ORIGINAL RESEARCH ARTICLE

Assessment of a sheltered euhaline area of the southeastern Bay of Biscay to sustain bivalve production in terms of phytoplankton community composition

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Abstract This study describes the phytoplankton community in sheltered euhaline waters of the Basque coast (southeastern Bay of Biscay). Phytoplankton composition, cell size, abundance, biomass and the presence of potentially toxic taxa, together with chlorophyll *a*, nutrients and hydrographic and optical conditions were measured, from August 2016 to August 2017, in the Mutriku port, with the main aim of assessing the suitability of the phytoplankton community as a food resource for bivalves. The water column in Mutriku showed the typical environmental conditions of Basque marine waters, with no significant nutrient enrichment caused by anthropogenic pressures. Haptophytes represented the greatest contribution to cell abundance (31—47%), and diatoms were the dominant group in terms of biomass (52—79%), which could favour mussel growth due to their high fatty acid content. In addition, the size

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structure of the phytoplankton community was suitable for mussel ingestion, since the predominant cell size was 2—20 μm. Regarding toxic phytoplankton, the genera that pose a risk for human health and those that affect negatively mussel physiology and survival were considered. Altogether, ten toxic phytoplankton taxa were identified, contributing in less than 5% to the total cell abundance of Mutriku. However, median chlorophyll *a* concentration was low (0.5 μg L^{-1}), reflecting the oligotrophic conditions of the area. Therefore, even if the composition of the phytoplankton community could be favourable for bivalve aquaculture, biomass values are low compared to other zones of bivalve production.

1. Introduction

Phytoplankton, as major primary producers in marine coastal systems, play an indispensable role in sustaining the pelagic food webs (e.g., Reynolds, 2006) and maintaining the ecosystem's healthy structure and functioning (e.g., Malone et al., 2016). In addition, phytoplankton communities exhibit large variations, mainly as a response to changes in abiotic factors driven by meteorological and hydrographic processes, but also because of biotic factors like grazing, competition, parasitism and microbial attack (Granéli and Turner, 2006).

Phytoplankton composition and dynamics are very important for shellfish aquaculture, being these organisms the main source of energy for filter-feeder bivalves like mussels (e.g., Petersen et al., 2008). However, not all phytoplankton species have either an appropriate cell size for ingestion or a good nutrient composition for the growth of bivalves (e.g., Brown, 2002; Marshall et al., 2010). The ingestion and retention efficiency of mussels depends on the cell size of the phytoplankton species, and although there is controversy about the most appropriate particle-size, most of the studies agree that a size range of 4—45 μm is the most suitable for high food depletion (Cranford et al., 2014). Moreover, several studies observed low retention of picophytoplankton (0.2—3.0 μm) in mussel rafts (Petersen et al., 2008) and long-line culture systems (Cranford et al., 2008). When it comes to nutrient composition, since lipids are the principal source of energy for bivalve larvae, the nutritional value of phytoplankton species principally belongs to their lipid content, especially essential fatty acids (EFA), which varies depending on the species or group (Marshall et al., 2010; Pettersen et al., 2010). In addition, several studies recognise that the nutritional value of a multispecies algal diet (at least one species of flagellate and one type of diatom) is higher than that of a monoculture, having synergistic effects on mussel growth (Pettersen et al., 2010; Stromgren and Cary, 1984).

On the other hand, phytoplankton can be problematic for shellfish aquaculture due to the potential for synthesising toxins that some phytoplankton species or strains have. When bivalves filter toxic phytoplankton, toxins are actively accumulated and concentrated in the hepatopancreas and get transferred to higher trophic levels, becoming a serious risk to humans and other consumers such as marine mammals or sea birds (e.g., Anderson, 2009; Davidson and Bresnan, 2009; Lawrence et al., 2011). Amnesic, paralytic and diarrheic shellfish poisonings (ASP, PSP and DSP, respectively) are some of the most common syndromes caused by phytotoxins in humans. In order to avoid the intoxication of consumers, closures of shellfish harvesting areas occur regularly all over the world, which leads to economic losses. In addition, there is evidence that toxic phytoplankton may affect filtration, feeding, growth, valve closure, byssus production, oxygen consumption, cardiac activity and survival in marine bivalves (Cao et al., 2018), which also affects the aquaculture industry negatively.

Globally, shellfish aquaculture production has undergone a major increase in recent years. In Spain, the overall aquaculture production was 293,000 t in 2015 and 77% of it (225,000 t) corresponded to mussel (*Mytilus galloprovincialis*) aquaculture (De la Figuera, 2017). More than a 95% of Spanish mussel production is carried out in Galicia (northwestern Spain), where mussels are cultivated in the coastal inlets (Rías) by means of floating rafts (Eurofish, 2016). The interaction between the coastal upwelling and the circulation patterns in the Rías leads to a massive response in the productivity of phytoplankton populations inside these inlets, even during weak upwelling events along the coast. This response of the phytoplankton provides the filter-feeding organisms with food of a high quality that determines high absorption efficiency, while the characteristics of the Rías maintain the seston concentrations at levels below the threshold of pseudo-faeces production (Figueiras et al., 2002). These favourable conditions make Galicia one of the largest mussel producers worldwide (Romalde et al., 2018).

Recently, in the Basque Country (north of Spain, southeastern Bay of Biscay), where hydrographic and physico-chemical conditions differ from Galicia, there is an increasing interest in shellfish aquaculture, and several studies have been conducted in order to establish this activity in offshore waters (Azpeitia et al., 2016, 2017). Thus, the commercial production of mussels began in 2019 with longlines in open marine waters (approximately 2 km offshore) in the area called "Mendexa". So far, bivalve production has not started in inshore areas, which are scarce, and most of them sustain activities incompatible with aquaculture. However, in 2016, a raft was installed in a sheltered area within a small port ("Mutriku") for the experimental culture of bivalves.
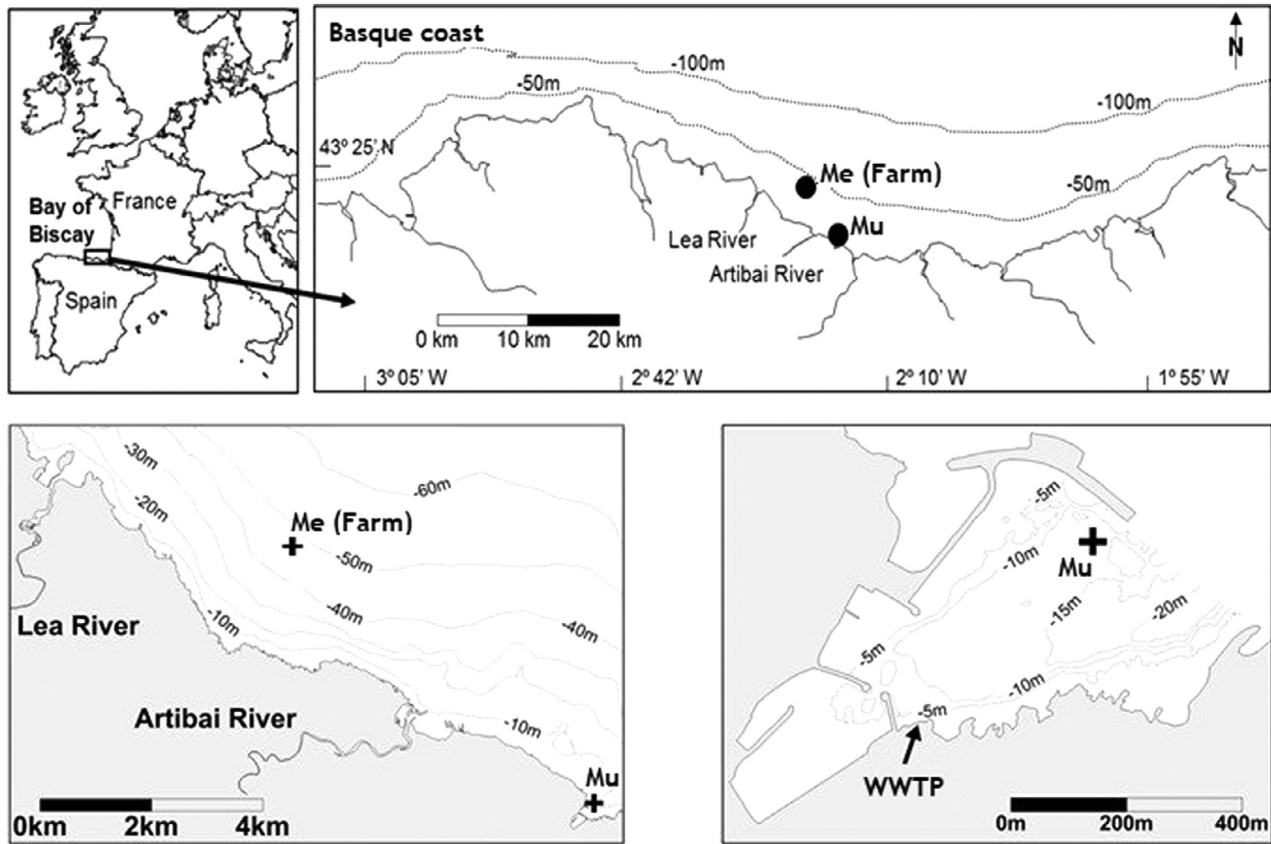
Figure 1   Study area and sampling station. On the top: the Basque coast, the location of the bivalve farm of "Mendexa" (Me) and "Mutriku" (Mu) station, within the context of the Bay of Biscay. Lower panels: the location of "Mendexa" (Me) and the station "Mutriku" (Mu); on the right, the Mutriku port is depicted with the sampling station and the discharge point of a Wastewater Treatment Plant (WWTP).

Several studies have been carried out that address phytoplankton communities in the southeastern Bay of Biscay. Most of these studies were focused on describing the phytoplankton communities' taxonomic composition and dynamics in estuaries and open marine waters (e.g., Batifoulier et al., 2013; Garmendia et al., 2011; Orive et al., 2010; Seoane et al., 2005; Seoane and Orive, 2012; Trigueros and Orive, 2000). However, when it comes to the study of phytoplankton in relation with bivalve aquaculture, just a few studies have been carried out, all of them in open marine waters (Muñiz et al., 2017, 2018, 2019) and just one that included an inshore area (Bilbao et al., 2020). Recently, Azpeitia et al. (2019) assessed the annual settlement and recruitment patterns of *Mytilus galloprovincialis* in several inshore areas on the Basque coast, concluding that they could be suitable for future seed gathering for the mussel aquaculture industry. This research addresses the phytoplankton community as a food resource for bivalves and as phytotoxins producers in an inshore area, the Mutriku port (Spanish Basque coast). This is relevant for the potential development of aquaculture in the southeastern Bay of Biscay, since the previous studies on this topic were mainly limited to open marine waters whose environmental conditions, such as light and nutrient availability for phytoplankton, could be different.

In this context, we have examined the composition, cell size, abundance and biomass of the phytoplankton commu-

nity and the presence of potentially toxic taxa, from August 2016 to August 2017, in the Mutriku port, with the aim of analysing, in detail, the suitability of this area to sustain shellfish aquaculture. In addition, we have compared the results with the studies conducted in open waters in this region, in terms of phytoplankton community composition. These results could contribute to a better knowledge of the phytoplankton community composition and dynamics in this type of systems (i.e., sheltered euhaline waters of the southeastern Bay of Biscay).

2. Material and methods

2.1. Study area

The Spanish Basque coast is placed in the southeastern Bay of Biscay and has an extension of ca. 100 km (Figure 1). The area is described as an exposed littoral coast of high energy, mainly erosional, with large cliffs (Cearreta et al., 2004). The climate is rainy, temperate and oceanic, with moderate winters and warm summers (Fontán et al., 2009). Consequently, the seasonal pattern of major climatic and hydrographic conditions corresponds to that of temperate sea areas: winters characterised by water column mixing, which leads to nutrient input from deep waters to the surface; springs with increases in surface water temperature

and relative stabilisation due to solar irradiance; summers with stratification resulting from greater solar irradiance; and autumn mixing processes induced by the cooling of surface waters and southerly and westerly winds (Fontán et al., 2008; Valencia et al., 2004).

Along the Basque coast, the tide is semi-diurnal, the mean tidal range is approximately 1.5 m at neap tides and 4 m at springs tides. The region is defined as 'low meso-tidal' during neaps and 'high meso-tidal' during spring tides (González et al., 2004). In addition, this coast is influenced by 12 rivers, which are torrential in character (Ferrer et al., 2009) and annually provide ca. 150 m^3 s^{-1} of fresh water to the coastal water bodies. This freshwater supply leads to alterations in the physico-chemical composition of the shallow waters and often an increase in nutrient concentration in inner shelf waters (Ferrer et al., 2009; Valencia et al., 2004).

This study was carried out at an experimental bivalve culture platform located in the port of Mutriku, on the Basque coast (Figure 1). The station is located in the outer part of a marina (43°18.7′N, 2°22.6′W), and it is protected from the wave action by a jetty. Its depth is 15 m, approximately, and although rivers do not discharge into this harbour, the station receives the effluents from a Wastewater Treatment Plant (WWTP) that serves the surrounding population (ca. 5,300 inhabitants).

2.2. Sampling/laboratory strategy and data acquisition

Samplings were carried out over 13 months (from August 2016 to August 2017), and samples were collected on a monthly basis at two depths (3 m and 10 m) (supplementary material Table A1).

Information on tidal conditions and water height is also presented in Table A1. Although the samplings were not conducted exactly at the same phase of the tide, this factor was controlled to some extent and it only caused a slight variation in the water height. Most of the samplings (10 out of 13) took place at neap or medium tides. Furthermore, 10 samplings were closer to low water time than to high water time.

The water height was measured using a GPS sounder (Garmin Ltd.) on boat. The high and low water times, as well as the tidal coefficient, were those provided by AZTI for Pasaia, a standard port located at about 40 km from Mutriku. The tidal coefficient was used to distinguish spring tides (> 0.85) from neap tides (< 0.55), following a criterion previously used for the Basque coast (González et al., 2004). This coefficient was calculated by dividing the tidal semi-amplitude by 1.967, which is the constant value of the Pasaia Port (Instituto Hidrográfico de la Marina, 1992).

Several measurements were undertaken *in situ* for the characterisation of the physico-chemical conditions. Secchi disk depth was measured in order to estimate water transparency, and a Seabird25 CTD was used for the measurement of temperature, salinity, density (Sigma Theta), Light Transmission (LT), Photosynthetically Active Radiation (PAR), chlorophyll *a*, oxygen concentration, oxygen saturation and pH at the studied depths. The CTD was calibrated, regularly, with water samples filtered through What-

man GF/F filters and analysed by spectrophotometry, after pigment extraction in acetone.

Water samples were collected with Niskin bottles at the two depths. These samples were employed for the analysis of Suspended Solids (SS), turbidity, Total Organic Carbon (TOC), dissolved inorganic nutrients and phytoplankton identification and counting.

In the laboratory, the concentration of SS was measured following the indications of Clesceri et al. (1989), after filtration of the water through Whatman GF/C filters. Turbidity of seawater was measured using a turbidimeter (2100 Turbidimeter, HACH; Loveland, Colorado, USA). For TOC, an analyser (TOC-V CSH/CSN, Shimadzu Corporation, Kyoto, Japan) was employed in non-purgeable organic carbon (NPOC) mode, as Grasshoff et al. (1983) described. When it came to nutrients (ammonium, nitrite, nitrate, silicate and phosphate), the measurements were carried out using a Continuous-Flow Autoanalyser (Bran + Luebbe Autoanalyser 3, Norderstedt, Germany), following the colourimetric methods described by Grasshoff et al. (1983). The quantification limit for ammonium, nitrate and silicate was 1.6 μmol L^{-1}, for nitrite, it was 0.4 μmol L^{-1} and for phosphate, it was 0.16 μmol L^{-1}. In order to calculate average concentrations, a quantity equal to 50% of the limit was assumed for the measurements that did not reach the quantification limit.

Moreover, in order to describe hydrographic conditions, two variables were estimated: the light extinction coefficient and the depth of the photic zone. The light extinction coefficient (k) was calculated graphically based on the general equation of the vertical extinction coefficient (see below), and that was obtained with the representation of the PAR measured by the CTD at every meter of the water column.

$$I_Z = I_0.e^{-kz}$$

where I_z [E m^{-2} d^{-1}] is the radiation received at a specific depth, I_0 is the radiation at a surface and z is the specific depth [m].

The k was then applied to estimate the depth of the photic layer using the following equation: photic zone [m] = 4.605/k.

The phytoplankton community was analysed according to cell concentration [cell L^{-1}] and biomass [μg C L^{-1}]. Water samples used for phytoplankton identification were fixed with acidic Lugol's solution (0.4% v/v), immediately after collection, and stored in 125 ml topaz borosilicate, in a dark and cool (4°C) place, until analysis. For taxonomic identification and cell counting, subsamples of 50 ml were analysed following the Utermöhl sedimentation method (Edler and El-brächter, 2010) under a Nikon diaphot TMD inverted microscope. The whole chamber was analysed at low magnifications (100 ×) to count the larger and less abundant taxa. For more abundant cells, transects at different magnifications (100 ×, 200 × or 400 ×) were analysed depending on the organism's abundance and size. For chain-forming taxa, cells were counted, not chains. Most of the diatoms and dinoflagellates were identified until genus level, and the nomenclature of the identified taxa was standardised according to AlgaeBase (Guiry and Guiry, 2018). However, some of them could not be identified to that level and were classified in the following groups: pennated diatoms, cen-

tric diatoms, thecated dinoflagellates, athecated dinoflagellates and flagellates.

In order to calculate the phytoplankton biomass, the biovolume was determined by assigning each taxon a mean equivalent spherical diameter (ESD), mostly based on Olenina et al. (2006), taking into account cells shape and size. The biovolume of the taxa that could not be accurately calculated from Olenina et al. (2006) was determined based on Muñiz et al. (2019). Then, the biomass was calculated using the equation reported for marine phytoplankton by Montagnes et al. (1994): Biomass $= 0.109 \times$ Volume$^{0.991}$, where biomass is expressed in pg C cell^{-1} and volume is expressed in μm^3.

In addition, in order to determine which taxa had to be considered potentially toxic, the Taxonomic Reference List of Harmful Micro Algae from the Intergovernmental Oceanographic Commission of the UNESCO was used as a checklist (Moestrup et al., 2009; http://www.marinespecies.org/hab/, accessed on 30 June 2018). As a measure of precaution, when a genus contained both toxic and non-toxic species, the whole genus was considered as potentially toxic if the identification could not reach the species level. Moreover, risk of shellfish poisoning was determined by applying alert levels of cell concentration to the genera causing the three main syndromes of concern in this study area (ASP, DSP and PSP): *Pseudo-nitzschia* spp., *Dinophysis* spp. and *Alexandrium* spp., respectively. The threshold levels employed were (Swan and Davidson, 2012) 50,000 cells L^{-1} for *Pseudo-nitzschia* spp., 100 cells L^{-1} for *Dinophysis* spp. and "presence" for *Alexandrium* spp.

2.3. Statistical analyses

Main statistical parameters (range, median and arithmetic mean) were calculated for physico-chemical and hydrographic variables and phytoplankton cell abundance and biomass, for the whole year and for each studied depth. The comparison between the studied depths was done in a descriptive way rather than statistically.

3. Results

3.1. Hydrographic and physico-chemical conditions

In order to describe the environmental conditions of the water that could most be related to phytoplankton communities, Figure 2 shows the intra-annual variability of some of the variables measured in Mutriku. The range (minimum—maximum) and the medians for the whole year can be consulted in Table B1 and the variability of additional variables in Figure B1 (supplementary material, Appendix B).

There were no parameters among the several hydrographic and physico-chemical conditions measured that showed consistent and remarkable differences between 3 m and 10 m (Figure 2 and Figure B1).

When it comes to the variations among samplings, water column depth varied in a range of 3 m (from 13 m to 16 m), with a median value of 14 m (Table B1). Salinity variations were small, it ranged from 34.28 to 35.47 at 3 m, and

from 34.36 to 35.52 at 10 m; therefore, Mutriku presented euhaline conditions throughout the study period.

Median seawater temperature was 15°C. This parameter showed a marked seasonal variability, with the highest temperatures being recorded in summer and the lowest in winter. Optical conditions also showed notable changes throughout the study period. Secchi disk depth, light transmission and the photic layer depth registered the highest values in spring and the lowest in winter. This was in accordance with the patterns of the light extinction coefficient (k) and turbidity, which, in contrast to the parameters named above, registered their maxima in winter.

Chlorophyll a concentration was low, with an average concentration of 0.5 ± 0.3 μg L^{-1} and 0.6 ± 0.3 μg L^{-1} at 3 m and 10 m respectively, and a median of 0.5 μg L^{-1} at both depths. Values ranged between 0.1 μg L^{-1} (in autumn, at 3 m) and 1.4 μg L^{-1} (in winter, at 10 m).

With regard to inorganic nutrients, although sporadic peaks could be observed, generally they did not show very high concentrations. Nitrate and silicate registered their maxima in winter (at 3 m), being 6.3 μmol L^{-1} and 3.1 μmol L^{-1}, respectively. Ammonium, on the contrary, registered its maximum in spring (at 10 m) with 6.4 μmol L^{-1}. Phosphate did not exceed 0.5 μmol L^{-1}, and nitrite was recorded above the quantification limit just twice (December and January) during the study period.

3.2. Phytoplankton composition, abundance and biomass

Concerning phytoplankton diversity, a total of 87 taxa were identified, 81 of them until genus level at least. Dinoflagellates represented the group with the highest number of taxa, with 46% of the total taxa identified, followed by diatoms, with 36%. The list of these taxa, their appearance frequency and their abundance range (minimum—maximum) can be consulted in the supplementary material (supplementary material Appendix C).

Phytoplankton total cell abundance ranged from 1.9×10^4 to 7.1×10^5 cells L^{-1}. Both cell abundance and biomass presented several peaks throughout the year, which, in general, were associated. At 3 m, the maximum cell abundance, which was also the absolute maximum, was observed in July, when the community was characterised by a large proportion of haptophytes and cryptophytes (Figure 3). However, at this depth, the maximum biomass (85.4 μg C L^{-1}) was recorded in October, coinciding with the dinoflagellate maximum abundance. At 10 m, on the contrary, the maximum phytoplankton abundance (6.2×10^5 cells L^{-1}) and biomass (94.4 μg C L^{-1}) were registered in the same month, February, with a community dominated by diatoms, *Thalassiosira* spp. concretely.

Regarding phytoplankton community composition (Figure 3), similar results were obtained for both depths. At 3 m, the groups that contributed the most to the total cell abundance were haptophytes and cryptophytes, with an average contribution of 37% and 36%, respectively. At 10 m, haptophytes were also the most contributing group (41%) in terms of abundance; however, the average contribution of diatoms was higher than at 3 m (24%), the same as cryptophytes. Nevertheless, the contribution of these groups
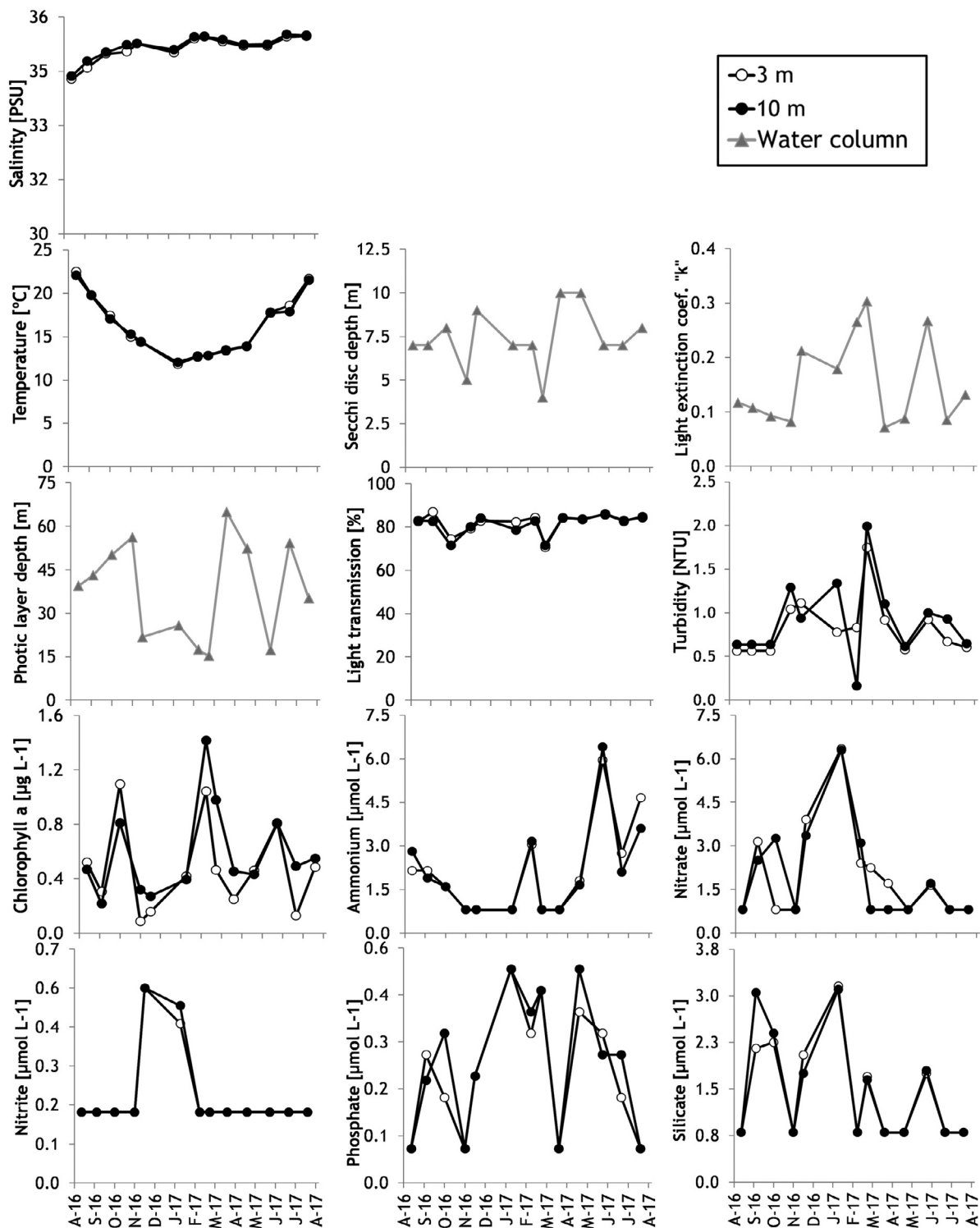
Figure 2   Intra-annual variability of the main hydrographic and physico-chemical conditions in Mutriku. In the case of nutrients, for values below the quantification limit, the value of half of the limit is represented. Some additional variables (suspended solids, total organic carbon and oxygen) are shown in supplementary material (supplementary material Appendix B).

to the total phytoplankton abundance differed from their contribution to the total biomass. Diatoms were, by far, the most contributing group in Mutriku terms of biomass (52% at 3 m and 79% at 10 m), followed by dinoflagellates (23% and 8%, at 3 m and 10 m, respectively). On the other hand, haptophytes and cryptophytes, two of the most abundant groups, only reached 7—8% of the contribution to the total phytoplankton biomass. Diatoms recorded their highest contribution values in winter, dinoflagellates in autumn and haptophytes and green algae in summer. The
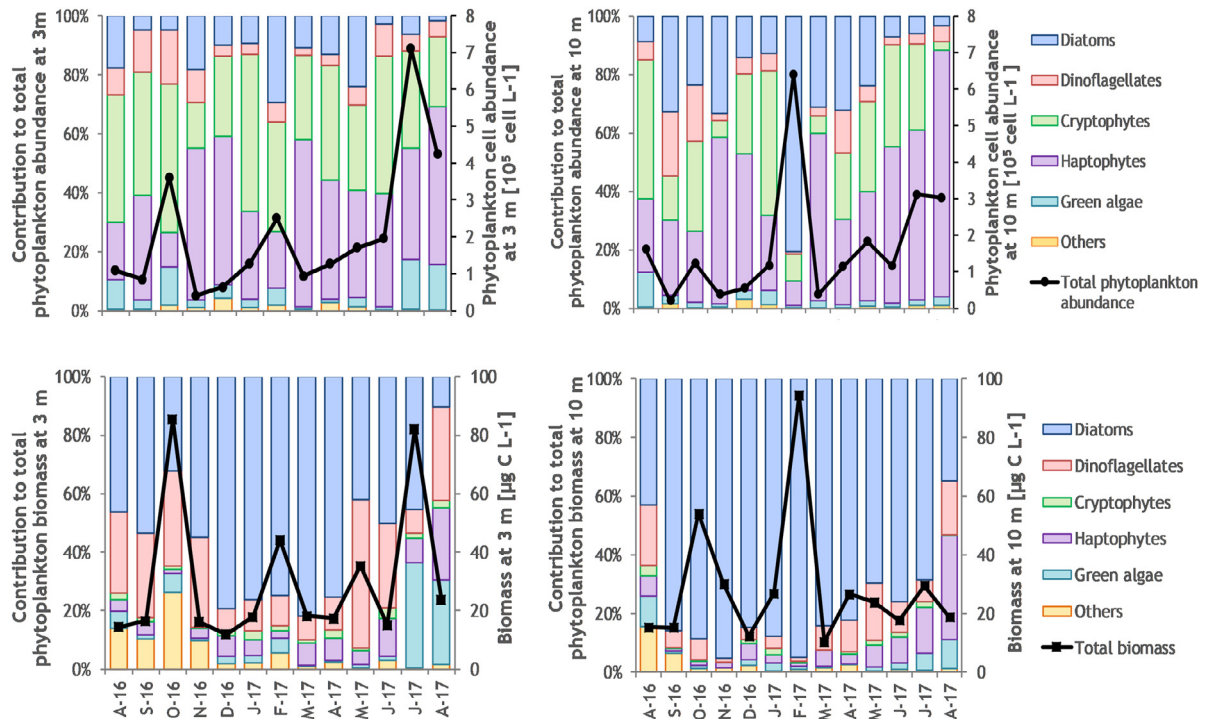
Figure 3   Contribution to total phytoplankton abundance (upper panels) and biomass (lower panels) of the main phytoplankton groups at both 3 m (left) and 10 m (right) depths.

Table 1   List of the most abundant taxa in each phytoplankton group at the two sampled depths, together with their appearance frequency throughout the year, maximum abundance and date when the maximum was registered.

Group	Depth [m]	Most abundant taxon	Appearance frequency [%]	Maximum abundance [cells L^{-1}]	Date of maximum abundance
Haptophytes	3	Prymnesiales	100	2.7×10^5	Jul. 2017
	10	Prymnesiales	100	2.5×10^5	Aug. 2017
Cryptophytes	3	*Plagioselmis* sp.	100	2.3×10^5	Jul. 2017
	10	*Plagioselmis* sp.	100	9.1×10^4	Jul. 2017
Diatoms	3	*Pseudo-nitzschia* spp. $< 3\ \mu$m	77	2.3×10^4	Feb. 2017
	10	*Thalassiosira* spp. $< 20\ \mu$m	8	4.2×10^5	Feb. 2017
Dinoflagellates	3	Athecated dinoflagellates $< 20\ \mu$m	100	3.8×10^4	Oct. 2016
	10	Athecated dinoflagellates $< 20\ \mu$m	92	1.5×10^4	Apr. 2017
Green algae	3	*Tetraselmis* sp.	100	5.1×10^4	Jul. 2017
	10	*Tetraselmis* sp.	100	1.9×10^4	Aug. 2016

only difference was recorded for cryptophytes, which, at 3 m, reached the maximum contribution in autumn and, at 10 m, in spring.

The most abundant haptophyte and cryptophyte taxa were Prymnesiales and *Plagioselmis* sp., respectively, regardless of the depth and season, and they registered their maximum abundances in the summer months of 2017 (Table 1). In addition, both of these taxa appeared in every

water sample analysed during this study. Among dinoflagellates, athecated cells ($< 20\ \mu$m) were the most abundant taxon at both depths and were observed in most of the samples. The maximum athecated cell abundance was registered at different months depending on the depth, in October at 3 m and in April at 10 m. Diatoms were the group showing the greatest variability in the dominant taxon, since while at 3 m, *Pseudo-nitzschia* spp. ($< 3\ \mu$m) was
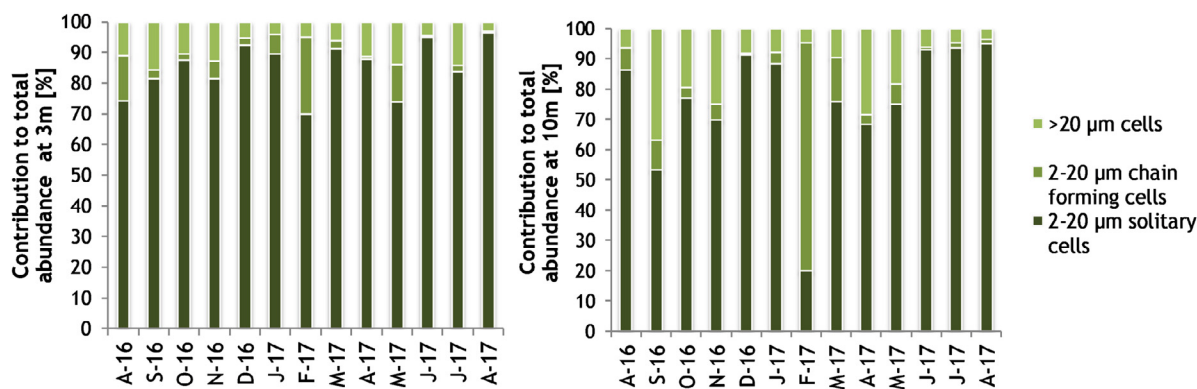
Figure 4   Contribution percentage of the two size-fractions considering the 2—20 μm at both the 3 m (left) and 10 m (right) depths during the study period and highlighting the 2—20 μm size-fraction's chain forming diatoms. Note: when referring to chain forming cells, the 2—20 μm size refers to each cell size, not the whole chain.

the most abundant diatom group, *Thalassiosira* spp. (< 20 μm) dominated at 10 m. When it comes to green algae, *Tetraselmis* sp. was the most abundant taxon, appearing in every analysed sample and registering its maximum values in summer.

When analysing the quality of the phytoplankton as a food resource for mussels, the observed community was assessed by considering cell size and toxicity. It was found that the community was dominated by cells ranging 2—20 μm (ESD) (Figure 4). The average contribution to the total phytoplankton cell abundance of this group along the study period was 90.9% at 3 m and 86.2% at 10 m. Nevertheless, part of this contribution corresponded to chain-forming diatoms, specifically 6.6% and 11.7% of the 2—20 μm size cells at 3 m and 10 m, respectively.

Regarding potentially toxic phytoplankton, ten taxa were registered. Among them, *Pseudo-nitzschia* spp. was the only potentially toxic diatom, whereas eight taxa belonged to the dinoflagellates group: *Alexandrium* spp., cf. *Azadinium* spp., *Dinophysis* spp., *Dinophysis acuminata*, *Goniaulax* cf. *spinifera*, *Ostreopsis* cf. *siamensis*, *Phalacroma* spp. and *Takayama* sp. From other groups, only one haptophyte (*Phaeocystis globosa*) was identified as a potentially toxic taxon. The average contribution of the potentially toxic taxa found in Mutriku to the total phytoplankton abundance was 3.6% at 3 m and 4.5% at 10 m.

Regarding the taxa responsible for producing the most concerning poisoning syndromes (*Pseudo-nitzschia* spp. of ASP; *Alexandrium* spp. of PSP; *Dinophysis* spp. and *Phalacroma* spp. of DSP), several differences were found between their appearance frequencies. While *Pseudo-nitzschia* spp. was the only taxon identified in every sample analysed, *Dinophysis* spp. appeared in 23% of the samples and *Alexandrium* spp. and *Phalacroma* spp. in 7.7% of them. In addition, these last two genera were only present at 3 m. When it comes to the exceedance of the abundance thresholds that determine risk for shellfish toxicity, alert limits for shellfish poisoning were registered in four of the 13 sampling days carried out in Mutriku, twice by *Alexandrium* spp. (at 3 m) and once by *Pseudo-nitzschia* spp. and *Dinophysis* spp. (at 10 m and 3 m respectively). Among the rest of the toxic taxa found in Mutriku, *Ostreopsis* cf. *siamensis* was the most frequent, appearing in 23% of the samples at 3 m

and in 31% at 10 m. *Goniaulax* cf. *spinifera*, *Takayama* sp., cf. *Azadinium* spp. and *Phaeocystis globosa* appeared in less than 16% of the samples, being cf. *Azadinium* spp. the most frequent (identified in 15.4% of the samples).

4. Discussion

Waters in Mutriku showed some of the classical environmental conditions and seasonal cycles of temperate coastal areas, which have previously been described for Basque marine waters (Valencia et al., 2004). Temperature, for example, showed a seasonal warming and cooling pattern, which is thought to be highly related to air temperature in the Basque shelf waters (Valencia et al., 2003) and has previously been recorded in several studies (e.g., Muñiz et al., 2019). Optical conditions in the area also varied a lot, and even if they did not show a clear seasonal pattern, turbidity registered its maximum values in winter. In this period, there is more water turbulence and, consequently, a higher chance for sediment resuspension, which is a common phenomenon in shallow waters and increases turbidity (González et al., 2004).

When it comes to the seasonal variation of dissolved nutrients, the effects of the thermal cycle and the succession of mixing and stratification conditions could be detected, as in other zones of the SE Bay of Biscay (Valencia and Franco, 2004). In Mutriku, nitrate and silicate registered their maximum values in winter, similarly to the patterns seen in Arcachon Bay, a shallow mesotrophic estuary (Glé et al., 2008), which can be attributed to the turbulent mixing processes and the subsequent input of nutrients to the surface waters from the deeper layer (Valencia and Franco, 2004). Ammonium, on the contrary, registered its maximum in spring and, together with nitrate, is one of the most abundant nutrients in Mutriku. It is known that sewage discharges are rich in ammonium and phosphate; nevertheless, the WWTP that discharges its waters to Mutriku performs the biological treatment, which targets residual organic matter and suspended solids present in wastewater after the primary treatment stage and includes the removal of dissolved nutrients (Carey and Migliaccio, 2009). In addition, according to the data available in the Basque

Water Agency (URA, https://www.uragentzia.euskadi.eus/u81-0002/es/, accessed on 2 April 2020), the nutrient concentrations registered in Mutriku were within the range of concentrations observed in nearby areas that are not subject to direct discharges from WWTPs, like the open marine waters of Sopelana ("L-N20" station) or Orio ("L-O10" station). Moreover, the annual average of ammonium, nitrate and phosphate concentrations in Mutriku were below the ones recorded by Revilla et al. (2009) along the Basque coast in a six-year-long survey for 13 different nearshore sites. Therefore, this might indicate that, in Mutriku, the effects of the WWTP were not significant when it comes to nutrient enrichment, as previously concluded by Bilbao et al. (2020).

Regarding the phytoplankton attributes studied here, the median value of chlorophyll a (chl a) concentration was 0.5 μg L^{-1}, with a maximum of 1.4 μg L^{-1}, reflecting the oligotrophic conditions of the area. Orive et al. (2004) indicated that the chl a sub-surface maximum in the Basque shelf waters was usually between 2 μg L^{-1} and 4 μg L^{-1}. Recent studies in the inner Bay of Biscay (Fanjul et al., 2017) registered similar chl a concentrations to Mutriku in Urdaibai estuary, where the annual mean concentration ranged from around 0.5 μg L^{-1} to 1 μg L^{-1} in most of the studied years (1998—2013). On the contrary, in Bilbao estuary, the annual mean of chl a concentration was above 1 μg L^{-1} in all the years of the study period (Fanjul et al., 2017). This might be mostly explained by the trophic conditions of these sites, since the Mutriku port presents oligotrophic conditions similar to those of Urdaibai and many other sites of the Basque coast, differing from the mesotrophic conditions reported for Bilbao estuary. In addition, the results obtained in Mutriku are also similar to those observed in the farm of Mendexa (located in an oligotrophic area as well), where the mean annual chl a value was 0.6 ± 0.4 μg L^{-1} (Muñiz et al., 2019). Moreover, chl a concentration in surface waters of Mendexa was below 0.6 μg L^{-1} for most of the studied year, only registering higher values from February to May, and Mutriku only registered values above 0.6 μg L^{-1} in 4 of the 13 sampled months (although these were detected at any season except summer). This makes both zones similar in terms of chl a, a proxy for phytoplankton biomass, for sustaining aquaculture production. Despite that, chl a in Mutriku was very small during the entire survey in comparison with other European aquaculture sites. Studies conducted in Galician Rías, where most of the Spanish mussel production is carried out, recorded mean values of chl a between 0.7 μg L^{-1} (winter) and 13.6 μg L^{-1} (spring) (Varela et al. 2005). In addition, Spyrakos et al. (2011) also found higher mean chl a concentrations in Rías Baixas than in Mutriku, with values lower than 1 μg L^{-1} in winter, close to 5 μg L^{-1} in summer and up to 8 μg L^{-1} during spring and autumn. Studies in the Eastern English Channel (France), where shellfish farming was one of the most important aquaculture industries of Europe, also showed higher chl a concentrations than in Mutriku, in the range of 1—25 μg L^{-1} according to Jouenne et al. (2007) and between 0.9 and 18.9 μg L^{-1} according to Klein et al. (2010). However, knowing that the total biomass is an important factor for the growth of bivalves, in field studies, Wall et al. (2013) observed that the growth rates of bivalves were more related to the community composition, especially the density of certain cellular types, than to the total phytoplankton biomass.

The total phytoplankton cell-abundance range registered in Mutriku was within the values previously found at nearshore stations along the Basque coast (Muñiz et al., 2017). In Mutriku the phytoplankton community was dominated by haptophytes and cryptophytes, in terms of cell concentrations, during most of the year. Haptophytes, concretely, were especially dominant from March to August. However, these groups are mainly composed of small nanoplankton, meaning that their contribution to total biomass was small. Haptophytes are known to be one of the main components of marine phytoplankton (Latasa et al., 2005; Not et al., 2005; Rodríguez et al., 2003), especially in oceanic waters, but they can also be abundant in coastal and estuarine waters (Dahl et al., 1998). Seoane et al. (2005, 2006, 2009) described the relative importance of haptophytes in terms of abundance in the Nervión River estuary (Bilbao estuary), concluding that this group was not among the most abundant since the estuary was mainly dominated by diatoms, chlorophytes and cryptophytes. Nevertheless, Muñiz et al. (2019) reported a high abundance of haptophytes, especially in spring and summer, in the neighbouring marine waters of Mendexa, where this group was 46% of the total cell abundance, exceeding the dominance of haptophytes in Mutriku (37—41%). This dominance of haptophytes in oligotrophic waters like Mutriku or Mendexa might be explained by their small cell size and mixotrophic ability (Hansen and Hjorth 2002; Jones et al., 1993; Lessard et al., 2005). In addition, nanophytoplankton also dominates the phytoplankton community in terms of abundance in other Atlantic areas, for example, the Western English Channel (Widdicombe et al., 2010). Besides that, a study of the phytoplankton distribution between 62 and 37°N in the northeastern Atlantic showed that, in surface waters between 62 and 50°N, haptophytes were the most abundant group (Gibb et al., 2001).

Diatoms were the most important group in Mutriku in terms of biomass during the whole year. This group was especially abundant at 10 m, becoming the second most abundant group after haptophytes, and reached their maximum values in winter at both depths. Diatoms are organisms that prefer turbulent and nutrient-rich conditions, and therefore, strong vertical mixing favours their dominance (Glibert, 2016; Margalef, 1978), conditions that typically appear in winter. In addition, diatoms are generally adapted to low light levels (Brahim et al., 2015), which favours their survival in turbid conditions (Lionard et al., 2005), and tend to have significantly higher maximum uptake rates of nutrients than any other group (Litchman et al., 2006), which might explain their predominance in late winter light and nutrient conditions. Moreover, since diatom cells are nonmotile (Ross and Sharples, 2007), the higher water turbulence that is usually found in winter enables them to keep re-suspending into the surface, where growth conditions are more favourable (Ross, 2006). Previous studies in the southern Bay of Biscay also described these late winter diatom peaks (Guillaud et al., 2008; Labry et al., 2001; Muñiz et al., 2019). Muñiz et al. (2019), in particular, described for Mendexa a diatom peak in March, when diatoms made up 50% of the total cell abundance; however, during the rest of the year, diatoms contributed in 13% of the total cell abundance, which is less than in Mutriku. Regarding the contribution to total biomass, diatoms only dominated the

community in Mendexa during winter, representing between 54% and 78% of the total biomass, being less important than in Mutriku as well.

The dominance of diatoms in biomass found in Mutriku suggests favourable phytoplankton nutritional quality for mussels. Several studies have reported a direct correlation between diatoms and bivalve growth (Pernet et al., 2012; Wall et al., 2013; Weiss et al., 2007). On the other hand, haptophytes dominate in terms of cell concentration, although their small size makes their contribution to total phytoplankton biomass smaller. While diatoms are known to be rich in eicosapentaenoic acid (EPA), some haptophytes are a rich source of docosahexaenoic acid (DHA) (Catarina and Xavier, 2012). EPA and DHA are two important essential fatty acids (EFA) for bivalves; the first one has an energetic function (Martínez-Pita et al., 2012; Sánchez-Lazo and Martínez-Pita, 2012), and the second one is known for promoting growth (Parrish, 2013). Due to this, diatoms and haptophytes (especially *Isochrysis galbana* and *Pavlova lutheri*) are frequently used as feed for shellfish worldwide (Catarina and Xavier, 2012; Eikrem et al., 2017; Volkman et al., 1989, 1991). Mixed microalgal diets of these two groups are common in bivalve hatcheries and considered highly nutritious in terms of polyunsaturated fatty acids (PUFAs) (Catarina and Xavier, 2012; Knuckey et al., 2002).

Apart from that, the presence of dinoflagellates in Mutriku has to be mentioned as well. Although it is not among the most abundant groups, the contribution of dinoflagellates to the total biomass (especially at 3 m) is notable. The presence of this group is beneficial to shellfish aquaculture since dinoflagellates also contain DHA (e.g., Azpeitia et al., 2016). Moreover, Trottet et al. (2008) found that dinoflagellates, together with diatoms, show some of the highest retentions in mussels compared to other phytoplankton groups.

When it comes to the suitability of cell size for efficient ingestion and retention, the composition of the phytoplankton community in Mutriku seems favourable for the correct growth of bivalves. While some researchers concluded that the most adequate cell size is 35—45 µm (Cranford et al., 2014; Strohmeier et al., 2012), some others set the size range at 15—20 µm (Lucas et al., 1987; Stenton-Dozey and Brown, 1992). However, as it has been mentioned before, most studies agree that the size range of 4—45 µm is the most suitable for high food depletion (Cranford et al., 2014). Therefore, taking into account that the predominant cell size in Mutriku was 2—20 µm, the size structure of the phytoplankton community was suitable for mussel ingestion. Similar results were obtained in a previous study of the Basque coastal waters (Muñiz et al., 2017).

The counterpoint to the benefits of phytoplankton growth lies in the presence of species that can be toxic and pose one of the main risks to shellfish aquaculture. Some potentially toxic phytoplankton taxa were found in Mutriku, all of them were previously described in the SE Bay of Biscay (e.g., David et al., 2012, 2013; Laza-Martinez et al., 2011; Muñiz et al., 2017; Orive et al., 2008, 2010; Revilla et al., 2009; Seoane et al., 2012). Mutriku registered abundances above the alert limits for shellfish poisoning four times during the study period, once for *Pseudonitzschia* spp. and *Dinophysis* spp. and twice for *Alexan-*

drium spp., which in general coincided with the frequencies found within open coastal waters of the Basque Country (Muñiz et al., 2017). However, when directly comparing the presence of toxic phytoplankton taxa and biotoxins in Mutriku and Mendexa, Bilbao et al. (2020) found that mussels that grew in Mendexa, where the offshore farm is located, presented a statistically higher amount of okadaic acid and a higher cell abundance of *Dinophysis* spp. than in Mutriku. Among the potentially toxic species found, special attention should be paid to *Dinophysis acuminata*, which is likely the species most responsible for the high concentrations of okadaic acid (OA) in oysters and mussels in the neighbouring West French coast (Batifoulier et al., 2013; Maurer et al., 2010). Although this genus generally appears in very low concentrations (< 100 cells L^{-1}) on the Basque coast (Muñiz et al., 2017), high concentrations of *D. acuminata* (maxima of 5×10^3 cells L^{-1}) have been previously registered in euhaline waters of this region (Bilbao et al., 2020; Seoane and Orive, 2012). Indeed, OA concentrations above the banning threshold have been recently reported for mussels growing in these waters (Bilbao et al., 2020).

Besides the potential shellfish poisoning risk, the presence of toxic taxa might also threaten the development of the aquaculture industry in Mutriku due to the negative effects of toxins in mussel physiology and survival. In several studies, negative effects on energy metabolism and neural function, combined with behavioural functions such as valve closure, feeding, cardiac activity and respiration, have been observed in different bivalve species exposed to harmful marine algae and their toxins (Basti et al., 2016; Estrada et al., 2007; Haberkorn et al., 2011; Moroño et al., 2001; Ramos and Vasconcelos, 2010). In addition, some types of toxins can lead to declined reproduction and growth rates in marine bivalves, which could be a major cause of mortality in natural populations (Blanco et al., 2006; Samson et al., 2008). Some of the toxic genera found in Mutriku and their toxins are among these threatening types. Nielsen et al. (2020) proved that DST (Diarrheic Shellfish Poisoning toxins) contained by *D. acuta* had a severe negative effect on the clearance rates of mussels (*Mytilus edulis*), reduced their feeding and, therefore, may cause low-quality mussels. The effects of PST (Paralytic Shellfish Poisoning toxins) produced by *Alexandirum catanella* have also been tested in seven different bivalve species (Shumway and Cucci, 1987). In this case, the responses included shell-valve closure, siphon retraction and mucus production in *M. edulis*, while the rest of bivalves showed different reactions. In addition, saxitoxins (produced by *Alexandrium* and *Gymnodinium*) may reduce growth, reproduction and survival rates of marine bivalves like green mussels (*Perna vidiris*) according to Shumway et al. (2006).

Finally, it has to be mentioned that phytoplankton abundance and community composition can vary in a very short timescale, even within the same day (e.g., Abreu et al., 2009; Li et al., 2009). Therefore, sampling on a monthly basis could impose constraints on the efficiency at which variability can be resolved and provides data of monthly variability that might have potential errors (Jassby et al., 2005). Considering this limitation, the present study is only intended to acquire a general knowledge of the phytoplankton community on an annual scale (e.g., Muñiz et al., 2019). If the results obtained were to be extrapolated to other

areas with similar hydrographic characteristics, it would be necessary to carry out additional studies to characterize the community more accurately. This could be done by increasing the sampling frequency with short-term studies (e.g., Madariaga 1995, 2002) or by maintaining a monthly frequency but with long-term studies (e.g., O'Brien et al., 2012).

5. Conclusion

The water column in the experimental bivalve culture platform located in Mutriku showed the typical environmental conditions previously described for Basque marine waters, with no remarkable anthropogenic pressures caused by the WWTP. The composition of the phytoplankton community of the area could be described as favourable for bivalve growth from the perspective of its composition due to the dominance of diatoms, the predominant adequate phytoplankton cell size for ingestion and retention and the relatively low presence of toxic phytoplankton. However, even if bivalve farms are now working in zones with similar phytoplankton community composition and biomass values (i.e., on the Basque coast, the open marine area of Mendexa), these values are low compared with classical zones of bivalve production (e.g., Galician Rías).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.oceano.2020.08.007

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