según especie
Biometry	25	Flesh condition	Enteros, vivos en agua o lodo según especie
QCA-PAHs, Plaguicidas y Metales pesados	25	PAHs	Enteros, vivos, bolsa QMCA
QCA-PAHs, Plaguicidas y Metales pesados	1 L	Agua química	Dos envases de ½ L

PLANIFICACIÓN GENERAL: / / / /

Participantes:

Material muestreos:

Por punto de muestreo y especie:

- 15 Crioviales etiquetados:
  - 15 para BIOLOGIA MOLECULAR (BM) de los cuales 5-GD 5-Br 5-M
- 25 casetes etiquetados para HISTOLOGIA, de los cuales:
  - 20 para cortes transversales.
  - 5 para GD+M+Br, órganos separados.
- 1 bolsa de plástico etiquetada para QMCA.
- 1 bolsa de plástico etiquetada con trozos de papel aluminio numerados para guardar las conchas.
- 3 cajas de 15L etiquetadas con el nombre del sitio para colectar los bivalvos.

Material de disección

- 1 caja poliespán con acumuladores de frío
- Material de disección (tijera, pinza y bisturí por persona)
- 2 cuchillos para ostras
- Lápiz y sacapuntas
- 2 Botes con fijador por especie (DAVIDSON, FORMALDEHIDO)
- 1 marcador permanente
- 1 regla
- espátula o cuchillo
- papel de filtro
- 3 bandejas
- etiquetas extra
- Guantes
- rNA later
- Bolsa para basura
- Balanza (g)

CAMBIOS A TENER EN CUENTA

1- Utilizaremos 2 fijadores: DAVIDSON, FORMALDEHIDO
2- Se guardan muestras para Metales, PHA y Plaguicidas a -40 hasta su proceso de liofilización.
3- Todas las conchas de todos los individuos se guardan.

¿QUÉ HAY QUE HACER?

1.- Es importante señalar que aún no están decididas las técnicas que se van a llevar a cabo sobre las muestras recogidas. Por eso las etiquetas se dividen en grupos: HISTOLOGIA, BIOLOGIA MOLECULAR y QUIMICA.

2.- En cada punto de muestreo se cogerán 85-110 individuos. En el caso de que los animales estén llenos de arena o fango procurar pasarlos por agua y quitar la mayor parte posible de arena.

3.- 85 bivalvos se meten en una caja de 15 L con agua de la estación de muestreo y se llevan al laboratorio. Al llegar al laboratorio, **30** de estos bivalvos se pondrán al aire en el lugar indicado para **stres on stres**, **25** se diseccionan se miden el peso total, concha y carne, todo el tejido blando del bivalvo se guarda -40ºC para su **liofilización** y posteriormente su análisis de **metales, PAHs, POPs** y cálculo de **índice de condición,** en el caso de que el proceso de liofilización y análisis químico se realice en otro centro, se deben seleccionar otros 25 bivalvos para el cálculo de índice de condición y secar a 105ºC por 24 hr.

4.- Para histología:

-Utilizaremos **20** bivalvos a los que haremos un corte transversal, que contenga la glándula digestiva, parte de la branquia y el manto (bivalvos 1-20). Los meteremos directamente en un casete para fijar en DAVIDSON.

-Diseccionaremos **5** bivalvos más. Meter la glándula digestiva, el manto y la branquia en un casete para fijar en FORMALDEHIDO (bivalvos 20-25).

-En total se utilizan 25 bivalvos para histología (1-25).

5.- Biología molecular

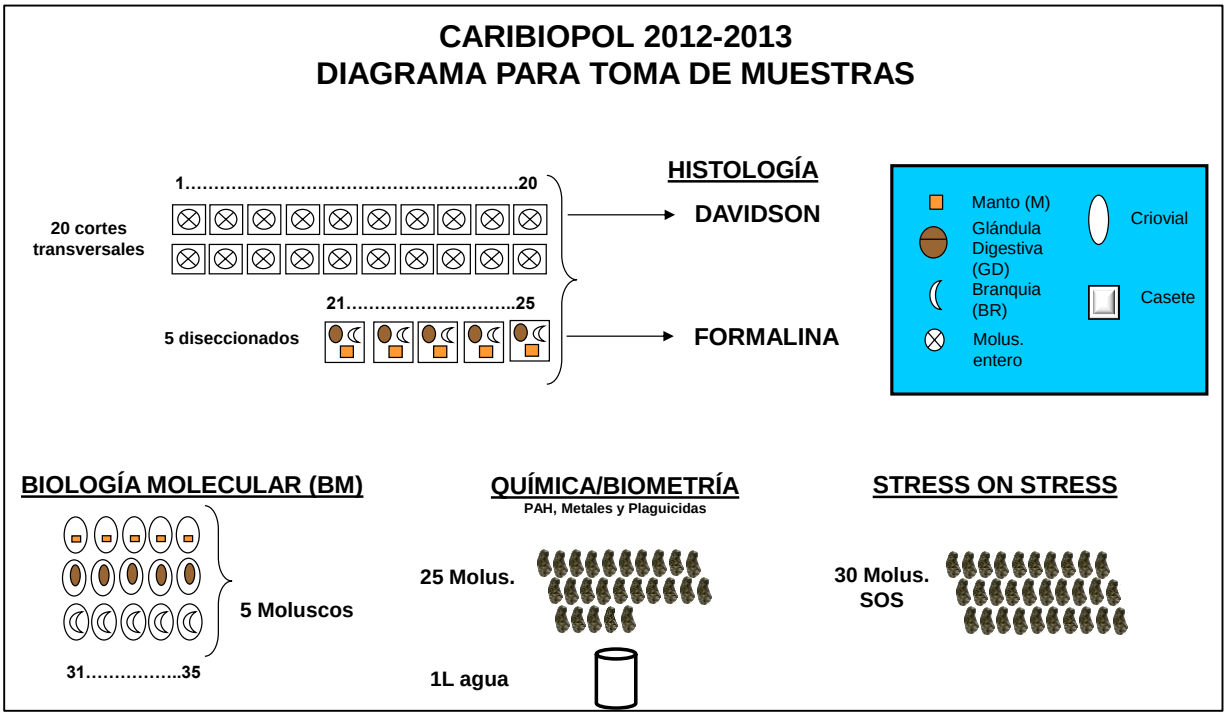
-A **cinco** bivalvos se les extraen ambas branquias de cada individuo y se introducen en los crioviales debidamente etiquetados, de igual manera la gónada y glándula digestiva. Los crioviales contienen 0.5 ml de rNA Later (inactivación rNasa) se traslada al laboratorio en una nevera con hielo azul (Ice pack) y luego se guardan a -80ºC.

6.-Guardar las conchas de cada individuo que se utilizó para su posterior análisis de biometría, con el código de la etiqueta en la parte interna de ambas valvas y envueltas en papel aluminio.

Importante:

1.- Al llegar de vuelta al laboratorio, el material refrigerado debe guardarse inmediatamente en un congelador a –80 ºC (Biología Molecular), limpiar, secar y preparar el material de disección para ser utilizarlo en el siguiente muestreo.

INCIDENCIAS: Anotar cualquier alteración que haya ocurrido en el proceso de muestreo, disección o transporte de muestras.



Anexo 2. Analysis of tissue concentrations of contaminants

Preprocessing and bottling: *t*he soft body of each bivalve was separated from the shell, homogenized (blender Black and Decker), pooled (25 bivalves per sample) and maintained at -40ºC during processing (2-3 d). Pooled samples were freeze-dried (between −46 and −52ºC) and at low pressure (between ~0.17 and 0.22 mbar) in a Cryodos T-50 (Telstar, Barcelona, Spain; Navarro et al., 2010). The samples was then homogenised in a Pulverisette 6 ball mill (Fritsch, Quebec, Canada) resulting in a fine powder (<125µm particle size), sieved with a 250µm mesh. A Laborette 27 rotary cone sample divider (Fritsch GmbH) was used to complete the homogenisation of the sample. This process was repeated twice to guarantee the material homogeneity. Then, the material was sub-sampled and distributed in amber bottles (~30g in each bottle) at −20 ˚C in the fridge until analysis. Metals tissue concentration of (silver, aluminium, arsenic, barium, cadmium, chromium, cobalt, copper, iron, mercury, lithium, magnesium, manganese, molybdenum, nickel, lead, antimony, selenium, tin, strontium, titanium, thallium, vanadium and tungsten) Ag, Al, As, Ba, Cd, Cr, Co, Cu, Fe, Hg, Li, Mg, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, Ti, TI, V and W, in whole soft tissue were determined by ICP–MS (Bartolomé et al., 2010; Navarro et al., 2010). Nevertheless, organic pollutants assessment was focused on Matrix Solid Phase Dispersion (MSPD) extraction method followed by gas chromatography couple to single mass spectrometry (GC–MS) to determine up to 41 non-polar or slightly non-polar organic pollutants (PAHs, PCBs, PBDEs, OCPs, OPPs and musk fragrances) (Ziarrusta et al., 2015).

Chemicals solution and reference material of metal: for the inductively coupled plasma-mass spectrometric (ICP-MS) determination, individual standard solutions of metals (1000µgmL^{-1} for Ag, Al, As, Ba, Cd, Cr, Co, Cu, Fe, Hg, Li, Mg, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, Ti, TI, V and W) was used. The Standard Reference Material SRM NIST-2977 (mussel tissue, *Perna perna*) was supplied by the National Institute of Standards and Technology (Gaithersburg, USA). The microwave-assisted extraction was carried out in a close microwave device MDS-2000 (CEM Corporation, Matthews, USA) equipped with 12 Teflon vessels and a pressure controller. The samples were evaporated in a TurboVap LV (Zymark, Barcelona, Spain).

Determination of metal tissue concentration: a liquots (0.2 g) were placed in an extration vessel with 15mL of 7% (v/v) nitric acid. The following digestion program was used: at first, the oven worked at full power (630W) in order to reach 30psi, then, the pressure was kept constant at 80% of power during 30min. After cooling, the extracts were filtered through syringe PTFE filters

(25mm,5µm,Waters, Milford, USA), diluted to 50mL with Milli-Q water and 100µL of the internal standard solution (5µgg^{-1}) was added. These extracts were kept in the dark in polyethylene vials at 4ºC until the analysis. The ICP-MS system (ELAN 9000; Perkin-Elmer SCIEX, Thornhill, Ontario, Canada) was used for the analysis: nebulizer, plasma and auxiliary flows 0.9, 15 and 1.2 Lmin^{-1}, respectively, sample flow 1mLmin^{-1}, radiofrequency power 1000W, integration time 1500ms and four replicates (Bartolomé et al., 2010; Navarro et al., 2010). The analytic procedure was evaluated by means of Standard Reference Material (SRM) and the use of laboratory reference materials. Tissue metal concentrations were expressed as µg metal/g tissue dry weight (Bartolomé et al., 2010; Navarro et al., 2010)

Chemicals solution and reference material of organic pollutants: the names of the target analytes and the isotopically labelled standards used as surrogates, the abbreviations and the purity of the standards are included in Table 1. PCB Mix-3 (CB 28, CB 52, CB 101, CB 118, CB 138, CB 153 and CB 180) was supplied by Dr. Ehren-storfer GmbH (Augsburg, Germany) and the individual standard of CB 52 by Sigma–Aldrich (St. Louis, MO, USA). Bromodiphenyl Ethers Lake Michigan Study mix (BDE 28, BDE 47, BDE 66, BDE 85, BDE 99, BDE 100, BDE 138, BDE 153 and BDE 154) was purchased from Isostandards Materials (Madrid, Spain) and the individual standard of BDE 100 from Sigma–Aldrich (St. Louis, MO, USA). SS TCL PAH Mix containing EPAs 16 priority PAHs was obtained from Supelco (Walton-on-Thames, UK). The surrogate standard PAH deut 5 containing 5 deuterated PAHs ([2 H8]-naphthalene, [2 H10]-acenaphthene, [2 H10]-phenanthrene, [2 H12]-chrysene and [2 H12]-perylene) was provided by Dr. Ehrenstorfer GmbH. The two polycyclic musks, tonalide (AHTN) and galaxolide (HHCB), were obtained from LGC Standards GmbH (Wesel, Germany), whereas [2 H15]-musk xylene was supplied by Dr. Ehrenstorfer GmbH. The four OCPs (o,p-dichlorodiphenyldichloroethane, p,p-dichlorodiphenyl-dichloroethylene, o,p-dichlorodiphenyl-trichloro-ethane, p,p-dichlorodiphenyl-trichloroethane) and four HCH isomers were supplied by Dr. Ehrenstorfer GmbH. The two OPPs, chlorpyriphos and chlorfenvinphos, as well as the deuterated analogue [2 H8]-1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, were provided by Sigma–Aldrich. [2 H66]-n-dotriacontane and [2 H46]-n-docosane were acquired from CDS Isotopes Inc. (Sainte-Foy-La-Grande, France). Individual stock solutions from each solid standard were dissolved to prepare ~1000 g g−1 stock solutions in 2-propanol (HPLC-grade, 99.8%, LabScan, Dublin, Ireland). These solutions were stored in amber vials at −20 ◦ C. 100 mg L−1 dilutions were prepared in 2-propanol monthly and more diluted stocks were prepared daily according to the experimentation. The solvents n-hexane

(95%), dichloromethane (DCM; 99.8%), ethyl acetate (EtOAc; 99.8%) and acetone (99.8%) used for elution were provided by LabScan. Empty polypropylene cartridges (10 mL capacity) were pur-chased from HSW Norm-Jet (Keltenstrasse, Germany), BD Discardit II (Huesca, Spain) and Omnifix-F (B. Braun Melsungen AG, Germany). Empty glass syringes of 6 mL and 10 mL capacity were acquired from Supelco (Bellefonte, PA, USA) and Rutherford Vintage (Ruthe, Portugal), respectively. 6 mL and 12 mL polyethylene frits were purchased from Supelco. For dispersion and clean-up purposes, different solid sorbents were used. Diatomaceous earth (acid washed not further calcined, 95% purity), Florisil and silica (high purity grade, 70–230 mesh) were provided by Sigma–Aldrich. EnviCarb (Supelclean 120/400) was acquired from Supelco, zeolite from Zeolyst International (Conshohocken, USA) and Plexa and octadecyl-functionalized silica (Bondesil-C18) from Agilent Technologies (Lake Forest, USA). Silica and Florisil were activated at 130 ˚C overnight and maintained in a dry atmosphere until their use for analysis purposes. When necessary, both phases were deactivated with controlled percentages of sulphuric acid (H_2 SO_4; 95–97%) purchased from Merck (Darmstadt, Germany). A Cryodos-50 laboratory freeze-dryer from Telstar Instrument (Sant Cugat del Valles, Barcelona, Spain) was used to freeze-dry the mollusc samples. Extracted fractions were evaporated at 20 ˚ C in a Turbovap LV Evaporator (Zymark, Hopkinton, MA, USA) using a gentle N_2 (99.999%, Messer, Vilaseca, Spain) blowdown.

Material cleaning: all the laboratory material was cleaned with abundant pure water (<0.2 S cm^{-1}, Millipore, Billerica, MA, USA) and without using detergent to avoid possible contamination. The material was sonicated in clean acetone (Q.P., Panreac Química, Spain) for an hour and rinsed with ultrapure water (<0.057 S cm^{-1}, Milli-Q model, Millipore). Finally, the glass material was dried in an oven at 400 ˚ C for 4 h. The glass syringes were sonicated in clean DCM for an hour and rinsed with ultrapure water before drying in an oven at 130 ˚ C for approx. 12 h.

MSPD extraction and clean-up: under optimised conditions, 0.30 g of freeze-dried mollusc sam-ple and 0.30 g of Florisil dispersant were manually blended for 2 min in a glass mortar using a glass pestle. A 10-mL glass syringe, containing a polyethylene frit at the bottom, was filled from bottom to top as follows: 0.60 g of deactivated silica, 4.00 g of activated silica and finally the blended material. Isotopically labelled surrogates were spiked at 50 ng g^{-1} in the blended material and a second frit was placed over. Subsequently, analytes were eluted with 25 mL of DCM (measured at collection) and the eluate obtained was evaporated to dryness using a gentle N_2 blowdown and reconstituted to a final volume of 140 L of n-hexane. The extracts were analysed

by means of GC–MS or GC–MS/MS. For optimisation and validation purposes freeze-dried fish hatchery mussels (obtained from a local market), a freeze-dried mussel tissue laboratory reference material (LRM) and the freeze-dried mussel tissue SRM-2977 (National Institute of Standards and Technology, Gaithersburg, MD, USA) were used.

GC–MS analysis: the MSPD extracts were analysed in an Agilent 7890A gas chromatograph coupled to an Agilent 7000 triple quadrupole mass spectrometer and using an Agilent 7693 autosampler (Agilent Technologies). 2 L of the extract was injected in the splitless mode (1.5 min) at 300 ˚C in an Agilent HP-5 ms capillary column (30 m × 0.25 mm, 0.25 m) with helium (99.9995%, Carburos Metálicos, Barcelona, Spain) as carrier gas at a constant flow of 1.3 mL min^{-1}. The following oven temperature programme was used for the separation of the 41 target compounds: 60 ˚C (held 1 min) to 140 ˚ C at 30 ˚ C min^{-1}, to 200 ˚ C at 3 ˚ C min^{-1} , to 240 ˚ C at 5 ˚ C min^{-1} and a final increase of 30 ˚ C min^{-1} up to 300 ˚ C, where it was held for 10 min. When LVI-PTV injection was considered, 10 L of the sample extract was injected at 50 ˚ C while the vent valve was opened for 3 min at a flow rate of 75 mL min^{-1} and a vent pressure of 2.9 psi. Subsequently, the analytes were focused to the column in splitless mode for 1.5 min while the temperature of the PTV injection port was increased at 12 ˚ C min^{-1} to 300 ˚ C and held for 5 min. Finally, the inlet was further cleaned at a purge flow of 50 mL min^{-1} before further injections. The mass spectrometer worked in the electron impact (EI) mode with electron energy of 70 eV. The temperature of the interface was kept at 310 ˚C, while the temperature of the ionisation source and the detector were maintained at 230 ˚C and 150 ˚C, respectively. The measurements were performed both in the selected ion moni-toring (SIM) and in the selected reaction monitoring (SRM) modes (see Table 1). In the latter, N_2 (99.9999%; Air Liquide, Spain) was required as collision gas at a flow of 1.5 mL min^{-1}. In the case of the SIM mode, the first ion was used as quantifier while the second ion was considered as qualifier. In the case of the SRM mode, one precursor ion and two product ions (one used as quantifier and the other one as qualifier) were monitored, as recommended by the identification requirements of the European guidelines (Commission Decision, 2002/657/EC and SANCO/10684/2009 Guideline). The sum of the dwell times within a window was maintained in 100 ms and longer dwell times were used for the target analytes compared to the corresponding labelled standards in order to improve the signal of the former (see Table 1). The MassHunter WorkStation Acquisition Software (Version B.05.02/Build 5.2.365.0, Agilent Technologies, 2008) was used for data acquisition and automatic integration and quantification of the results.

Compounds analyzed by MSPD–GC–MS according to Ziarrusta et al., 2015.

Abbreviation	Name	Abbreviation	Name
Polycyclic aromatic hydrocarbons		***Polychlorinated biphenyls***	
Acy	Acenaphthylene	**CB 28**	2,4,4′-Trichlorobiphenyl
Ace	Acenaphthene	**CB 52**	2,2′,5,5′-Tetrachlorobiphenyl
Flu	Fluorene	**CB 101**	2,2′,4,5,5′- Pentachlorobiphenyl
Phe	Phenanthrene	**CB 118**	2,3′,4,4′,5- Pentachlorobiphenyl
Ant	Anthracene	**CB 138**	2,2′,3,4,4′,5′-Hexaachlorobiphenyl
Flr	Fluoranthene	**CB 153**	2,2′,4,4′,5,5′-Hexachlorobiphenyl
Pyr	Pyrene	**CB 180**	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl
B[a]A	Benzo[a]anthracene	***Polybrominated diphenyl ethers***	
Chr	Chrysene	**BDE 28**	2,4,4′-Tribromodiphenyl ether
B[b]F	Benzo[b]fluoranthene	**BDE 47**	2,2′,4,4′-Tetrabromodiphenyl ether
B[k]F	Benzo[k]fluoranthene	**BDE 66**	2,3′,4,4′-Tetrabromodiphenyl ether
B[a]P	Benzo[a]pyrene	**BDE 85**	2,2′,4,4′,6-Pentabromodiphenyl ether
B[ghi]P	Benzo[g,h,i]perylene	**BDE 99**	2,2′,3,4,4′-Pentabromodiphenyl ether
Ind	Indene[1,2,3-cd]pyrene	**BDE 100**	2,2′,4,4′,5-Pentabromodiphenyl ether
D[ah]A	Dibenzo[a,h]anthracene	**BDE 153**	2,2′,4,4′,5,5′-Hexabromodiphenyl ether
Isotopically labelled compounds		**BDE 154**	2,2′,4,4′,5,6′-Hexabromodiphenyl ether
[2H10]-Ace	[2H10]-Acenaphthene		
[2H10]-Phe	[2H10]-Phenanthrene		
[2H10]-Chr	[2H10]-Chrysene		
[2H12]-Pyr	[2H12]-Perylene		
[2H15]-MX	[2H15]-musk xylene		
[2H8]-4,4′ -DDT	1,1,1-Trichloro-2,2- bis(4-chlorophenyl)ethane-d8		
[2H46]-n-docosane			
[2H66]-n-dotriacontane			
Organophosphorous			
Chlor	Chlorpyriphos		
Chlorf	Chlorfenvinphos		
Organochlorine pesticides			
δ-HCH	**δ**-Hexachlorocyclohexane		
α-HCH	**α**-Hexachlorocyclohexane		
β-HCH	**β**-Hexachlorocyclohexane		
γ -HCH	γ-Hexachlorocyclohexane		
4,4′ -DDE	p,p′-Dichlorodiphenyl- dichloroethylene		
2,4′-DDD	o,p′- Dichlorodiphenyldichloroethane		
2,4′ -DDT	o,p’-Dichlorodiphenyl- trichloro-ethane		
4,4′-DDT	p,p′-Dichlorodiphenyl- trichloroethane		
Polycyclic musks			
HHCB	Galaxolide		
AHTN	Tonalide		

Anexo 3. Descripción de disección según la especie de bivalvo y procesos de preparación de tejido.

Preparación de tejido y disección de Ostra: Para abrir la ostra se introduce un cuchillo de ostras en la parte del ligamento entre los umbos. Una vez roto el ligamento, se cortan los músculos aductores presente en la parte media de la ostra. Una sección transversal de 5 mm de espesor de tejido se elimina de la ostra con un bisturí o unas tijeras, con la finalidad que la segunda sección contenga todos los órganos. La segunda sección de tejido se obtiene de tal manera que la cara dorsal-ventral pasa a través de la glándula digestiva, tejido de las branquias y gónada. Veinte secciones se colocan inmediatamente en un casete de tejido y el casete se introduce en un recipiente lleno de fijador de Davidson (Tabla 1) durante 48 horas. Después de 48 horas, el fijador se elimina y se añade etanol al 70% y los tejidos se dejan reposar hasta su procesamiento. Otras cinco secciones se colocan inmediatamente en un casete de tejido y el casete se introduce en un recipiente lleno de fijador de Formaldehido al 10% (Tabla 2) durante 24 horas. Después de 24 horas, el fijador se elimina y se añade etanol al 70% y los tejidos se dejan reposar hasta su procesamiento.

Tabla 1. Reactivos y proporcione para preparación histológica del fijador Davidson (Kim et al., 2006).

Secuencia de mezcla	Compuesto	Formula	Peso molecular (g/mol)	Marca	1L
1	Ácido acético glacial (ml)*	$C_2H_4O_2$	60.05	Fluka 45731	100
2	Formaldehido 40% (ml)		30.03	Panreac 211328	200
3	Etanol 96º (ml)				300
4**	Cloruro de sodio (g)				7.5
	Agua destilada (ml)				300
5	Glicerina (ml)	$C_3H_8O_3$	92.09	Sigma G-6279	100

Nota: * ácido acético ≥ 99.8%, las proporciones están dadas para un volumen total de 1 litro.
****** La mezcla de la sal y agua destilada se prepara aparte y luego se mezcla.

Preparación de tejido y disección de Berberecho y Almeja: Para abrir los berberechos y almejas se introduce un cuchillo en la parte del ligamento entre los umbos, hasta romper éste y se cortan los músculos aductores. Una sección transversal de 5 mm de espesor de tejido se elimina del bivalvo con un bisturí, con la finalidad que la segunda sección contenga todos los órganos. La segunda sección de tejido se obtiene de tal manera que la cara dorsal-ventral pasa a través de la glándula digestiva, tejido de las branquias y gónada. En ésta segunda sección, debemos de limpiar un poco la glándula digestiva del musculo del píe, ya que a la hora de realizar las secciones en el micrótomo nos daña la cuchilla al igual que la sección a extraer. Otra forma de hacer la toma de muestra es extraer de cada bivalvo la glándula digestiva, branquias y manto por separado. Veinte secciones se colocan inmediatamente en un casete de tejido y el casete se introduce en un recipiente lleno de fijador de Davidson (Tabla 1) durante 48 horas. Después de 48 horas, el fijador se elimina y se añade etanol al 70% y los tejidos se dejan reposar hasta su procesamiento. Otras cinco

secciones se colocan inmediatamente en un casete de tejido y el casete se introduce en un recipiente lleno de fijador de Formaldehido al 10% (Tabla 2) durante 24 horas. Después de 24 horas, el fijador se elimina y se añade etanol al 70% y los tejidos se dejan reposar hasta su procesamiento.

Tabla 2. Reactivos y proporcione para preparación histológica del fijador Formaldehido 10% (Martoja y Martoja-Pierson, 1970).

Secuencia de mezcla	Compuesto	Formula	Peso molecular (g/mol)	Marca	1L
1	Formaldehido 40% (ml)		30.03	Panreac 211328	100
2	Fosfato disódico 12H2O (g)	$Na_2H_2PO_412H_2O$	358.14	Fluka 71650	28.92
3	Fosfato monosódico 1H2O (g)	$NaH_2PO_4H_2O$	137.99	Panreac 131965/Fluka 71506/Probus 183710	2.56
4	Agua destilada (ml)	Rellenar hasta alcanzar un litro			1L

Nota: Si no se cuenta con el fosfato monosódico y disódico, se puede utilizar agua marina del sitio de muestreo y formaldehido en la proporción 9:1 (formaldehido al 10%).

Preparación de portas

Deshidratación e inclusión de muestras de tejido de bivalvos: Las muestras de tejido individuales se preparan para ser incluidas en parafina, utilizando un protocolo de deshidratación (Tabla 3). Las soluciones usadas para la deshidratación, limpieza e inclusión se cambian con frecuencia para mantener la pureza de la solución. Todo éste proceso se puede hacer manualmente moviendo los tejidos, siguiendo la secuencia de las soluciones. La parafina se funde a una temperatura de 60-75 ° C. La parafina derretida siempre se debe utilizar en la infiltración final y pasos de inclusión. Después de que los tejidos están en las cubetas de infiltración de parafina lista para su inclusión, se extrae la muestra de tejido de los casetes y se ponen en un molde de plástico o acero inoxidable. Se procede a rellenar de parafina fundida para su inclusión, se coloca en la parte superior del molde el casete que contiene la etiqueta de la muestra y se traslada a una placa fría del sistema de inclusión. A medida que el tejido / parafina se enfría endurece. Se debe tener cuidado de usar parafina suficiente para cubrir el tejido después de esta contracción. El molde se deja en la placa fría hasta que se retira el bloque de tejido-parafina 1hr o de 1-2 hr a temperatura ambiente. El bloque se almacena a temperatura ambiente 32-35ºC.

Corte de tejido: Los bloques de parafina se cortan utilizando un micrótomo, iniciamos a 20 micras hasta exponer el tejido en toda la sección transversal, luego se cortan las secciones a 5 micras. Las secciones de tejido pueden ser cortadas individualmente o en secciones continuas. Las secciones se colocan en la superficie de un baño maría de agua destilada, a temperatura de 45-50 ° C y se dejan expandir. Una vez que las secciones se expanden a su tamaño completo, son pescadas con un portaobjetos en un ángulo de 90º, el que se desliza bajo una o más de las secciones de tejido, el portaobjeto contiene albúmina de huevo para mejorar la fijación del tejido al cristal.

Las secciones ya puestas en el portaobjeto son secadas en un horno a 37 ºC por 24 hr o a temperatura ambiente toda la noche. Éstas se almacenan a temperatura ambiente 32-35ºC, hasta su proceso de tinción.

Tabla 3. Protocolo de deshidratación de muestras de bivalvos, adaptado para Nicaragua CARIBIOPOL 2012-2013, modificado de Kim et al., (2006).

Pasos	Deshidratación de muestras en Formaldehido y Davidson	
	Compuestos	Tiempo (h)
1	Etanol 70%	1
2	Etanol 96%	1
3	Etanol 96%	1
4	Etanol absoluto 100%	1
5	Etanol absoluto 100%	1
6	IMS	1
7	Xilol	1
8	Xilol	1
9	Parafina	2
10	Parafina	2
11	Inclusión	
Tiempo total		12

Nota: IMS (proporción 1:1 de alcohol absoluto y xilol). El alcohol etílico desnaturalizado al 99º es considerado absoluto.

Tinción de tejidos: Las secciones son desparafinadas e hidratadas utilizando una serie de xiloles y etanoles (Tabla 4). Después de la hidratación, los portaobjetos se tiñen en Hematoxilina de Harris moficada 4 minutos. En éste paso es conveniente respetar el tiempo de tinción que recomienda cada tipo de solución de hematoxilina. Dependiendo de la fórmula de preparación el tiempo puede variar de 3-20 minutos. Luego se procede a lavar en agua corriente y diferenciar el tejido con alcohol ácido 0.25% para eliminar el exceso de colorante hasta que los núcleos y componentes basófilicos de las células sea lo único que permanezcan teñidos. Virar al color azul, empleando Carbonato de litio 1.3%. Aplicamos el colorante de Eosina 2 minutos, el cual pude modificarse según el tipo de tejido y tiempo de uso del tinte. Posteriormente se procede a la deshidratación de las secciones en una serie de etanoles en escala ascendente y finalmente se diafanizan o aclaran empleando xilol. Los tiempos requeridos para cada paso son flexibles, tanto en los procedimientos de tinción descritos aquí y en el protocolo de inclusión anterior. Diferentes tipos de tejido pueden requerir diferentes momentos. Todas las soluciones, especialmente los xilenos y etanoles queridos, deben cambiarse con frecuencia. Los portaobjetos que contienen las secciones, no debemos permitir que se sequen durante las transferencias entre cubetas si el proceso es manual.

Montaje de tejido: Este procedimiento consiste en colocar encima de la sección o corte teñido y diafanizado una gota de una sustancia adherente, diluida, generalmente en *xilol (DPX medio de montar para histología) o resina natural como el bálsamo de Canadás*, cuyos índices de refracción son similares a los del vidrio, luego se pone una lámina de cubreobjetos encima del tejido, cuidando que no queden burbujas de aire entre la resina. A continuación se deja que el xilol se evapore y la resina adquiera solidez suficiente; para ello las láminas se colocan en una bandeja horizontal por 12 hrs y estarán listas para ser observadas.

Tabla 4. Protocolo de tinción de Hematoxilina –Eosina adaptado para "Bivalvos de Nicaragua", modificada de Kim et al., (2006).

Paso	Estación	Reactivo	Tiempo en minutos	Exacto
1	1	Xilol	10:00	-
2	2	Xilol	10:00	-
3	3	Alc. absoluto	02:00	-
4	4	Alc. absoluto	02:00	-
5	5	Alc. 96º	02:00	-
6	6	Alc. 70º	02:00	-
7	7	Agua destilada	05:00	-
8	8	**Hematoxilina**	**04:00**	SI
9	Wash 5	Agua corriente	04:00	-
10	9	Alcohol ácido	00:10	SI
11	Wash 4	Agua corriente	05:00	-
12	10	Carbonato de litio	00:10	SI
13	Wash 3	Agua corriente	01:00	-
14	11	**Eosina**	**02:00**	SI
15	Wash 2	Agua corriente	00:01	-
16	Wash 1	Agua corriente	00:50	-
17	13	Alc. 70º	00:30	SI
18	14	Alc. 96º	00:30	SI
19	15	Alc. absoluto	01:20	SI
20	16	Alc. absoluto	01:20	SI
21	17	Xilol	01:00	-
22	18	Xilol	01:00	-
23	Exit	Xilol	01:00	-

Nota: El tiempo de la EOSINA (aumenta en relación al tiempo de uso del tinte). La intensidad del color de la eosina disminuye si aumentamos el tiempo de la salida de los alcoholes (pasos 17;18;19).

Carbonato de Litio 1.3%: Para hacer una dilución de carbonato de litio en agua se busca la saturación de ésta sal en ella, y después se filtra. El nivel de saturación es de 1.3 g cada 100 ml de agua destilada.

Alcohol ácido al 0.25%: Añadir 0.25 ml de ácido clorhídrico en 100 ml de alcohol al 70%.

Anexo 4. Análisis de tejidos teñidos con Ematoxilina-Eosina.

Las diapositivas preparadas son examinadas individualmente bajo el microscopio con un objetivo de 10X. Si cualquier tejido necesita ser examinado más de cerca, un objetivo 20X o 40X puede ser utilizado para un examen más detallado de las sospechas de patologías o parásitos. Los principales tipos de tejidos examinados incluyen branquias, manto, gónadas y gonoductos, túbulos de glándula digestiva, estómago y tejido conectivo. A medida que el análisis histopatológico se realiza en conjunto con el análisis gonadal, los bivalvos son examinados generalmente empezando por las gónadas para determinar el sexo y la etapa de desarrollo gonadal. Luego se examinan las branquias y la masa visceral (Kim et al., 2006).

Tabla 5. Estados reproductivos en ostras, modificado de Kim et al., (2006); Ortiz-Zarragoitia y Cajaraville, (2010).

Fase gonadal	Índice gonadal	Estado	Características
1	0	Sexualmente indiferenciado	Poco o ningún tejido gonadal visible.
2	1	Desarrollo inicial	Los folículos comienzan a expandirse.
3	2	Desarrollo medio	Los folículos se expanden y empiezan a fusionarse; no hay gametos maduros presentes.
4	4	Desarrollo avanzado	Folículos expandidos en gran medida, y unidos, el tejido conectivo es considerable; algunos gametos maduros presentes.
5	5	Desarrollo total	La mayoría de los son gametos maduros, poco tejido conjuntivo restante.
6	3	Desove	Gametos visibles en gonoductos.
7	2	Desovado	Reducción del número de gametos, algunos gametos maduros que aún quedan, evidencia de actividad reproductiva renovada.
8	1	Desovado	Poco o ningún gameto visible, tejido gonadal atrofiado.

Un examen histopatológico también se puede llevar a cabo en paralelo al análisis gonadal (Kim et al., 2006; Garmendia et al., 2011). Se necesita un examen cuidadoso de las etapas tempranas del desarrollo gonadal para distinguir entre machos y hembras, el cual puede ser indiferenciado. La etapa del ciclo gametogénico se asigna en función de la madurez de los folículos y gametos, un valor numérico es asignado como se describe en las Tablas 5 y 6. El cálculo de la proporción sexual, se determina Nºhembras/Nºmachos.

Tabla 6. Estado reproductivo en *Polymesoda arctata*, *Anadara tuberculosa* y *Larkinia grandis*, modificado de Kim et al., (2006); Ortiz-Zarragoitia y Cajaraville, (2010).

Fase gonadal	Índice gonadal	Estado	Característica
I	0	Reposo	Inactivo o indiferenciado.
II	1	Desarrollo	La gametogénesis comienza; no hay gametos maduros visibles.
	2	Desarrollo	Gametos maduros presentes; gónadas desarrolladas a cerca de 1/3 de su tamaño final.
III	3	Desarrollo	Gónada desarrollada a cerca de 1/2 de su tamaño final de condición completamente madura; cada folículo contiene, en términos de área, aproximadamente proporciones iguales de gametos maduros y en desarrollo.
	4	Desarrollo	Gametogénesis sigue avanzando, los folículos contienen gametos maduros, principalmente.
IV	5	Maduro	Gónadas completamente maduras, las primeras etapas de la gametogénesis muy poco presentes; folículos dilatados con gametos maduros, óvulos compactados en configuraciones poligonales; espermatozoides con colas visibles.
V	4	Desovado	La emisión activa ha comenzado, la densidad de esperma se ha reducido; los óvulos dejan de ser tan redondos debido a que la presión dentro de los folículos se reduce.
	3	Desovado	Gónada vacía de aproximadamente la 1/2.
	2	Desovado	Zona gonadal reducida; aproximadamente 1/3 del folículo está lleno de gametos maduros.
VI	1	Desovado	Sólo quedan gametos residuales; algunos están en citólisis.

En el caso de las alteraciones histopatológicas como parásitos, enfermedades o patologías de tejidos se asigna un valor según la intensidad usando una escalas cuantitativas, semi cuantitativas y presencia-ausencias, según la patología (Tabla 7) (Kim et al 2006). Se debe anotar cada incidencia para evitar volver a examinar el tejido escaneado o la muestra varias veces para cada categoría. En la escala semi-cuantitativa pueden requerir un re-escaneo del tejido para cada categoría a evaluar dependiendo de la magnitud de la infección.

En éste apartado incluiremos un listado de los parásitos y patologías encontradas durante el programa piloto CARIBIOPOL. La lista no tiene la intención de incluir a todos los parásitos y patologías conocidas de Ostras, Almejas y Berberechos, sino solo los parásitos y patologías encontradas durante el proyecto. Con frecuencia, en el examen de rutina, no tratamos de diferenciar a un bajo nivel taxonómico entre parásitos, debido a que la intensidad de la infección encontrada fueron bajas para la mayoría de especies de parásitos.

La información obtenida a partir del análisis taxonómico no justifica el tiempo empleado en la identificación. Más bien, hemos agrupado las distintas especies en categorías superiores (por ejemplo, todos los cestodos, todos los ciliados). En éste proyecto no se analizaron muestras en

fresco de parásitos para su identificación. Cuando se necesita una mayor diferenciación, lo primero es diferenciar el tejido donde se produzca, porque la mayoría de las especies tienen preferencias según el tipo de tejido. En casi todos los casos, este nivel de diferenciación ha sido adecuado para la estimación de la prevalencia y la intensidad de la infección.

Tabla 7. Lista de categorías cuantitativas y semi cuantitativas utilizada por taxón de bivalvo en Nicaragua, modificada de Kim et al., (2006).

Categorías	Especies			
	C. rhizophorae	*P. solida*	*A. tuberculos*	*A. grandis*
Categoría cuantitativa				
Inclusion de procariontе	x	x		
Gregarinas	x	x	x	
Ciliados	x	x	x	
Cestodos	x			
Trematodo metacercaria	x		x	x
Turbelarian o nemertinos	x	x	x	
Copepodos	x		x	x
Infiltración hemocítica en tejidos	x			
Granulocitoma			x	
Celulas pardas	x	x	x	x
Categoria Semi-cuantitativa				
Neoplasia hemocítica	x			
Atrecia gonadal	x	x		
Esporoquiste de tremado			x	

Los resultados obtenidos se utilizan para calcular los siguiente parámetros: Prevalencia = NH / NS, y la intensidad = SP / NH, donde NH es el número de especímenes que hospedan, parásitos o patologías, NS es el número de muestras analizadas por sitio, SP es la cantidad correspondiente a cada parásito y la patología registrada. Prevalencia e intensidad proporcionan información acerca de la incidencia de cada parásito y condiciones del tejido (Kim et al., 2006; Garmendia et al., 2011). Entre las categorías semi cuantitativas, solo se describiremos como evaluar infección por esporoquistes de trematodos, neoplasia y alteraciones en el desarrollo gonadal Tabla 8 y 9.

Tabla 8. Escala semi-cuantitativa para infección de esporoquistes de trematodos (Kim et al., 2006).

Valor	Descripción
0	No infectado.
1	Presente solo en la gónada (permanece presente en algunos gametos).
2	Gónada completamente llena (no hay presencia en gametos); pueden estar presente en glándula digestiva o en branquias de forma muy esporádico.
3	Gónada completamente llena, la infección se ha extendida a la glándula digestiva o branquias.
4	Gónada completamente llena, sustancialmente llena la glándula digestiva o branquias, aparecen individuos en sacos de esporoquistes.

Tabla 9. Escala semi-cuantitativa para medir las alteraciones hemocíticas (neoplasia) y desarrollo anormal en gónada (Kim et al., 2006).

Valor	Descripción
0	No infectado
1	Menos del 25% de los túbulos están infectados
2	25% de los túbulos están infectados
3	50% de los túbulos están infectados
4	Mas del 50% de los túbulos están infectados

Anexo 5. Equipos y material de laboratorio

Equipamiento básico de campo

- 7 Equipos de disección (tijeras y pinzas)
- 2 Bisturí por persona
- 1 Caja de guantes de latex # L, M, S
- 1 Litro de fijador de Davidson y 1L de Formaldehido al 10%
- 3 Botes plásticos de 2L para colecta de muestras
- 4 Acumuladores de frios (Ice pack)
- 2 Cuchillos de ostras
- 4 Bandejas grandes para disección y stress on stress
- 3 Pares de guantes de buceo para colecta de ostras
- 1 Caja de poliespán o termo para conservar muestras
- 1 Bolsa con marcadores, lápices, borrador, saca puntas y regla
- 10 Pliego de papel filtro o dos rollo de papel de cocina
- Bolsa de basura
- Balanza granataria (g)
- 25 casetes para histología
- 17 crioviales para histoquímica y biología molecular
- 2 botellas de 500ml para colecta de muestras de agua
- 1 Rollo de papel aluminio
- 1 Caja de bolsas ziploc o cierre fácil

Unidad de histología

- 1 Espacio para realizar tinción de tejidos y stress on stress
- 4 Canastillas con capacidad 10 placas para realizar tinción de tejido
- 12 Cubetas para realizar tinción
- 1 Dispensadores de parafina
- 1 Incubadora 60°C
- 1 Baño de parafina
- 1 Baño maría
- Casetes de histología
- 200 Moldes para inclusión de tejido
- Cuchillas para micrótomo
- 1 Juego de pinceles
- Porta objetos con borde esmerilado
- Cubreobjetos 24x50 mm
- 1Micrótomo
- 1 Nevera
- 1 Congelador -40ºC
- 1 Congelador -70ºC
- 1 Balanzas de precisión (g)

- 1 Microscopios binocular
- 1 Microscopio binocular con cámara
- Equipo menor (cristalería)

Otros equipos

- Bombas de vacío y materiales para el filtrado de agua.
- Liofilizador
- Balones de cristal para liofilizar
- Agitador magnético
- Magneto para homogenizar las soluciones

Referencias

Garmendia L, Soto M, Vicario U, Kim Y, Cajaraville MP, Marigómez I (2011). Application of a battery of biomarkers in mussel digestive gland to assess long-term effects of the Prestige oil spill in Galicia and Bay of Biscay: Tissue-level biomarkers and histopathology. J Environ Monit 13: 915-932.

Kim Y, Ashton-Alcox KA, Powell EN (2006) Histological Techniques for Marine Bivalve Molluscs: Update. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 27, 76 pp.

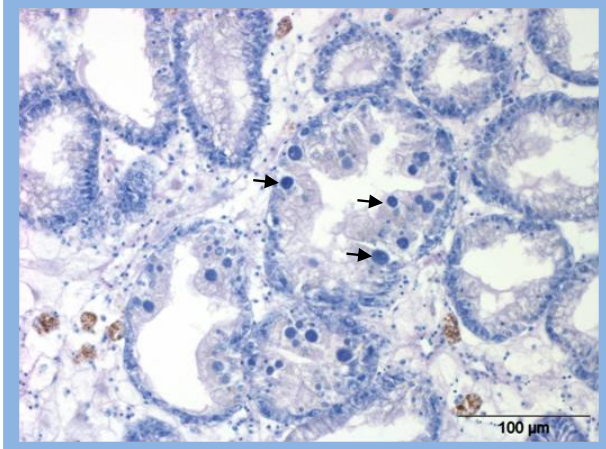
Martoja R, Martoja-Pierson M (1970) Técnicas de Histología Animal, Toray Masson SA Barcelona, p 350.

Bartolomé L, Etxebarria N, Martínez-Arkarazo I, Raposo JC, Usobiaga A, Zuloaga O (2010) Distribution of organic microcontaminants, butyltins, and metals in mussels from the Estuary of Bilbao. Arch Environ Contam Toxicol 59: 244–254.

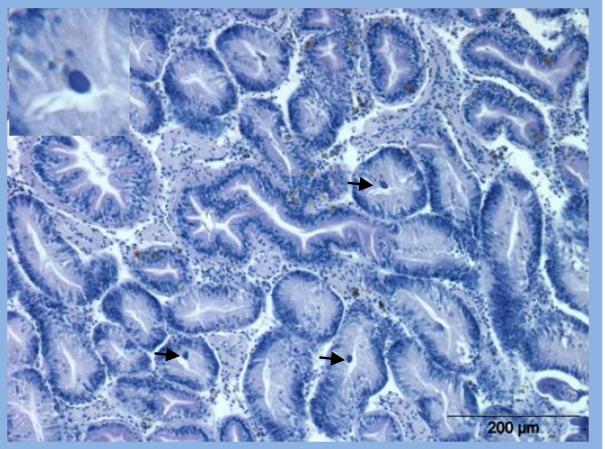
Navarro P, Bartolomé L, Raposo JC, Zuloaga O, Arana G, Etxebarria N (2010) Preparation of a reference mussel tissue material for polycyclic aromatic hydrocarbons and trace metals determination. Anal Chim Acta 675: 91–96.

Ziarrusta H, Olivares M, Delgado A, Posada-Ureta O, Zuloaga O, Etxebarria N (2015) Multiscreening determination of organic pollutants in molluscs using matrix solid phase dispersion. J Chromat 1391A: 18–30.

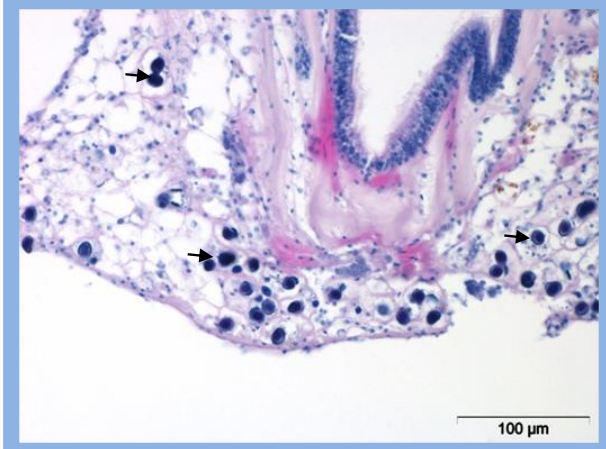
Anexo 6. Figuras de alteraciones histopatológicas encontradas en ostras, almejas y berberecho de Nicaragua.



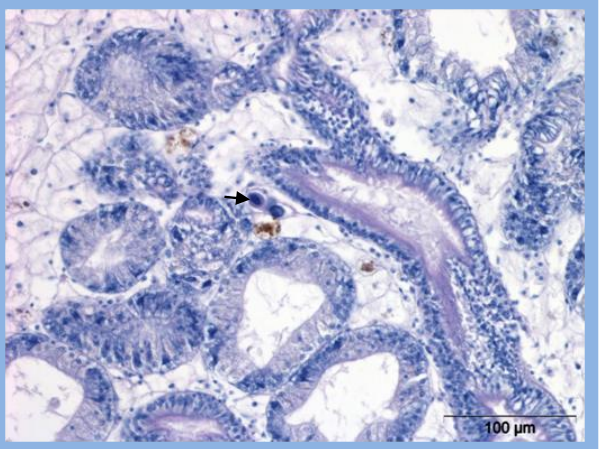
Protista indiferenciado en túbulos de Ostras (*Crassostrea rhizophorae*)



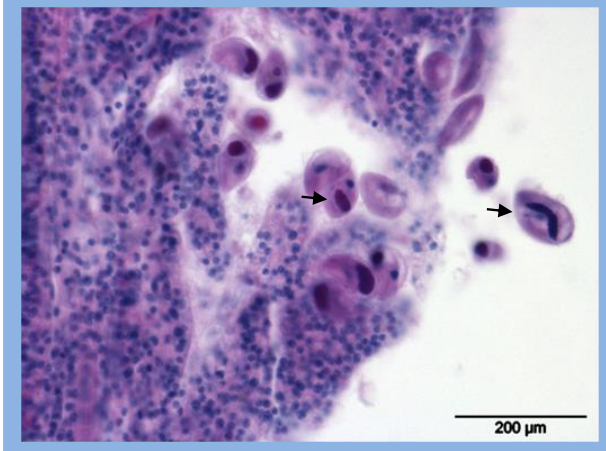
Rikettsia/Clamidia como organismo en túbulos de Almeja (*Polymesoda solida*)



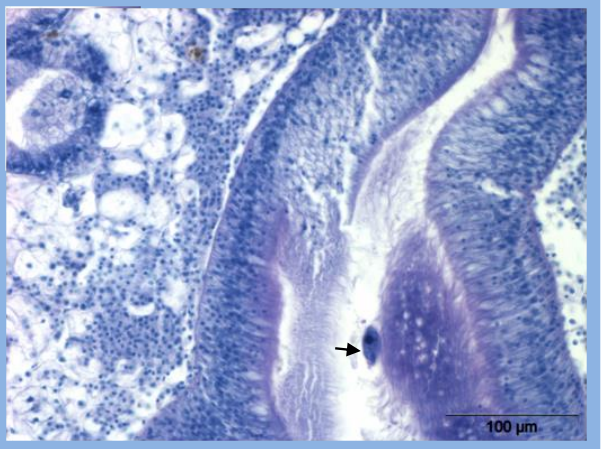
Gregarinas, *Nematopsis* sp en tejido conectivo de gónada en Ostras (*Crassostrea rhizophorae*)



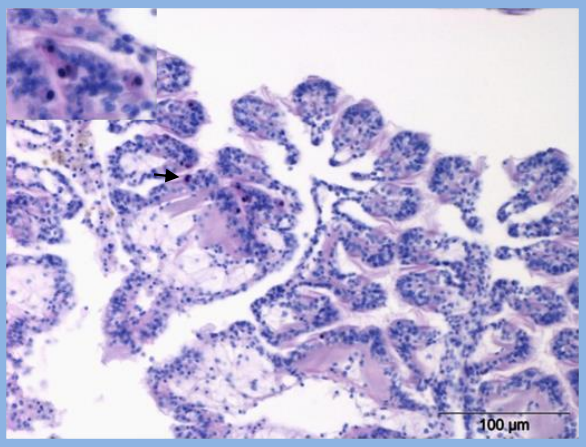
Gregarinas, *Nematopsis* sp en tejido conectivo de glándula digestiva en Ostras (*Crassostrea rhizophorae*)



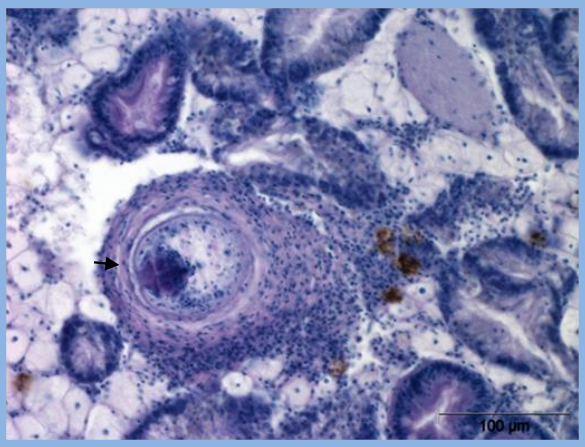
Ciliado, Trichodina sp en la parte basal en branquias de Ostras (*Crassostrea rhizophorae*)



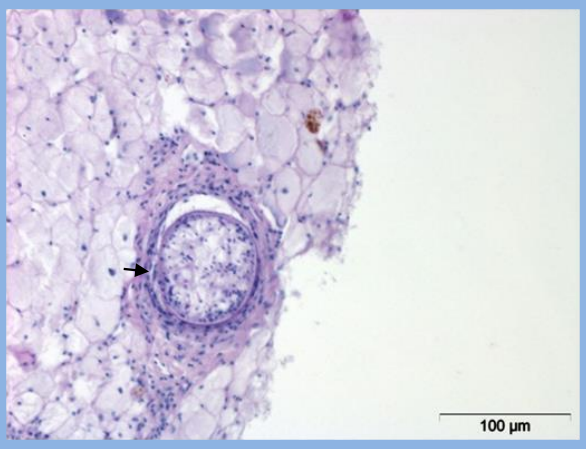
Ciliado, *Ancistrocoma* sp en conducto de glándula digestiva en Ostras (*Crassostrea rhizophorae*)



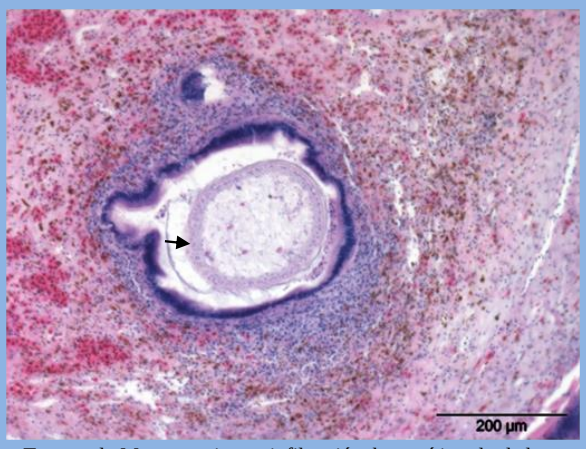
Ciliado, Rynchodida en branquias de Ostras (*Crassostrea rhizophorae*)



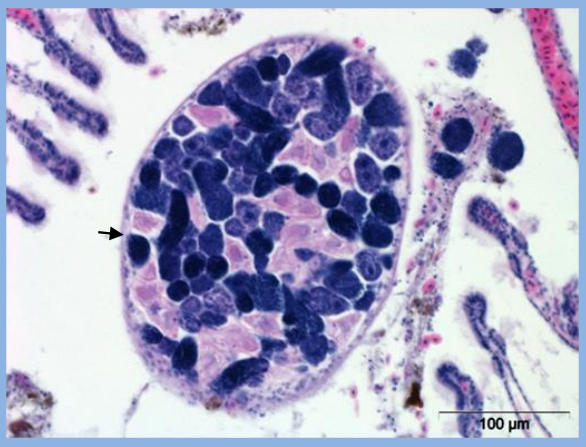
Cestodo, *Tylocephalum* sp con infiltración hemocitica en tejido conectivo de glándula digestiva en Ostras (*Crassostrea rhizophorae*)



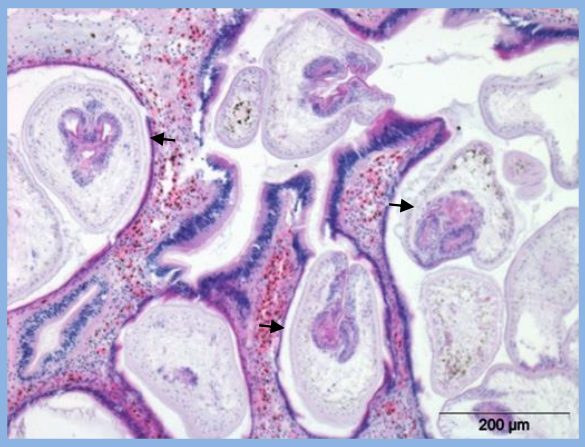
Trematodo, Metacercaria con infiltración hemocitica en tejido conectivo de gónada en Ostra (*Crassostrea rhizophorae*)



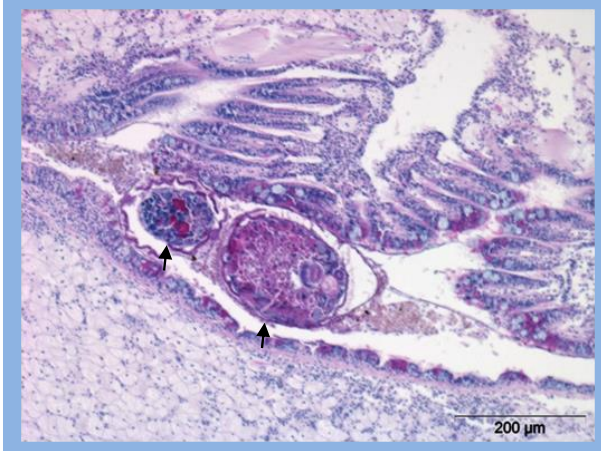
Trematodo Metacercaria con infiltración hemocítica alrededor del túbulo de glándula digestiva en berberecho (*Anadara tuberculosa*)



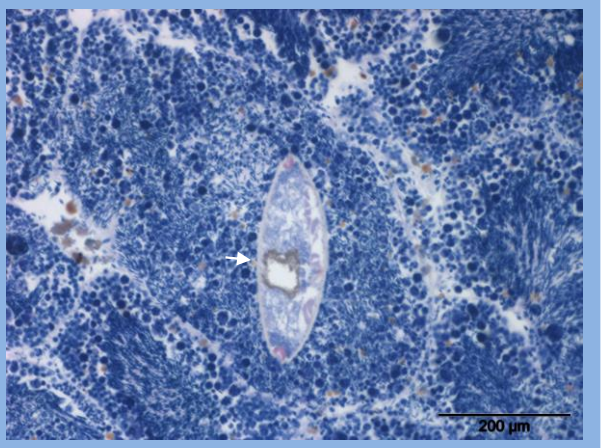
Trematodo, *Bucephalus sp* en branquias de berberecho (*Anadara tuberculosa*)



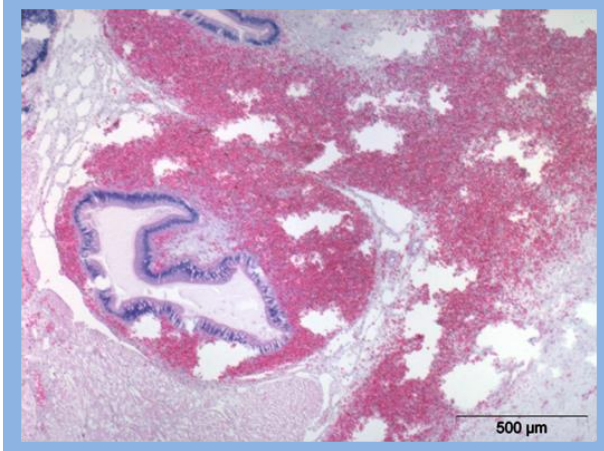
Trematodo indiferenciado en gónada y túbulo de glándula digestiva de *Anadara tuberculosa*



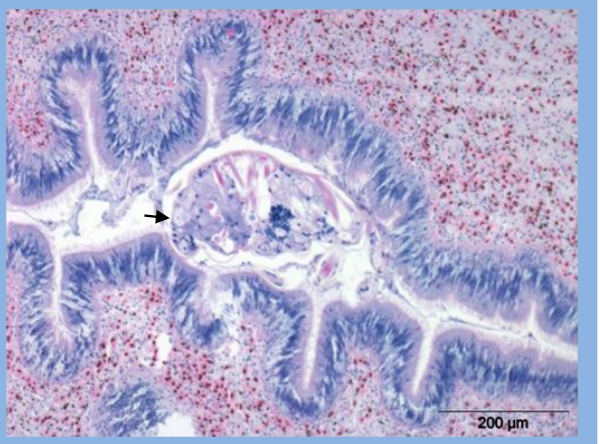
Turbelarian, *Urastoma* sp en banquías de *Crassostrea rhizophorae*



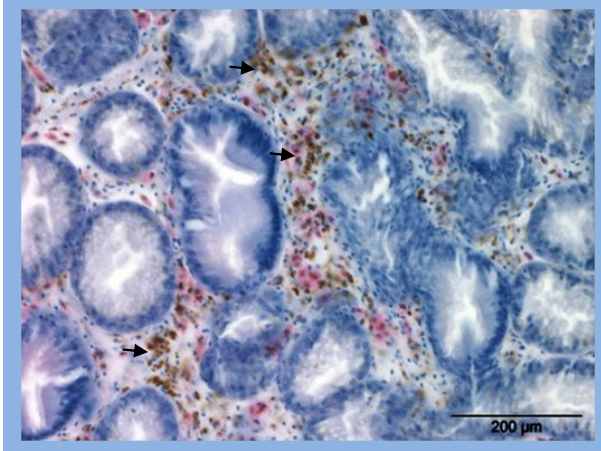
Turbelarian indiferenciado en gónada de *Polymesoda solida*



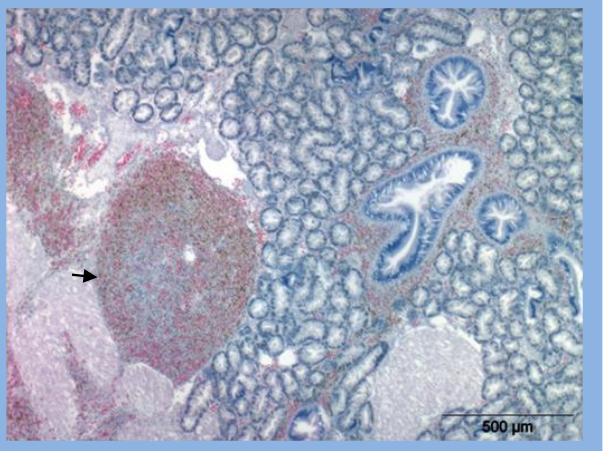
Infiltración hemocítica difusa alrededor de túbulo de glándula digestiva y conectivo de gónada de *Anadara tuberculosa*



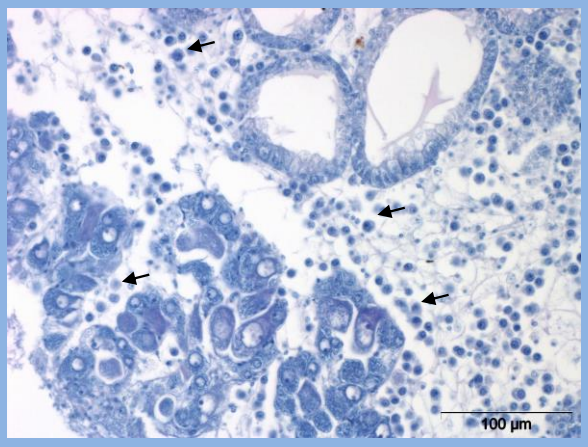
Copépodo indiferenciado en túbulo de glándula digestiva de *Anadara tuberculosa*



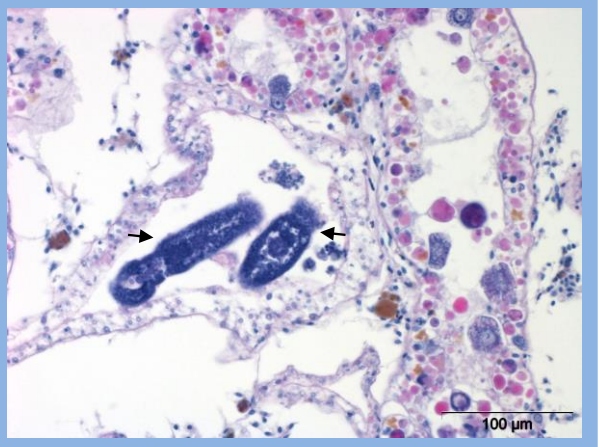
Células pardas en tejido conectivo de glándula digestiva en *Anadara tuberculosa*



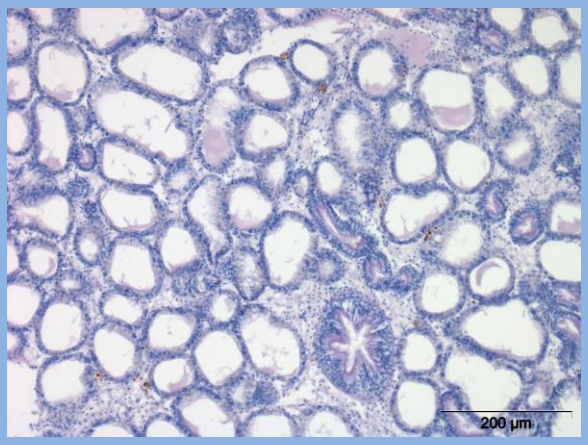
Granulocitoma entre glándula digestiva y conectivo de gónada en *Anadara tuberculosa*



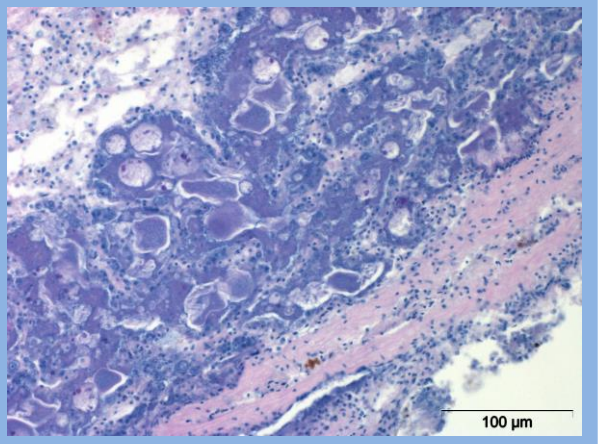
Células hemocíticas con neoplasia en tejido conectivo de glándula digestiva y gónada en *Crassostrea rhizophorae*



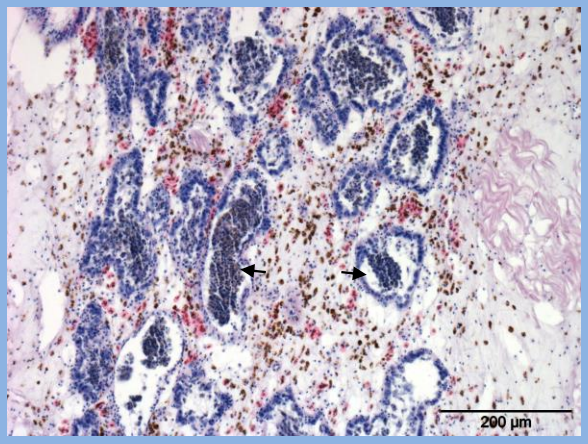
Células germinales de esporoquistes de *Bucephalus* sp en conectivo de gónada en *Polymesoda solida*



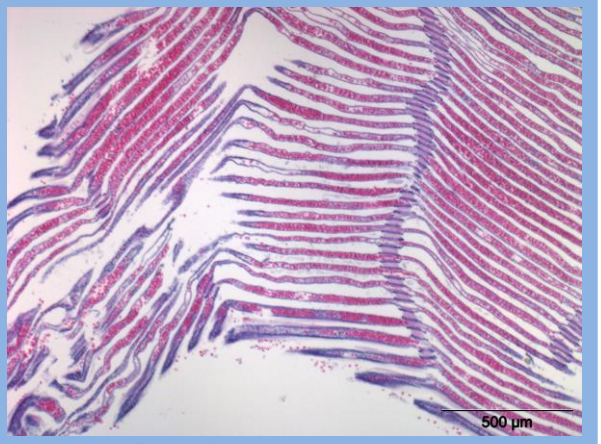
Atrofia en túbulos de glándula digestiva en *Crassostrea rhizophorae*



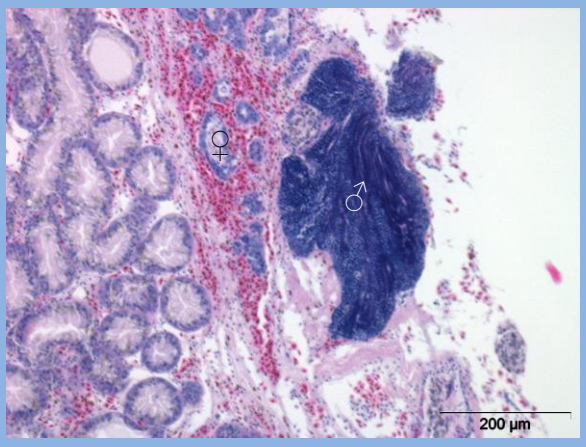
Atresia en ovocitos de gónada en *Crassostrea rhizophorae*



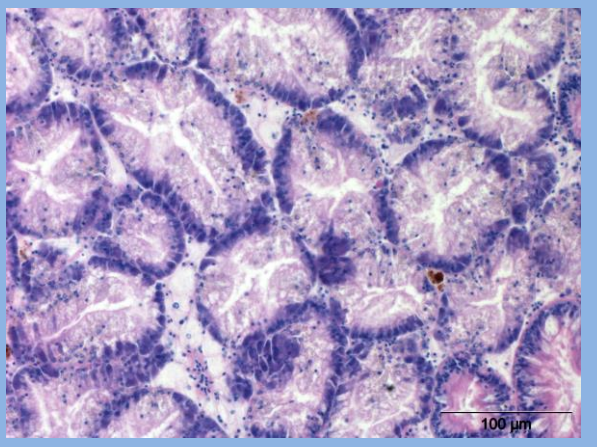
Apoptosis en folículos de gónada en *Anadara tuberculosa*



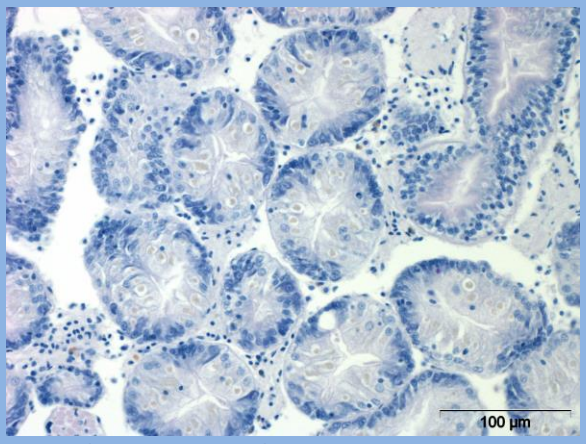
Infiltración hemocítica en branquias de *Anadara tuberculosa*



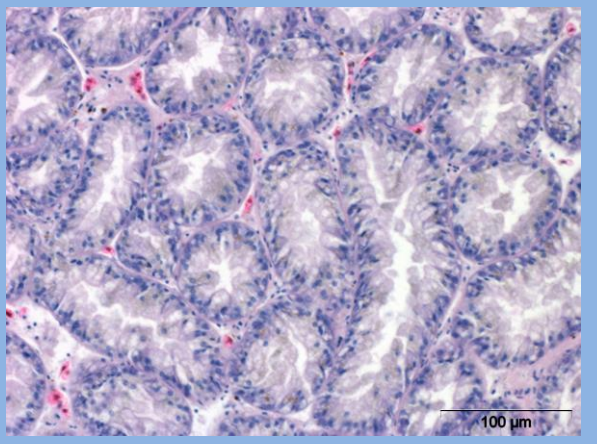
Intersex en gónada: células germinales de hembra y macho en *Anadara tuberculosa*



Túbulos casi ocluidos (glándula digestiva normal) de *Crassotrea rhizophorae*



Túbulos casi ocluidos (glándula digestiva normal) de *Polymesoda solida*



Túbulos casi ocluidos (glándula digestiva normal) de *Anadara tuberculosa*

Anexo 7. Figuras de los sitios de muestreo e investigadores que participaron en la primera colecta de muestras en el Caribe (Nicaragua y Colombia) y Pacífico de Nicaragua.

Fig. 1. Fotos de los sitios de muestreo en el Caribe de Nicaragua. De izquierda a derecha y de arriba abajo: a) En Bluefields: Half Way Cay; Punta San Gabriel y Punta Lora; b) en Laguna de Perla: Pigeon Cay.

Fig. 2. Fotos de los sitios de muestreo en el Caribe de Colombia. De izquierda a derecha y de arriba abajo: a) En Santa Marta: Taganga y Marina de Santa Marta; b) en Cartagena: Barú; Isla Brujas e Isla Maparadita.

Fig. 3. Fotos de los sitios de muestreo en el Pacífico de Nicaragua. De izquierda a derecha y de arriba abajo: a) En la Reserva Natural de Padre Ramos: Estero Venecia y Estero La Virgen; b) en Corinto: Isla Machuca.

Fig. 4. Fotos del primer muestreo de campo (época de lluvia) en el Caribe de Colombia. Equipo de investigadores que participaron: N Etxabarria, L Garmendia, M Ortiz-Zarragoitia, A Luna, S Casseres y L Villamil.

Fig. 5. Fotos del primer muestreo de campo (época de lluvia) en el Caribe de Nicaragua. Equipo de investigadores que participaron: E Rivas, L Garmendia, O Gonález, M Ortiz-Zarragoitia, N Etxabarria.

Fig. 6. Fotos del primer muestreo de campo (época de lluvia) en el Pacífico de Nicaragua. Equipo de investigadores que participaron: J Trinidad, O Gonález, M Navarrete, D Padilla.