On the spatial and temporal variations of zooplankton in Northeast Atlantic shelf waters

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List of publications

Part of the present PhD thesis has been presented in four international conferences and published in the following scientific articles:

- **Fanjul, A.**, Villate, F., Uriarte, I., Iriarte, A., Atkinson, A., & Cook, K. (2017). Zooplankton variability at four monitoring sites of the Northeast Atlantic Shelves differing in latitude and trophic status. *Journal of Plankton Research*, *39*(6), 891-909. https://doi.org/10.1093/plankt/fbx054.

- **Fanjul**, **A.**, Iriarte, A., Villate, F., Uriarte, I., Atkinson, A., & Cook, K. (2018). Zooplankton seasonality across a latitudinal gradient in the Northeast Atlantic Shelves Province. *Continental Shelf Research*, *160*, 49-62. https://doi.org/10.1016/j.csr.2018.03.009.

Resumen

El zooplancton, componente animal del plancton, está compuesto por organismos heterótrofos que viven en estado de flotación en las masas de agua, a merced de la corriente. Estos organismos pueden clasificarse de acuerdo a diversos criterios, como es su tipo de ciclo vital. Por un lado, nos encontramos aquellos organismos que pasan toda su vida viviendo en estado de flotación y formando parte del plancton, los denominados organismos holoplanctónicos. Por otro lado, están aquellos que, tras un periodo de vida planctónica, mayormente en estado larvario, pasarían a convertirse en necton o nadadores activos o a formar parte de la fauna bentónica, como organismos móviles o como organismos sésiles adheridos a algún sustrato, estos serían los organismos meroplanctónicos. Otro tipo de clasificación se realiza en torno a su tamaño, que oscila desde las pocas micras (nanoplancton o microplancton), hasta varios centímetros o incluso metros (macroplancton o megaplancton), siendo el mesozooplankton la fracción intermedia. Dicha fracción engloba a todos aquellos organismos comprendidos entre las 200 micras y los 2 centímetros, incluyendo los copépodos, grupo mayoritario del zooplancton, así como a la mayoría de larvas meroplanctónicas. Debido a su composición, el mesozooplancton es de gran relevancia en la literatura científica, siendo ampliamente muestreado en programas de monitoreo y series temporales.

El zooplancton es una parte esencial de las redes tróficas marinas, siendo productores secundarios que se alimentan de fitoplancton (componente vegetal del plancton y productores primarios), haciendo de enlace trófico con organismos de niveles superiores como son los peces pelágicos. Entre otras importantes funciones de estos organismos estaría su participación en la recirculación del nitrógeno mediante su excreción y también en la bomba de carbono, ayudando al transporte de este elemento a las profundidades de los océanos mediante sus migraciones verticales. La composición y abundancia del zooplancton afecta directamente al reclutamiento de peces que dependen de ellos como fuente de alimento, las cuales pueden tener relevancia pesquera, siendo importantes recursos económicos y alimentarios para el ser humano, y cuyo estado actual, tras décadas de sobreexplotación, requiere de una cuidadosa atención, comprensión y manejo.

Actualmente, se está dando una situación en la el cambio climático producido por el hombre tiene profundas implicaciones en los ecosistemas marinos, así como en los sistemas sociales y económicos que son sustentados por los mismos. A día de hoy, prácticamente la mitad de las áreas marinas se encuentran afectadas por la acción de diferentes factores, cuyo origen común son las acciones antropogénicas. Los organismos zooplanctónicos, debido a sus características, se constituyen como buenos centinelas de dicho cambio climático. Son organismos poiquilotermos altamente sensibles a cambios de temperatura, que afecta a su fisiología,

además, gracias a sus rápidos ciclos vitales, son capaces de responder en escalas de tiempo cortas a las variaciones de aquellos factores ambientales que les afectan. Asimismo, debido a que por norma general estos organismos no son explotados de forma comercial, sus patrones de cambio a largo plazo no son confundidos con tendencias de explotación. Comprender como la composición y abundancia del zooplancton varía en función de la acción de factores extrínsecos sigue siendo una tarea compleja, pero de gran interés, ya que permitiría comprender sus propiedades ecológicas y ayudar a predecir los efectos potenciales del cambio climático.

La temperatura del agua, la salinidad y la concentración de clorofila *a* (como indicador de abundancia de fitoplancton), se encuentran entre las variables ambientales que más afectan al zooplancton y que, además, son medidas de forma rutinaria en los programas de monitoreo. La temperatura, entre otros efectos, ejerce un fuerte control en el ciclo estacional del zooplancton, actuando como punto de señalización temporal para los procesos recurrentes producidos en dicha escala temporal; la salinidad afecta a la distribución de las diferentes especies debido a las tolerancias fisiológicas de las mismas a los cambios de esta variable; la clorofila *a* se encuentra contenida en el fitoplancton, principal fuente de alimento de aquellas especies herbívoras o de alimentación omnívora que dependen de esta fuente de alimento para su supervivencia. Por otro lado, además de estas variables medidas *in situ*, se encuentran las teleconexiones o índices climáticos, que son "*anomalías a gran escala, tanto espacial como temporal, que afectan a la variabilidad de la circulación atmosférica*". Existen múltiples de estos índices, pero en el océano Atlántico los de mayor importancia son los de la "NAO" (North Atlantic Oscillation Index), la "EA" (Eastern Atlantic pattern) y la "AMO" (Atlantic Multidecadal Oscillation). Los registros de variabilidad de estos índices, muestran una alternancia entre fases que ha podido ser relacionada con cambios en la abundancia de organismos zooplanctónicos, además de interaccionar con las variables ambientales modificando sus patrones y, a su vez, afectando a los ecosistemas marinos.

Existen diferentes tipos de variación que determinan la abundancia y estructura de la comunidad zooplanctonica. Un ejemplo son las variaciones temporales, la estacional o ciclo que ocurre a lo largo de un año, es de gran importancia debido a la cantidad de cambios bióticos y abióticos involucrados. Por otro lado, se encuentra el gradiente espacial, donde la distribución de zooplancton puede mostrar variaciones en función de las diferentes zonas climáticas englobadas dentro de un gradiente latitudinal. Además, gradientes como el costero-oceánico, también ha sido relacionado con cambios en la estructura de la comunidad, con especial influencia de las intrusiones de aguas oceánicas en ecosistemas de plataforma. Los ecosistemas situados a diferentes latitudes están sujetos a distintos ciclos anuales de temperatura y longitud diurna, mostrando patrones estacionales característicos, desde el ecuador a los polos. La acción de factores que actúan en escalas mucho menores también puede causar cambios en los ecosistemas marinos costeros y de la plataforma continental, como es la descarga de sistemas fluviales o el afloramiento costero. Además, también existen factores de estrés ambiental de origen antropogénico, como el vertido de aguas residuales, susceptibles de modificar los ciclos normales del zooplancton.

Existe la posibilidad de que se produzcan cambios en los anteriormente mencionados patrones estacionales del zooplancton, o lo que sería lo mismo, alteraciones en su fenología (momento de la ocurrencia de los fenómenos biológicos de ritmo periódico). Estas alteraciones causadas principalmente por la influencia de factores ambientales, pueden tener repercusiones como el posible desacople entre presas (zooplancton) y depredadores, afectando negativamente mediante un control de abajo hacia arriba, o lo que sería lo mismo, organismos de niveles inferiores afectando a la productividad de los organismos de niveles superiores. A escalas mayores de la anual, las variaciones interanuales en la abundancia de zooplancton, pueden producirse en respuesta a los cambios naturales de las variables abióticas, causando patrones de cambio en los organismos que pueden ser progresivos o bruscos. Cambios progresivos producen tendencias de cambio gradual o ajuste de la comunidad de zooplancton al seguir esta poco a poco la variabilidad ambiental. Los cambios bruscos, pueden producir fenómenos denominados cambio de régimen, causando una alteración de la composición y abundancia de la comunidad, evento producido al cruzarse un límite de tolerancia de los organismos o al cruzar un umbral ambiental que lo desencadene.

Todos los estudios que se realizan sobre los distintos modos de variabilidad del zooplancton, con mención especial de aquellos de gran escala temporal, son posibles gracias a la existencia de programas de monitoreo a largo plazo. Estos programas, mediante la recolección periódica y sistemática de muestras, acumulan extensas bases de datos que permiten seguir la variabilidad temporal del zooplancton y evaluar si los factores ambientales, que potencialmente pueden causar un efecto en las comunidades biológicas, explican los cambios observados. Existen diferentes estrategias de monitoreo continuado de zooplancton marino, por un lado, estarían aquellos programas de muestreo que cubren un área extensa, como es el “Continuous Plankton Recorder” o CPR, aparato arrastrado por buques mercantes en rutas comerciales, en activo desde 1931 y que cuenta con cuarenta zonas de acción en el Atlántico Norte. Por otro lado, están los puntos de monitoreo espacialmente fijos en aguas costeras o de plataforma continental, donde, además de muestras biológicas, se realizan mediciones periódicas de variables ambientales. Actualmente existen sesenta y dos puntos de muestreo con estas características en el Atlántico Norte, cubriendo un amplio rango de ambientes hidrográficos. Las series temporales de zooplancton obtenidas de estos programas de monitoreo contienen información muy extensa, pero aun así actualmente siguen estando poco utilizadas para la realización de estudios comparativos y, en los pocos casos que han sido utilizadas, solo se han evaluado las dinámicas temporales de unas pocas especies.

La Provincia de Plataformas del Atlántico Noreste, clasificación realizada de acuerdo a diferencias entre los patrones temporales del zooplancton y de las variables observadas en áreas contrastantes, se extiende desde el norte de la Península Ibérica hasta el Canal de las Islas Faroe-Shetland y la Fosa Noruega, y posee programas de monitoreo fijo dispuestos a lo largo de un amplio gradiente latitudinal.

De acuerdo a lo anteriormente mencionado, queda claro que los organismos zooplanctónicos tienen la capacidad de actuar como buenos centinelas del cambio climático, por lo que el conocimiento de sus respuestas ante factores ambientales y en diferentes escalas de variación, puede ser muy útil para predecir los posibles cambios ecosistémicos en el futuro y las potenciales respuestas al cambio climático en el que actualmente se encuentra el planeta. A partir de los estudios realizados hasta la fecha, existe una comprensión limitada de los patrones de cambio de la comunidad zooplanctonica en áreas extensas y del efecto de sus variables ambientales. Esto se ha debido principalmente al empleo de periodos de tiempo diferentes o al uso de una cobertura espacial y/o taxonómica limitada. Por lo tanto, muchas cuestiones permanecen sin resolver en cuanto a la coherencia de patrones temporales dentro de gradientes latitudinales, entre diferentes taxones dentro de la comunidad de zooplancton y del efecto que ejercen las variables ambientales, todo ello hablando dentro del contexto geográfico de la Provincia de Plataformas del Atlántico Noreste. Debido a esto, series temporales de abundancia de zooplancton, así como de valores de variables ambientales procedentes de cuatro sitios de monitoreo situados a lo largo de un gradiente latitudinal dentro de la Provincia de Plataformas del Atlántico Noreste (desde el golfo de Bizkaia con los sitios denominados U35 y B35, pasando por el Canal de la Mancha, con el sitio L4 y hasta el Mar del Norte, con el sitio SH) y para el periodo de tiempo 1999 – 2013, fueron empleados para la realización de diferentes análisis comparativos. Dos de estos sitios, los situados en la misma latitud dentro del Golfo de Bizkaia (B35 y U35), encontrándose bajo un efecto de influencia antropogénica y estado trófico diferente. Es importante recalcar, que, con el objetivo de tener un punto de vista holístico de las dinámicas de población, los análisis realizados en el presente trabajo se llevaron a cabo tanto al nivel de taxones individuales como de comunidad.

La presente tesis partió de la siguiente **hipótesis principal**:

"La estructura de la comunidad del zooplancton, la abundancia de sus componentes y sus patrones estacionales e interanuales de variabilidad, dentro de la Provincia de Plataformas del Atlántico Noreste, presentan diferencias causadas por la latitud y/o estado trófico"

Siendo los **cinco objetivos** específicos de la presente tesis los siguientes:

1. Extraer y comparar la magnitud de las variaciones del mesozooplancton a escala interanual, estacional y residual, y sus patrones interanual y estacional durante el periodo 1999 – 2013 en cuatro sitios de monitoreo que difieren en latitud y estado trófico, dentro de la Provincia de Plataformas del Atlántico Noreste.

2. Evaluar y comparar el papel de los factores ambientales en el ciclo estacional de la comunidad de mesozooplancton durante el periodo 1999 – 2013 en los cuatro sitios de monitoreo.

3. Evaluar y comparar los cambios en la fenología de los taxones zooplanctónicos, además de la influencia de los factores ambientales durante el periodo 1999 – 2013 en los cuatro sitios de monitoreo.

4. Comprobar la existencia de cambios graduales o bruscos de la comunidad de mesozooplancton durante el periodo 1999 – 2013 en los cuatro sitios de monitoreo y evaluar la influencia de las variables ambientales en dichos cambios.

5. Describir y comparar la estructura de la comunidad del mesozooplancton durante el periodo 1999 – 2013 en los cuatro sitios de monitoreo y determinar los principales factores responsables de las diferencias observadas.

En el **primer capítulo**, se han analizado las diferentes escalas y los distintos patrones de variabilidad, en función de la localización latitudinal y el estado trófico. Para ello se utilizó un modelo multiplicativo que descompone las series temporales en tres componentes: un componente estacional, un componente interanual y un componente residual, referido a eventos puntuales. Los resultados mostraron que el sitio con mayor componente de variabilidad estacional fue el situado más al norte, SH. El sitio con mayor componente de variabilidad interanual fue U35, asociado a un evento atípico de altas abundancias durante el año 2012. Los dos sitios que presentaron mayor componente de variabilidad residual fueron B35 y U35, hecho relacionado con una frecuencia de muestreo inferior a los sitios de SH y L4 (mensual frente a semanal) y también asociado al estrés natural y el producido por las acciones antropogénicas que existe en estos sitios. La variabilidad interanual de los taxones no fue coherente entre los distintos sitios de estudio, sugiriendo un mayor efecto de las variables ambientales locales que de los factores a gran escala. Sin embargo, la mayoría de los taxones si presentaron coherencia en su ciclo estacional entre los sitios de estudio, siendo el retraso del máximo anual la característica mas común. Las diferencias entre los taxones de primavera-verano fueron principalmente relacionadas con la biomasa de fitoplancton, a su vez relacionado con diferencias latitudinales y el enriquecimiento en nutrientes de origen antrópico. El retraso temporal del enfriamiento del agua, correlacionado positivamente con la latitud, dio cuenta de las diferencias observadas entre sitios para aquellos taxones que aumentan en abundancia durante la segunda mitad del año.

En el **segundo capítulo** se investigó la estacionalidad del zooplancton y los factores ambientales que la controlan mediante el uso de métodos multivariantes (análisis de redundancia o RDA). Para ello se dividió la comunidad en dos grupos, (i) grupos o taxones mayoritarios, que aglutinan varias especies y géneros y (ii) especies y géneros más relevantes de copépodos y cladóceros. También se utilizaron las variables ambientales: WT, Chl *a* y Sal, además de los índices climáticos EA, AMO y NAO. La temperatura fue la variable que mejor explicó los cambios estacionales observados en la comunidad para los cuatro sitios de estudio. Sin embargo, diferencias debidas al estado trófico y a la latitud fueron observadas en la estacionalidad de la comunidad de zooplancton, principalmente en los patrones mostrados por los taxones que alcanzan su máxima abundancia durante los meses de primavera-verano. Por otro lado, el patrón estacional de fitoplancton fue relacionado con el de aquellos taxones que alcanzan su máximo durante los meses de otoño e invierno. El porcentaje de variabilidad explicada por los factores ambientales aumentó con la latitud y el estado trófico, probablemente debido a un incremento en la covariación de la temperatura y la clorofila *a*, además de por un incremento en la regularidad de sur a norte del patrón estacional entre años. También se produjo un aumento en la regularidad de la clorofila *a* con el estado trófico. Cladóceros y larvas de cirrípedo en B35 y U35, así como larvas de equinodermo en L4 y SH fueron los taxones con la mayor contribución al patrón de la comunidad de zooplancton, el cual mostró un retraso estacional con la latitud y con el estado trófico.

En el **tercer capítulo** se evaluó, para los diferentes taxones de la comunidad de zooplancton, los cambios en su fenología, la cual se ha sugerido como cambiante debido al calentamiento global, durante el periodo 1999 – 2013 y en los cuatro lugares de monitoreo de este estudio. Además, se evaluó la influencia en estos cambios de índices climáticos (NAO, EA y AMO) y variables ambientales a nivel local (temperatura del agua, salinidad y clorofila *a*). En general, la variabilidad en la fenología del zooplancton fue mayor en los sitios situados mas al sur y mas cercanos a la costa (B35 y U35) que en los situados mas al norte y alejados de la costa (L4 y SH). Muy pocos taxones de zooplancton presentaron tendencias lineales significativas de cambio, pero mayormente se observaron tendencias opuestas en los cambios fenológicos entre los sitios situados más al sur (tendencia a ocurrir más tarde a lo largo del periodo de estudio) y los sitios situados mas al norte (tendencia a ocurrir antes a lo largo del periodo de estudio). En cada sitio, se encontraron grupos de taxones (cuya composición difirió entre sitios) con patrones interanuales de cambio fenológico similares, siendo los máximos anuales de dichos taxones coincidentes en la misma estación del año. Los índices climáticos (principalmente EA y AMO en B35 y U35, EA y NAO en SH) o la temperatura del agua (en L4) mostraron correlaciones significativas con las variaciones fenológicas de la mayoría de las agrupaciones de taxones sincrónicos, estando en muchos casos, además, correlacionadas con la disponibilidad de biomasa de fitoplancton. En el caso en que una única especie dominó su género en todos los sitios de estudio (p.ej. *Acartia clausi*), se observaron tendencias interanuales opuestas en la variación de su fenología entre los sitios situados más al sur y los sitios situados más al norte.

En el **cuarto capítulo** hemos modelado las posibles tendencias y cambios de tendencia mostradas por la comunidad zooplanctonica en los cuatro sitios de estudio durante el periodo 1999 – 2013. Para ello, compendiamos el estado de la comunidad de zooplancton mediante el uso de análisis multivariantes (componentes principales) y posteriormente modelamos las variaciones temporales del zooplancton a través de modelos generales aditivos mixtos (General Additive Mixed Models (GAMMs)), incluyendo como covariables en los mismos la temperatura del agua, la salinidad y la clorofila *a*. El primer componente principal capturó las dinámicas de aquellos taxones de zooplancton cuyo máximo de abundancia ocurre durante el periodo principal de crecimiento, esto es de primavera a verano. El segundo componente principal capturó las dinámicas de aquellos taxones cuyo máximo ocurre durante el final del año (verano – otoño) en contraposición con las de los taxones de desarrollo más temprano. Se detectaron tendencias graduales de cambio en L4 para el colectivo de especies cuyo máximo ocurre durante el periodo principal de crecimiento, mientras que para los otros tres sitios se encontraron tendencias graduales de cambio entre los taxones tardíos y tempranos La tendencia de cambio estuvo restringida al periodo 1999 – 2005 en SH, probablemente en relación a un cambio de régimen iniciado previamente a nuestro periodo de estudio. Dentro de los taxones seleccionados para su modelado individual, tendencias de incremento y disminución fueron observadas, pero ningún taxón mostró el mismo patrón en los cuatro sitios de estudio. Es remarcable el caso de *Acartia*, que mostró tendencias opuestas entre el sitio situado más al norte (disminución en SH) y los sitios situados más al sur (aumento en B35 y U35), pero no mostró tendencia en el sitio intermedio de L4. La selección de variables ambientes por los modelos indicó que la dinámica del zooplancton fue principalmente modulada por la temperatura del agua, a pesar de que los cambios producidos por esta variable difirieron entre sitios. La interacción entre la temperatura y el mes fue seleccionada como la mayor causa de cambio en la estructura de la comunidad en la mayoría de los casos, con la excepción de U35, y en la abundancia de los taxones seleccionados en L4. La salinidad y la clorofila *a*, de forma independiente o mediante interacción, mostraron un papel mas variable dependiendo del sitio, y fueron principalmente seleccionadas para explicar los cambios del zooplancton en B35 y SH, respectivamente. La práctica ausencia de variables ambientales seleccionadas por los modelos en U35 se atribuyó a la baja coherencia de los patrones ambientales estacionales observados en este sitio.

En el **quinto capítulo** se estudió la estructura de la comunidad zooplanctonica presente en cada uno de los sitios de estudio durante el periodo 1999 – 2013, determinando, además, los factores ambientales que la afectan. Para ello se utilizaron análisis multivariantes (RDAs), además de modelos regresivos ortogonales polinómicos, con el objetivo de describir la relación entre la abundancia de cada taxón y la temperatura. Para ello, en este trabajo se analizaron por separado la comunidad de zooplancton a nivel de grupos taxonómicos amplios y la comunidad constituida por cladóceros y copépodos a nivel de género. Para los grupos taxonómicos amplios, la latitud fue el factor que mejor explicó las diferencias entre los diferentes sitios, diferencias a las cuales el meroplancton contribuyó en mayor medida que el holoplancton. Dichas diferencias latitudinales fueron puestas principalmente de manifiesto en la abundancia de doliolidos (más abundantes en los sitios situados a baja latitud) y briozoos y larvas de poliquetos (más abundantes en el sitio situado a mayor latitud). Los doliolidos, además, fueron los mejores indicadores de las diferencias latitudinales dependientes de la temperatura. La interacción entre la latitud y la distancia de la costa o la interacción entre salinidad y biomasa de fitoplancton, explicaron una parte más pequeña de la variabilidad observada. Las mayores diferencias para las comunidades de cladóceros y copépodos reflejaron la influencia oceánica, apareciendo *Corycaeus* y *Oncaea* mayormente asociados a L4, como indicadores de intrusiones de masas de agua oceánicas, y el copépodo nerítico *Acartia* como dominante en SH, U35 y B35. Los

cladóceros *Podon* y *Evadne*, mostraron una disminución de sur a norte, mostrando diferencias latitudinales mas relacionadas con la salinidad que con la temperatura. A pesar de que la consistencia en la identificación de especies limitó la resolución taxonómica de este trabajo, en el caso del género *Acartia* (casi exclusivamente *Acartia clausi* en todos los sitios), se observó una relación común con la temperatura, consecuente con un nicho térmico fijo.

Thesis structure

The present work is organized in eight parts. The first part is a **general introduction** where we introduce relevant aspects of the zooplankton, time series and the state of the art. It is accompanied by the main **hypothesis** of the present work and five individual **objectives**. The second part is the **study area**, **sampling methodology** and **data pretreatment** of this thesis, which is common for all the chapters, so with the aim of not repeating this information it only appears once.

Following, the next five parts are different chapters where different objectives are covered. In the **first chapter**, we aimed to analyze the different patterns of variability of zooplankton (seasonal, interannual and residual). In the **second chapter**, we assessed the environmental factors effect on the zooplankton seasonal cycle. In the **third chapter**, we appraised the patterns in zooplankton phenology. In the **fourth chapter**, we explored the interannual changes produced in the zooplankton community. In the **fifth chapter**, we inspected the structural differences of the zooplankton community and the influence of the environmental factors.

Finally, the eighth part of the present thesis, consists on the **general conclusions** of this work.

General introduction

Zooplankton definition and classification

Zooplankton is the animal component of the plankton which, according to their mode of nutrition, are an assemblage of all phagotrophic organisms (Lenz, 2000). The term zooplankton derives from the ancient Greek words "*zoon*" and "*planao*", meaning, respectively, animal and errant, drifter or wanderer. This term envelopes those organisms floating and drifting in the water, which do not hold sufficient locomotion abilities to bear water masses currents and turbulence. Notwithstanding, almost all zooplankton species have developed some means to move or, at least, move their vertical position within the water column (Lenz, 2000).

Zooplankton can be classified as a function of their life cycle. Zooplankton species that spend their whole life in the pelagic realm as plankton, are known as holoplankton, whilst those that only float and drift in the sea during a certain part of their life cycle being part of the plankton are known as meroplankton. Among the latter, we can distinguish between those species that switch from plankton to nekton (active swimmers) during their juvenile stage, and those that migrate from the pelagic zone to the benthos. Marine zooplankton are an abundant and diverse group which includes around seven thousand described species from up to fifteen different phyla (Bucklin *et al*., 2010), which, if we include meroplanktonic organisms this number grows up to several ten-thousands species (Lenz, 2000).

Another classification could be done according to the organism size (Omori and Ikeda, 1992). The size of the zooplankters ranges widely, the smallest ones belonging to the nanoplankton (2 – 20 µm), with heterotrophic flagellates and ciliates (Rassoulzadegan and Sheldon, 1986). Pluricellular zooplankton would start at the microplankton (20 – 200 µm) fraction, with small crustaceans (i.e. copepod nauplii; Beers *et al*., 1980), together with large protozoans. On the other side of the spectrum we find the largest zooplankters, which belong to the macro (2 – 20 cm) and megaplankton fraction (> 20 cm), with organisms such as jellyfish, ctenophores, cephalopods, amphipods and tunicates, such as pyrosomes (Craig *et al*., 2009). Right in the middle of this size-based classification we find the mesozooplankton fraction (0.2 – 20 mm; **Figure 1**). This fraction is the only size class that covers two orders of magnitude, which is caused by the size spectrum of the organisms captured by a mesh size of 200 – 330 µm (Lenz, 2000). Within the range of this size class we find also the size range of copepodites and adult copepods, which comprise the most numerous zooplankton group, covering also the size range of most meroplanktonic larvae as well. Mesozooplankton is of great relevance in scientific literature, as it is one of the most extensively sampled in monitoring programs and zooplankton time series (Kamburska and Fonda-Umani, 2009; Eloire *et al*., 2010).

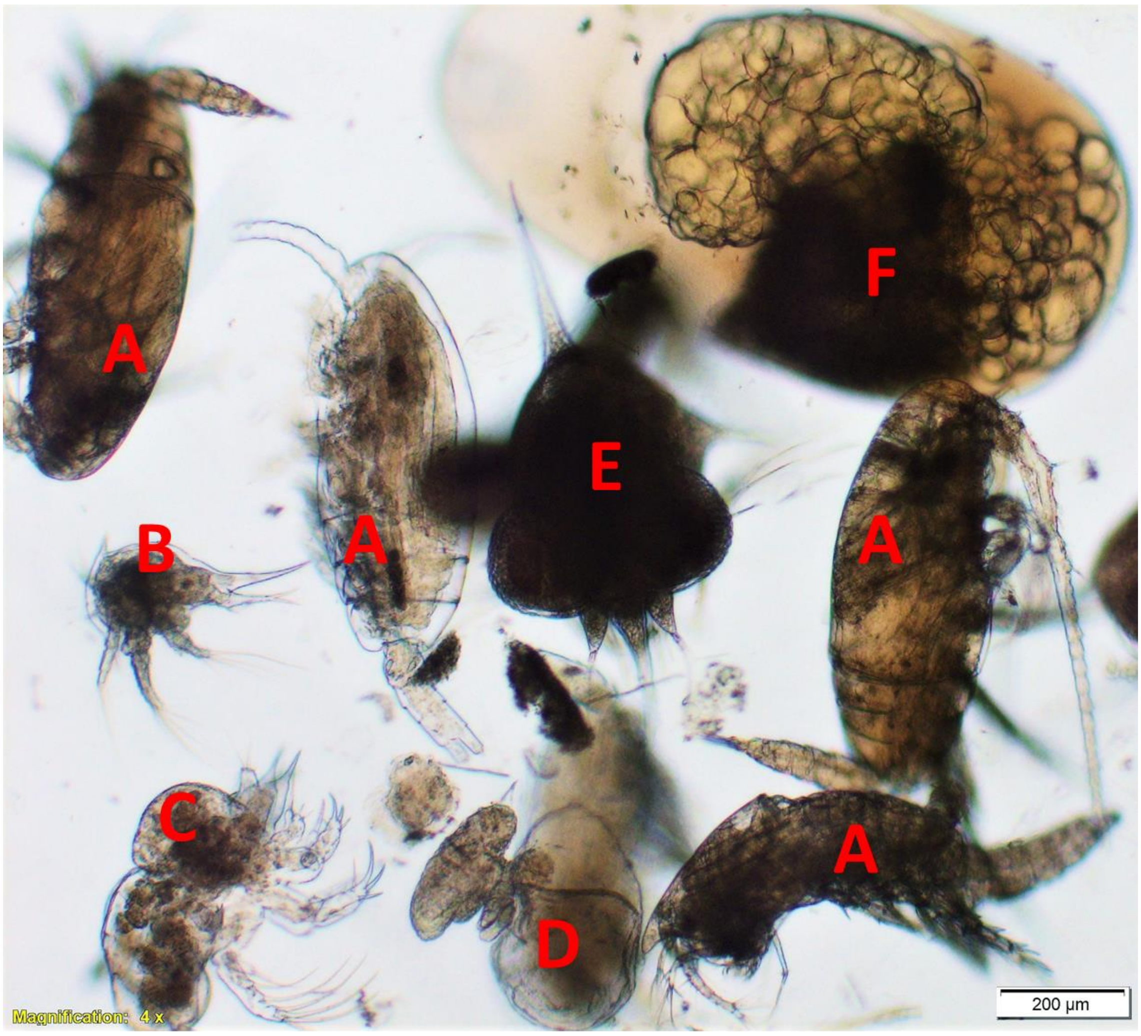

Figure 1. Example of the mesozooplankton community, showing different organisms: copepods (A), cirripede nauplius larvae (B), cladoceran (C), appendicularian head (D), decapod larvae (E), fish egg (F). Photograph by *Ibon Uriarte*.

Zooplankton role in pelagic systems

Zooplankton are an essential part of the ocean food webs, contributing to their functioning due to their role as secondary producers that link primary production (phytoplankton) with higher trophic level consumers (Dam, 2013). Zooplankton organisms are great in abundance and they play important ecosystem roles, such as their participation in the nitrogen cycle, which is released and recirculated by them through excretion, supporting bacterial and phytoplankton production (Smith and Whitledge, 1977) and, thus, helping the proper functioning of the food web. Zooplankton also takes part on the carbon biological pump, which is a global process where the oceans behave as a sink for atmospheric CO_2. Zooplankton organisms help to move large quantities of carbon from the ocean's surface to deeper layers. They acquire it by feeding on phytoplankton, which are CO_2 fixing organisms through photosynthesis, then, by moving vertically in the water column, going down, which is a predation avoiding strategy (Richardson, 2008).

Furthermore, the temporal variability in composition and abundance of zooplankton can affect larval survival and recruitment success of fish, which depend on the presence of suitable zooplankton prey during their spawning and development, that is to say, on a good match between prey and predator (e.g. match-mismatch hypothesis; Cushing, 1990). Environmental changes can cause mismatches in the occurrence of prey and predator and this can affect commercially important fisheries (Beaugrand *et al*., 2010) because of inadequate larval fish foraging (Chick and Avyle, 1999). Fisheries, after decades of mishandling and overexploitation (63% of the stock worldwide requires rebuilding; Worm *et al*., 2009) require better understanding of how the ecosystems that sustain them work in terms of their response to environmental variables and to trophic interactions.

Zooplankton response to environmental changes

Human actions have been proved to be inducing a global climate change. It has profound implications for marine ecosystems and also on the social and economic systems that depend upon them (Hoegh-Guldberg and Bruno, 2010). The world has run out of unaffected and pristine areas due to human actions, and the percentage of those which are strongly affected by multiple drivers are more than forty percent (Halpern *et al*., 2008). There are several reasons that make zooplankton excellent sentinels of the mentioned climate change. They are poikilothermic organisms highly sensitive to temperature, as it greatly affects their physiology. They also have short lives and fast life cycles, which makes them tightly coupled to climate (Hays *et al*., 2005). Most zooplankton organisms are usually not commercially exploited, meaning that their long-term patterns of change would be caused by trophic interactions and climate variations and not confounded with exploitation trends (Richardson, 2008).

Understanding how zooplankton abundance and composition vary in time and space and identifying which are the drivers of change is an issue of great interest. Untangling the role of the extrinsic factors is still one of the biggest challenges when trying to understand the ecological properties of pelagic ecosystems and their potential responses to a changing environment (Pepin *et al*., 2015), which can help predict the ecological effects of environmental change (Stenseth *et al*., 2002).

Main drivers of zooplankton dynamics

Water temperature, salinity and chlorophyll *a* concentration, the latter as a proxy of phytoplankton biomass, are among the main environmental variables affecting zooplankton and they are routinely measured in zooplankton monitoring programmes. Many studies have suggested that water temperature plays a key role in zooplankton spatio-temporal changes (Reygondeau and Beaugrand, 2011; Rombouts *et al*., 2010). It has been shown to be a major driver of the seasonal cycle of zooplankton, since it affects the rates of egg development, feeding, production, respiration and other metabolic processes (Ambler *et al*., 1985; Ikeda, 1985; Peters and Downing, 1984) and may act as a timing cue for different seasonally recurrent processes (Mackas *et al*., 2012).

Salinity is an environmental constraint and it has been shown to play an important role in some regime shifts of marine ecosystems (Weijerman *et al*., 2005). Salinity-induced stratification can alter the exchange of nutrients and suspended particulate matter affecting zooplankton distribution (Cross *et al*., 2015). It seems to be a limiting factor for the presence and penetration of shelf/coastal zooplankton into estuarine waters and estuarine zooplankton into shelf waters, i.e. cladocerans abundance decreased as a function of salinity in the estuary of Bilbao (Bay of Biscay) (Uriarte and Villate, 2004; Villate *et al*., 2017). Salinity has been shown to affect the speed of growth and biological functions of different zooplankton taxa e.g. house renewal of appendicularians (Flores-Coto *et al*., 2010) or oxygen consumption rates copepods (Gaudy and Thibault-Botha, 2007).

Zooplankton are primary consumers of phytoplankton. Grazer zooplankters, such as appendicularians, highly depend on phytoplankton biomass as food source (Alldredge, 1981; Tomita *et al*., 2003), and so do many species of copepods and cladocerans (Tirelli and Mayzaud, 2005; Xiong *et al*., 2012; Tiselius *et al*., 2013). Phytoplankton biomass has been found to be a factor triggering the release of meroplanktonic larvae, which greatly depend on phytoplankton for their survival (Starr *et al*., 1991; Highfield *et al*., 2010). Therefore, phytoplankton availability can ultimately affect their recruitment.

In addition to local environmental conditions, there are large scale processes that affect marine ecosystems, in general, and zooplankton in particular, such as atmospheric teleconnections. These are "*spatially and temporally large-scale anomalies that influence the variability of the atmospheric circulation*" (https://www.ncdc.noaa.gov/teleconnections). There are different teleconnection patterns that affect the North Atlantic, which can be potential drivers of changes in marine ecosystems and zooplankton communities. The he North Atlantic Oscillation (NAO) index, the Atlantic Multidecadal Oscillation (AMO) index and the East Atlantic pattern (EA) have been suggested to have a relevant effect on marine ecosystems in the Northeast Atlantic.

Piontkovski *et al*. (2006) found a relationship between the positive phase of the NAO index and increased zooplankton abundance across the mid-Atlantic, and the opposite for negative NAO years. Similarly, Beaugrand and Reid (2003) found an increase of the calanoid copepod *Calanus helgolandicus* during positive phases of the NAO index in the Northeast Atlantic. Fromentin and Planque (1996) found an indirect effect of the NAO index (through west wind stress intensity and temperature) on *Calanus finmarchicus* abundance in the Eastern Atlantic and the North Sea, associated effects on west wind stress and spring primary production, and also to *C. helgolandicus* with a less clear relationship. Cotton *et al*. (2005) explored beyond the positive relationship between the NAO and zooplankton, looking for bottom-up control in the distribution of higher trophic level organisms, such as basking sharks, in southwestern Britain, where zooplankton density and the NAO were partially related to.

Kane (2011) found that increasing stocks of up to eleven zooplankton taxa in the Middle Atlantic Bight, were associated with the AMO index and rising surface temperatures. Beaugrand *et al*. (2009) found a relationship between this index and changes in the distribution of calanoid copepods in the Northeast Atlantic. In the estuary of Bilbao (Bay of Biscay), AMO index made a significant contribution to explain zooplankton variability (Uriarte *et al*., 2016). Furthermore, zooplankton community composition in northeast US seems to be significantly affected by the AMO (Morse *et al*., 2017).

The phase of the EA pattern can have a significant impact on the NAO structure, including changes to the location and strength of its centers of action, affecting the position and intensity of its temperature anomaly (Moore and Renfrew, 2012), affecting also the predictability of the climate on certain parts of Europe (Woollings, 2010). Furthermore, NAO and EA interactions have been related to climate patterns over Iberian Peninsula (Sanchez-López *et al*., 2016). Concretely, in the southeastern Bay of Biscay the positive phase of the EA index appeared to be related to southwesterly winds and downwelling (Borja *et al*., 2008). Thus, variations in this index may cause alterations in the patterns of environmental variables such as temperature, precipitation, or even affect the upwelling/downwelling process, which could cause a direct effect on zooplankton (Llope *et al*., 2006).

Global vs. local impacts on zooplankton

However, the relationship between environmental drivers and plankton appears to be difficult to generalize (Beaugrand *et al*., 2000) because zooplankton can show a multifactor effect, driven by variables acting at different ecosystem levels and with different timing (Medellín-Mora *et al*., 2016). One of the questions of interest for which there is little understanding yet is the relative importance of macroscale processes as opposed to local environmental factors on the distribution and abundance of zooplankton and, therefore, whether synchronous variations in zooplankton over large spatial areas are widespread in the marine environment (Pepin *et al*., 2015). Also, zooplankton abundance and composition is highly variable at different spatial and temporal scales because the environmental factors to which they respond operate across a wide range of spatial and temporal scales (Haury *et al*., 1978; Villate *et al*., 2016). Therefore, the relative importance of environmental drivers is expected to vary as a function of the spatial and temporal scale of variation considered.

We can identify different types of spatial gradients across which zooplankton abundance and community structure can vary. Differences in zooplankton distribution can be found across latitudinal gradients spanning different climate zones (Xu *et al*., 2016). Inshore-offshore gradients in zooplankton community structure across shelf waters have also been documented, with oceanic water intrusions onto the shelf being very influential (Pepin *et al*., 2015) and, in general, regional hydrography may also play a primary role in shaping the zooplankton community (Gluchowska *et al*., 2017). Moreover, zooplankton community differences can also be observed related to differences in salinity and/or the level of nutrient enrichment and pollution of estuarine and nearshore coastal waters (Uriarte and Villate, 2004; David *et al*., 2016).

Temporal scales of zooplankton variability in response to environmental changes

Regarding temporal variations of zooplankton, the seasonal cycle is a scale of key importance because of the large physical and biotic variations involved (Mackas *et al*., 2012). Ecosystems located at different latitudes are subject to different seasonal cycles of temperature and daylength and these, in turn, greatly affect the seasonal cycles of plankton. Accordingly, different types of seasonal cycles have been observed for oceanic zooplankton from different latitudes. In terms of total zooplankton, at lower latitudes, where conditions only change slightly during the year, temperature being quite stable and daylength close to twelve hours regardless of the season, no clear seasonal pattern is observed, as zooplankton dynamics may respond to other factors, such as small changes in solar radiation shifting the inter-Tropical Convergence Zone and associated meteorological and oceanographic changes (McClanahan, 1988). At intermediate latitudes, where variations in the daylength and temperature are moderate, since from summer to winter daylength shortens and temperature decreases noticeably, zooplankton usually shows a bimodal cycle, with a spring bloom and a secondary peak in autumn. At higher latitudes, the differences in temperature and daylength between seasons are even more marked, causing zooplankton to show a large-amplitude single summer peak (Heinrich, 1962). It is important to note, that apart from these main drivers of zooplankton change, their seasonal cycles can be altered by other variables acting at medium or small scales. In shallow shelf seas local natural processes, such as river discharge and coastal upwelling, plus anthropogenic stressors, such as wastewater inputs and other human activities, substantially modify the standard plankton cycles (Cloern, 1996; Jamet *et al*., 2001; D'Alcalà *et al*., 2004).

The seasonal cycle of zooplankton taxa may show interannual variability (Richardson, 2008; Ji *et al*., 2010) or, in other words, alterations in its phenology (i.e. the timing of annually recurring life cycle events). This can be assessed through the use of phenological indices (i.e. Edwards and Richardson, 2004), being very relevant as this could cause the above-mentioned predator-prey mismatches, with the possibility of causing cascade type of effects up the trophic chain. These changes may be caused by changes in key environmental factors, such as water temperature. In many regions zooplankton that peak early in the year have been claimed to occur "earlier when warmer", whereas late summer and autumn taxa have been reported to occur "later when warmer", acting water temperature as a timing cue for zooplankton (Mackas *et al*., 2012). It has been observed that in marginal seas of the Northeast Atlantic the between-site similarity in year-to-year zooplankton phenology variations decreases in a scale of a thousand kilometres or less (Mackas *et al*., 2012). Phytoplankton biomass is another factor which could be of great importance controlling zooplankton phenology, as Falk-Petersen *et al*. (1999) described in the marginal ice zone of the Barents Sea, where they found that the occurrence of new cohorts of copepods coincided with the onset of the phytoplankton bloom, suggesting that spawning relies on stored energy.

In addition to interannual variations in phenology, interannual variations in the abundance of zooplankton taxa can have a strong impact on the abundances of higher trophic level consumers. In marine ecosystems of the North Atlantic synchrony between the interannual patterns of weather and abundances of organisms from different trophic levels have been reported (Aebischer *et al*., 1990).

Zooplankton abundance over long periods of time can be affected by natural changes in abiotic conditions, like intensity of winter winds seemed to indirectly increase zooplankton abundance (Brodeur and Ware, 1992; Sugimoto and Tadokoro, 2003). Anthropogenic activities in coastal and estuarine areas, such as agriculture, industrial activities and sewage outfalls, could also affect the numbers of zooplankton taxa. Agriculture, appeared to be a positive factor for zooplankton abundance, while industry and sewage discharge affected negatively by inducing environmental stress (Vecchione, 1989). Additionally to the previously mentioned factors, interactions between taxa, such as the competition which *C. finmarchicus* faces against *Calanus glacialis* when moving northwards (Kwasniewski *et al*., 2012) and predation, like chaetognaths and euphausiids predating on copepods off Northumberland coast (Roff *et al*., 1988) could also negatively affect the interannual abundances of zooplankton taxa.

A possible response of zooplankton taxa is to track the underlying gradual unidirectional changes of their environmental drivers, showing temporal trends of change (Molinero *et al*., 2013; Reygondeau *et al*., 2015). Apart from environmental drivers, trophic interactions as bottom-up processes, where there is a positive correlation between the biomass of predators and the abundance of zooplankton preys and vice versa, could play a role too in shaping these trends (Kane, 2007). Another possible response pattern to unidirectional changes, are abrupt deviations from the temporal trend i.e. a regime shift. The definition of a regime shift should include at least the following terms: "sudden, high-amplitude, infrequent events, which are detectable in multiple aspects of the physical and biological components and on large spatial scales" (Lees *et al*., 2006).

These events have recently attracted a lot of interest and are often related to long term climate dynamics (Ayón *et al*., 2004), which cause major changes in the Earth's biophysical systems all around the globe (Reid *et al*., 2016). Regime shifts and long-term trends could potentially end with irreversible changes in species composition and community functioning (Clare *et al*., 2017; Spencer *et al*., 2012). Beaugrand *et al*. (2002) found a relationship between the NAO index (governing climatic factors such as the state of wind and temperature) and the interannual variations of plankton abundance in the English Channel for the 1960 – 1999 period. Beaugrand and Ibañez (2004), using data of the 1958 – 1999 period, described a regime shift in the North Sea plankton ecosystem, which resulted from the conjunction of both local and regional hydro-climatic forcing and a change in the location of an oceanic biogeographical boundary in the Northeast Atlantic Ocean. Möllmannn *et al*. (2008), found that climate-induced changes in hydrography in the Central Baltic Sea caused an ecosystem regime shift, where the dominance of the copepod *Pseudocalanus acuspes* was replaced by *Acartia* spp. due to reduced salinity and increased temperature during the 1974 – 2005 period. Alvarez-Fernandez *et al*. (2012) used CPR data for the 1970 – 2008 period and found three zooplankton regime changes in the North Sea. Verheye and Richardson (1998) found, for the 1951 – 1996 (with a 20-year hiatus) period, a significant increase of crustacean zooplankton abundance, accompanied by a shift in the community size structure, which could be related to a long-term intensification of coastal upwelling and a reduction in predating pelagic fish.

Zooplankton time series: a powerful tool to analyse variability scales

All these studies on the different modes of zooplankton variability, particularly the large temporal scale ones, have been possible thanks mainly to the existence of long-term monitoring programs. These programs, by obtaining long data sets over long periods of time, help to track temporal variability in natural biological assemblages and assess whether the potential environmental drivers can explain the observed patterns (Magurran *et al*., 2012). There are different types of long-term monitoring strategies. On the one hand, we have those that cover an extensive sampling area, for which the sampling points are not fixed in time, such as the continuous plankton recorder (CPR). The CPR monitoring has been active for 86 years now (since 1931), operated by the Sir Alister Hardy Foundation for Ocean Science (SAHFOS). There are 40 CPR standard areas in the North Atlantic and the resulting time series have been used widely in long term series comparisons (Beaugrand *et al*., 2002; Beaugrand and Ibañez, 2004; Hátún *et al*., 2009; Alvarez Fernandez *et al*., 2012; Edwards *et al*., 2013). On the other hand, in contrast to sampling strategies such as the CPR, zooplankton are being regularly monitored at multiple fixed sites in shelf waters around the world (Mackas and Beaugrand, 2010; O'Brien *et al*., 2013), where in addition to biological samples, basic environmental variables are measured periodically. There are sixty-two zooplankton monitoring sites in the North Atlantic (ICES Zooplankton status report 2010/2011), covering a broad range of hydrographic environments. The zooplankton time-series from these fixed monitoring stations thus contain a large amount of information, but they are still largely underused for comparisons between sites (Mackas *et al*., 2012; Bresnan *et al*., 2015), and a lot of these between-site comparative studies have only dealt with the temporal dynamics of a few species (Bonnet *et al*., 2007; Castellani *et al*., 2016), lacking a more holistic view of the zooplankton community dynamics.

The extent of the differences in the temporal patterns of zooplankton at contrasting areas has been one of the features used to build ecological classifications of pelagic ecosystems on a geographical basis. For example, Longhurst (1998) distinguished 4 marine biomes and 57 provinces. The Northeast Atlantic Shelves Province (NECS) extends from northern Spain to the Faroe-Shetland Channel and the Norwegian Trench, and is one of the biogeographical provinces for which there are several zooplankton fixed monitoring sites extending over a relatively large latitudinal gradient.

Considerations for planning the present study

From the above premises, it is clear that zooplankton may be good sentinels of ecosystem change, so, knowledge about how zooplankton respond to environmental drivers at different scales of variation could be very helpful to predict future marine ecosystem changes, which could be a response to the ongoing global change. From the studies conducted so far there is limited understanding of the patterns of change of zooplankton community over broad areas and their environmental drivers, mainly because the time windows of data analyses in the different studies have been different and most studies have had a limited spatial coverage (some have had limited taxonomic coverage too), but climate and other environmental changes are not homogeneous in time and space. Therefore, many questions, particularly regarding the coherence of temporal patterns of variation across large spatial gradients or across different taxa within the zooplankton community, as well as the relative importance of different environmental drivers (local versus macroscale) at different scales of variation remain unclear. The present study aimed to contribute to resolve these questions by assessing spatio-temporal patterns of variation of mesozooplankton community composition and abundance and the influence of environmental factors in these variations across the Northeast Atlantic Shelves Province (NECS), defined by Longhurst (1998). To that purpose, time-series of zooplankton and environmental factors from four fixed monitoring sites located along a longitudinal gradient in the NECS province from the Bay of Biscay to the North Sea for the 1999 – 2013 period have been used. The two Bay of Biscay sites are located at the same latitude but differ in the level of anthropogenic influence and trophic status. It is important to highlight that data analyses have been conducted both at the level of individual taxa and at the whole community level, thus providing also a more holistic view of the community dynamics.

Hypothesis and objectives

The main hypothesis of this thesis was:

"Zooplankton taxa abundance and community structure and their seasonal and interannual patterns of variability in coastal waters of the Northeast Atlantic Shelves Province show differences related to latitude and/or trophic status."

The specific **objectives** set in this thesis were the following:

1. To extract and compare the magnitude of mesozooplankton variability at interannual, seasonal and residual scales, and their interannual and seasonal patterns during the 1999 – 2013 period at four monitoring sites within NECS that differ in latitude and trophic status.

2. To assess and compare the influence of environmental factors in the mesozooplankton community seasonal cycles during the 1999 – 2013 period at the four monitoring sites.

3. To assess and compare mesozooplankton taxa phenology changes and the influence of environmental drivers during the 1999 – 2013 period at the four monitoring sites.

4. To test for interannual trends and shifts in mesozooplankton community and representative taxa abundance at the four monitoring sites, during the 1999 – 2013 period and assess the influence of environmental factors in the observed patterns.

5. To describe and compare mesozooplankton community structure and determine the main factors responsible for the observed differences during the period 1999 – 2013 at the four monitoring sites.

Study area and sampling methodology

The time series of zooplankton (> 200 µm) abundance, water temperature (WT), salinity (Sal) and chlorophyll *a* concentration (Chl *a*) used in this study, spanned a 15-year period (1999 – 2013). They were obtained from the ongoing monitoring programmes carried out at the Bilbao 35 (B35) and Urdaibai 35 (U35) sites by the Zooplankton Ecology Research Group of the University of the Basque Country (UPV/EHU), at Plymouth L4 (L4) by researchers from the Plymouth Marine Laboratory (UK) and at Stonehaven (SH) site by researchers from Marine Scotland Science, UK (**Figure 2**). B35 and U35 are located close to each other on the Basque coast, inner Bay of Biscay, at the southern limit of the NECS (Longhurst, 1998), but they differ substantially in their trophic status (Iriarte *et al*., 2010). L4 is located off the southwest coast of England, in the western English Channel, at an intermediate latitude, and SH is off the eastern Scottish coast, in the northwest North Sea, near the northern limit of the same geographical province.

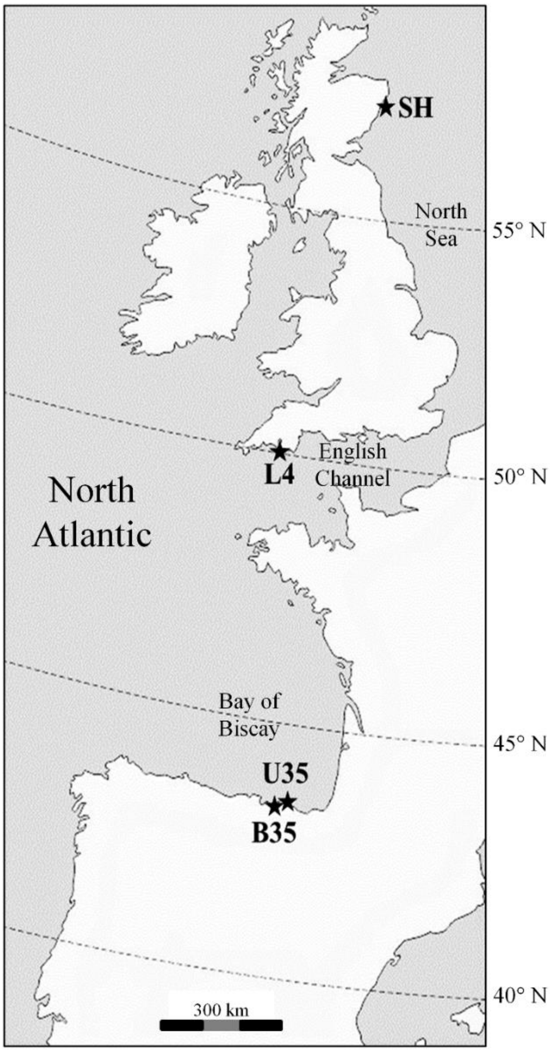

Figure 2. Map showing the location of the sampling sites.

The **SH** site (56° 57.8′ N, 02° 06.2′ W), with a mean depth of ca. 48 m, is located 5 km offshore from Stonehaven, where the impact of freshwater inputs of the rivers Dee and Don (outflowing at Aberdeen, 15 miles north) is reduced (Bresnan *et al*., 2015). This is a dynamic site, well-mixed for most of the year due to strong tidal currents, and a weak thermal stratification is usually restricted to neap tides in the summer months. In this site sampling is carried out weekly, weather permitting. Water temperature was measured using a digital reversing thermometer fitted to the Niskin sampling bottle, salinity was measured using a CTD and chlorophyll *a* (Chl *a*) was determined fluorometrically as described in Bresnan *et al*. (2015). In the present study surface values were used for the latter three parameters. Zooplankton samples were taken by vertical hauls from 45 m to the surface using 200 µm mesh Bongo nets (40 cm diameter but 30 cm during January, February and March 1999) and preserved in 4% borax buffered formaldehyde. Larger zooplankton were identified and counted from the whole sample. Subsamples (of variable volume depending on density of animals, but a minimum of 2.5% of the whole sample) were taken for the identification and counting of smaller zooplankton, so that at least 100 individuals were counted. More details on the methodology used for data acquisition can be found in Bresnan *et al*. (2015).

L4 is located about 13 km southwest of Plymouth (50°15′ N, 4°13′ W), and 6.5 km away from the nearest land (Litt *et al*., 2010). This is a transitionally mixed site (Southward *et al*., 2004) with a mean depth of 54 m. It receives periodic freshwater inputs from the rivers Plym and Tamar outflowing at Plymouth and it is influenced by oceanic water during periods of strong south west winds (Rees *et al*., 2009). Sampling at this site carried out weekly (weather permitting). Water temperature was initially measured using a thermometer placed inside a stainless-steel bucket and with a CTD since 2000. More details on the methodology used for data acquisition can be found in Atkinson *et al*., (2015). Chl *a* was determined by using reversed-phase HPLC and surface values for water temperature, salinity and Chl *a* have been used. Two replicate vertical net hauls (WP2 net, 200 µm mesh size, 0.57 m diameter) from 50 m to the surface were used to collect zooplankton samples. These samples were stored in 5% formalin. Subsamples were taken with a stempel pipette for identifying and counting smaller organisms, whilst larger and rarer organisms were counted in subsamples, often one-half to one-eighth, taken with a Folsom splitter.

B35 (43° 20.9' N, 3° 1.6' W) is a coastal site (< 1 km offshore) influenced by the plume of the estuary of Bilbao (high nutrient enrichment and pollution; Ferrer *et al*., 2009). It is partially mixed and has a mean depth of 13 m. **U35** (43° 24.2' N, 2° 41.7' W) is a shallower (mean depth of 4.5 m), well-mixed coastal (< 1 km offshore) site, that bears the influence of the Urdaibai estuary, but because of high tidal flushing, the estuarine influence is much lower than at B35. It also is under much lower human pressure than the estuary of Bilbao. At the two sites, samplings were performed monthly. Water temperature and salinity were measured using portable multiparameter meters and Chl *a* was determined spectrophotometrically according to the monochromatic method with acidification (Lorenzen, 1967). Water temperature, salinity and Chl *a* data used in this study correspond to subsurface measurements at B35 (around 4 m depth) and U35 (around 2 m depth). Zooplankton were sampled by horizontal tows at mid-depth, below the halocline (when present), of a 200 µm mesh size ring net (mouth diameter 0.25 m) equipped with a flowmeter and preserved in 4% borax buffered formalin seawater solution. For the systematic and quantitative analysis, the original zooplankton samples were diluted in filtered sea water to a volume of 50 – 500 mL, depending on organism density, and sub-sampled into aliquots after gentle mixing to distribute organisms randomly. Subsamples were analysed in Bogorov-type counting chambers under an inverted microscope (40× – 400× magnification). One or more subsamples were analysed from each sample until 100 individuals of the most abundant taxonomic category and more than 30 individuals of the following most abundant one, or at least more than 30 individuals of the three most abundant categories were counted (Olivar *et al.*, 2010).

For the present study period, on the basis of Chl *a* criterion (Molvær *et al.*, 1997; Smith *et al.*, 1999), B35 and U35 may be classified as mesotrophic and oligotrophic, respectively, the trophic status of L4 and SH being more similar to that of U35 than to that of B35. Chl *a* values and other relevant features of these sites are summarized on **Table 1**.

Table 1. Main characteristics of the study sites and summary of their sampling features.

Characteristic		**B35**	**U35**	**L4**	**SH**
Distance offshore (km)		< 1	< 1	6.5	5
Water depth (m)	mean	13.0	4.5	54.0	48.0
Stratification/mixing		Partially mixed	Mixed	Transitionally mixed/stratified in summer	Mixed/weak stratification in summer
Salinity	mean (range)	34.8 (32.9-35.5)	35.0 (30.3-35.6)	35.0 (34.0-35.4)	34.5 (33.8-34.9)
Temperature (°C)	mean (range)	16.0 (11.3-23.7)	16.2 (10.8-24.9)	12.6 (7.6-19.9)	9.5 (4.5-13.9)
Chlorophyll *a* (µg L^{-1})	mean (range)	2.19 (0.08-31.33)	0.82 (0.04-7.91)	1.24 (0.23-6.29)	1.29 (0.09-5.96)
Samplings/month		1	1	4	4
Tows/timepoint		1	1	2	2
Reference of sampling and analytical methods		(Aravena *et al.*, 2009)	(Chapter 1, this work)	(Atkinson *et al.*, 2015)	(Bresnan *et al.*, 2015)

Data pretreatment

As B35 and U35 were sampled monthly, usually during, or close to, the last week of the month, whereas L4 and SH were generally sampled weekly, the mean of all values for each month were calculated for L4 and SH. Missing values (<5%) in the monthly data sets were filled by data interpolation using the mean values of the previous month and the following month. To ensure data consistency in zooplankton series, taxonomic homogenization was undertaken.

In the present study total zooplankton and selected zooplankton taxa (**Figure 3**) belonging to (i) the herein termed zooplankton Group level (ZG), a broad level consisting of six holoplankton categories (copepods, cladocerans, appendicularians, chaetognaths, siphonophores and doliolids) and nine meroplankton categories (cirripede larvae, decapod larvae, gastropod larvae, bivalve larvae, polychaete larvae, fish eggs and larvae, bryozoan larvae, echinoderm larvae and hydromedusae, in which some very much less abundant holoplanktonic forms, such as *Liriope tetraphylla*), were included and (ii) the copepod and cladoceran genera level (CCGen), consisting of genera or genera-assemblages (exceptionally family) of cladocerans and copepods: *Evadne* and *Podon* genera for the cladocerans and *Acartia*, *Centropages*, *Temora*, *Oithona*, *Oncaea*, *Corycaeus* (former genus that represents mainly the present genus *Ditrichocorycaeus* at the four sites) genera, the "PCPC-calanus" genera assemblage (this includes *Paracalanus*, *Clausocalanus*, *Pseudocalanus* and *Ctenocalanus*), and the family Calanidae for the copepods.

Some of the above genera include recently separated new genera that were not originally considered in the series, such as the genera *Mesocalanus* and *Calanoides* within Calanidae, the genus *Monothula* within *Oncaea*, the genus *Ditrichocorycaeus* within *Corycaeus*, the genus *Pseudevadne* within *Evadne* and the genus *Pleopis* within *Podon*. Holoplanktonic gastropods such as *Limacina retroversa* and *Gymnosomata* and holoplanktonic polychaetes such as *Tomopteris helgolandica* were included in the gastropod larvae and polychaete larvae categories respectively, because they were not always distinguished from meroplanktonic species in routine identifications.

Detailed information of the components identified and their contribution to the total in each selected taxon are shown in **Annexes**, **tables A.1.**, **A.2.** and **A.3.**, for holoplankton groups, meroplankton groups and cladoceran-copepod genera, respectively.

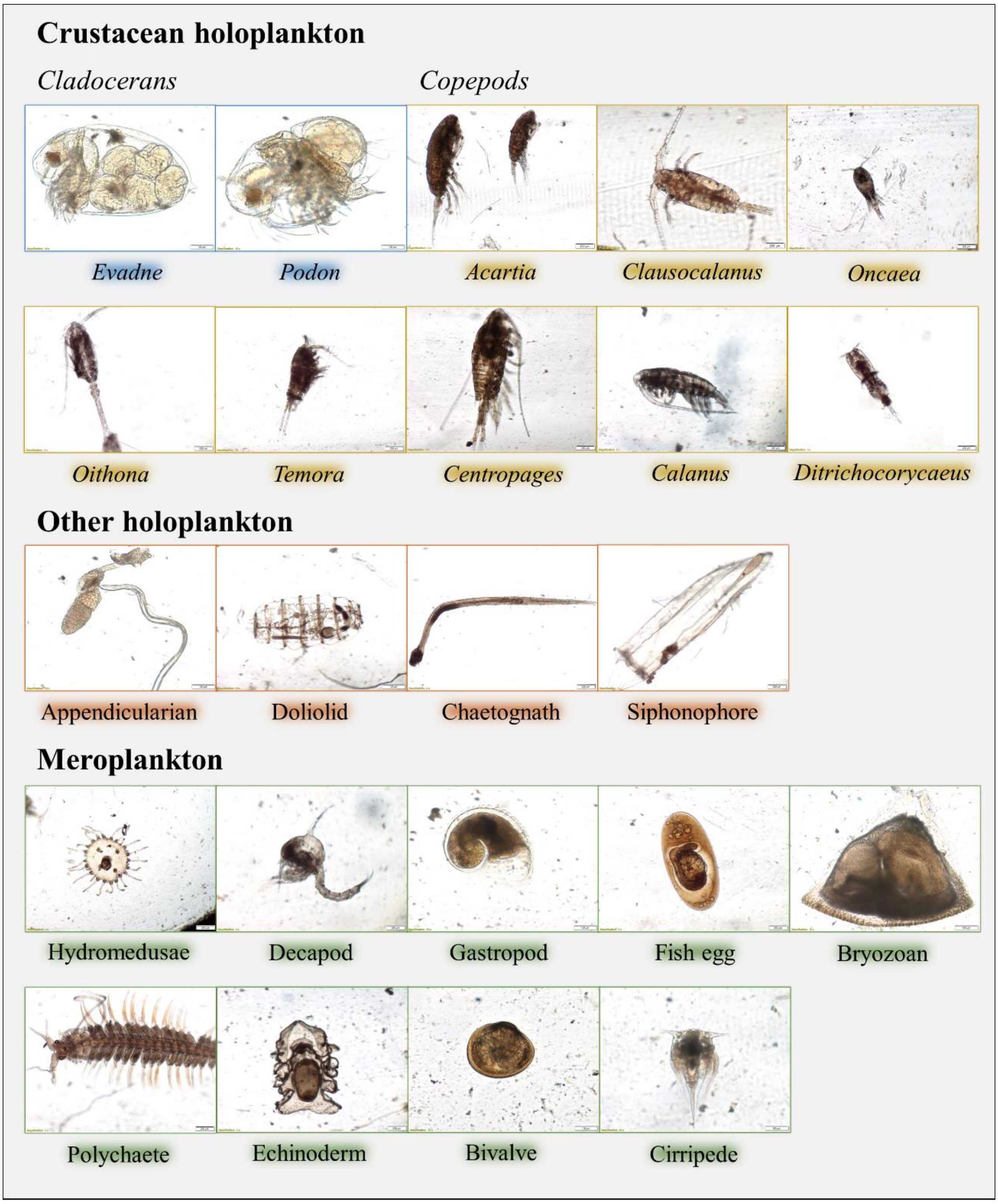

Figure 3. Example photographs of the main zooplankton taxa which we focused on in the present study. They are classified in three different groups: Crustacean holoplankton (copepods and cladocerans); Other holoplankton (taxa other than cladocerans and copepods), and meroplankton. Photographs by *Ziortza Barroeta*.

References

Aebischer, N. J., Coulson, J. C., Colebrookl, J. M. (1990). Parallel long-term trends across four marine trophic levels and weather. *Nature*, *347*(6295), 753–755. https://doi.org/10.1038/347753a0.

Alvarez-Fernandez, S., Lindeboom, H., Meesters, E. (2012). Temporal changes in plankton of the North Sea: Community shifts and environmental drivers. *Marine Ecology Progress Series*, *462*, 21–38. https://doi.org/10.3354/meps09817.

Ambler, J. W., Cloern, J. E., Hutchinson, A. (1985). Seasonal cycles of zooplankton from San Francisco Bay. *Hydrobiologia*, *129*, 177–197. https://doi.org/10.1007/BF00048694.

Aravena, G., Villate, F., Uriarte, I., Iriarte, A., Ibáñez, B. (2009). Response of *Acartia* populations to environmental variability and effects of invasive congenerics in the estuary of Bilbao, Bay of Biscay. *Estuarine, Coastal and Shelf Science*, *83*, 621–628. https://doi.org/10.1016/j.ecss.2009.05.013.

Atkinson, A., Harmer, R. A., Widdicombe, C. E., McEvoy, A. J., Smyth, T. J., Cummings, D. G., Sommerfield, P. J., Maud, J. L., McConville, K. (2015). Questioning the role of phenology shifts and trophic mismatching in a planktonic food web. *Progress in Oceanography*, *137*, 498–512. https://doi.org/10.1016/j.pocean.2015.04.023.

Ayón, P., Purca, S., Guevara-Carrasco, R. (2004). Zooplankton volume trends off Peru between 1964 and 2001. *ICES Journal of Marine Science*, *61*, 478–484. https://doi.org/10.1016/j.icesjms.2004.03.027.

Beaugrand, G., Edwards, M., Legendre, L. (2010). Marine biodiversity, ecosystem functioning, and carbon cycles. *Pnas*, *107*(22), 10120–10124. https://doi.org/10.1073/pnas.0913855107/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.0913855107.

Beaugrand, G., Ibañez, F., Reid, P. C. (2000). Spatial, seasonal and long-term fluctuations of plankton in relation to hydroclimatic features in the English Channel, Celtic Sea and Bay of Biscay. *Marine Ecology Progress Series*, *200*, 93–102. https://doi.org/10.3354/meps200093.

Beaugrand, G., Ibañez, F. (2004). Monitoring marine plankton ecosystems. II: Long-term changes in North Sea calanoid copepods in relation to hydro-climatic variability. *Marine Ecology Progress Series*, *284*, 35–47. https://doi.org/10.3354/meps284035.

Beaugrand, G., Luczak, C., Edwards, M. (2009). Rapid biogeographical plankton shifts in the North Atlantic Ocean. *Global Change Biology*, *15*, 1790–1803. https://doi.org/10.1111/j.1365-2486.2009.01848.x.

Beaugrand, G., Reid, P. C. (2003). Long-term changes in phytoplankton, zooplankton and salmon related to climate. *Global Change Biology*, *9*, 801–817. https://doi.org/10.1046/j.1365-2486.2003.00632.x.

Beaugrand, G., Reid, P. C., Ibañez, F., Lindley, J. A., Edwards, M. (2002). Reorganization of North Atlantic marine copepod biodiversity and climate. *Science*, *296*, 1692–1694. https://doi.org/10.1126/science.1071329.

Beers, J. R., Reid, F. M. H., Stewart, G. L. (1980). Microplankton population structure in Southern California nearshore waters in late spring. *Marine Biology*, *60*, 209–226. https://doi.org/10.1007/BF00389164.

Bonnet, D., Harris, R., Lopez-Urrutia, A., Halsband-Lenk, C., Greve, W., Valdes, L., Hans-Jürgen, H., Engel, M., Alvarez-Ossorio, M. T., Wiltshire, K. (2007). Comparative seasonal dynamics of *Centropages typicus* at seven coastal monitoring stations in the North Sea, English Channel and Bay of Biscay. *Progress in Oceanography*, *72*, 233–248. https://doi.org/10.1016/j.pocean.2007.01.007.

Borja, A., Fontán, A., Sáenz, J., Valencia, V. (2008). Climate, oceanography, and recruitment: The case of the Bay of Biscay anchovy (*Engraulis encrasicolus*). *Fisheries Oceanography*, *17*(6), 477–493. https://doi.org/10.1111/j.1365-2419.2008.00494.x.

Bresnan, E., Cook, K. B., Hughes, S. L., Hay, S. J., Smith, K., Walsham, P., Webster, L. (2015). Seasonality of the plankton community at an east and west coast monitoring site in Scottish waters. *Journal of Sea Research*, *105*, 16–29. https://doi.org/10.1016/j.seares.2015.06.009.

Brodeur, R. D., Ware, D. M. (1992). Long-term variability in zooplankton biomass in the subarctic Pacific Ocean. *Fisheries Oceanography*, *1*(1), 32–38. https://doi.org/10.1111/j.1365-2419.1992.tb00023.x.

Bucklin, A., Nishida, S., Schnack-Schiel, S., Wiebe, P. H., Lindsay, D., Machida, R. J., Copley, N. J. (2010). A Census of Zooplankton of the Global Ocean. In *Life in the World's Oceans: Diversity, Distribution, and Abundance* (247–265 pp.). Wiley-Blackwell. https://doi.org/10.1002/9781444325508.ch13.

Chick, J. H., Van Den Avyle, M. J. (1999). Zooplankton Variability and Larval Striped Bass Foraging : Evaluating Potential Match / Mismatch Regulation. *Ecological Applicaitons*, *9*(1), 320–334.

Clare, D. S., Spencer, M., Robinson, L. A., Frid, C. L. J. (2017). Explaining ecological shifts: the roles of temperature and primary production in the long-term dynamics of benthic faunal composition. *Oikos*, *126*, 1–11. https://doi.org/10.1111/oik.03661.

Castellani, C., Licandro, P., Fileman, E., Di Capua, I. Mazzocchi, M. G. (2016). *Oithona similis* likes it cool: evidence from two long-term time series. *Journal of Plankton Research*, *38*(3), 703–717.

Cloern, J. E. (1996). Phytoplankton bloom dynamics in coastal ecosystems: A review with some general lessons from sustained investigation of San Francisco Bay, California. *Reviews of Geophysics*, *34*(2), 127–168. https://doi.org/10.1029/96RG00986.

Cotton, P. A., Sims, D. W., Fanshawe, S., Chadwick, M. (2005). The effects of climate variability on zooplankton and basking shark (*Cetorhinus maximus*) relative abundance off southwest Britain. *Fisheries Oceanography*, *14*(2), 151–155. https://doi.org/10.1111/j.1365-2419.2005.00331.x.

Craig, J., Jamieson, A. J., Heger, A., Priede, I. G. (2009). Distribution of bioluminescent organisms in the Mediterranean Sea and predicted effects on a deep-sea neutrino telescope. *Nuclear Instruments and Methods in Physics Research, Section A*, *602*, 224–226. https://doi.org/10.1016/j.nima.2008.12.043.

Cross, J., Nimmo-Smith, W. A. M., Hosegood, P. J., Torres, R. (2015). The role of advection in the distribution of plankton populations at a moored 1-D coastal observatory. *Progress in Oceanography*, *137*, 342–359. https://doi.org/10.1016/j.pocean.2015.04.016.

Cushing, D. H. (1990). Plankton production and year-class strength in fish populations: An update of the match/mismatch hypothesis. *Advances in Marine Biology*, *26*. 249–293. https://doi.org/10.1016/S0065-2881(08)60202-3.

D'Alcalà, M. R., Conversano, F., Corato, F., Licandro, P., Mangoni, O., Marino, D., Mazzocchi M. G., Modigh, M., Montesor, M., Nardella, M., Saggiomo, V., Sarno, D. Zingone, A. (2004). Seasonal patterns in plankton communities in a pluriannual time series at a coastal Mediterranean site (Gulf of Naples): an attempt to discern recurrences and trends. *Scientia Marina*, *68*, 65–83. https://doi.org/10.3989/scimar.2004.68s165.

Dam, H. G. (2013). Evolutionary Adaptation of Marine Zooplankton to Global Change. *Annual Review of Marine Science*, *5*, 349–370. https://doi.org/10.1146/annurev-marine-121211-172229.

David, V., Selleslagh, J., Nowaczyk, A., Dubois, S., Bachelet, G., Blanchet, H., Goilleux, B., Lavesque, N., Leconte, M., Savoye, N., Sautour, B., Lobry, J. (2016). Estuarine habitats structure zooplankton communities: Implications for the pelagic trophic pathways. *Estuarine, Coastal and Shelf Science*, *179*, 99–111. https://doi.org/10.1016/j.ecss.2016.01.022.

Edwards, M., Bresnan, E., Cook, K., Heath, M., Helaouet, P., Lynam, C., Raine, R., Widdicombe, C. (2013). Impacts of climate change on plankton. *Marine Climate Change Impacts Partnership*, *1*, 98–112. https://doi.org/10.14465/2013.arc12.098-112.

Edwards, M., Richardson, A. J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature*, *430*, 881–884. https://doi.org/10.1038/nature02808.

Eloire, D., Somerfield, P. J., Conway, D. V. P., Halsband-Lenk, C., Harris, R., Bonnet, D. (2010). Temporal variability and community composition of zooplankton at station L4 in the Western Channel: 20 years of sampling. *Journal of Plankton Research*, *32*(5), 657–679. https://doi.org/10.1093/plankt/fbq009.

Eurgain, J. H., Batten, S. D., Harris, R. P., Hays, G. C. (2001). Comparison between zooplankton data collected by the Continuous Plankton Recorder survey in the English Channel and by WP-2 nets at station L4, Plymouth (UK). *Journal of Sea Research*, *46*(3–4), 223–232. https://doi.org/10.1016/S1385-1101(01)00085-5.

Falk-Petersen, S., Pedersen, G., Kwasniewski, S., Hegseth, E. N., Hop, H. (1999). Spatial distribution and life-cycle timing of zooplankton in the marginal ice zone of the Barents Sea during the summer melt season in 1995. *Journal of Plankton Research*, *21*(7), 1249–1264. https://doi.org/10.1093/plankt/21.7.1249.

Ferrer, L., Fontán, A., Mader, J., Chust, G., González, M., Valencia, V., Uriarte, A., Collins, M. B. (2009). Low-salinity plumes in the oceanic region of the Basque Country. *Continental Shelf Research*, *29*(8), 970–984. https://doi.org/10.1016/j.csr.2008.12.014.

Flores-Coto, C., Sanvicente-Añorve, L., Sánchez-Ramírez, M. (2010). Distribución y diversidad de apendicularias en el sur del Golfo de México. *Revista Mexicana De Biodiversidad*, *81*, 123–131. http://dx.doi.org/10.22201/ib.20078706e.2010.001.192.

Fromentin, J.-M., Planque, B. (1996). *Calanus* and environment in the eastern North Atlantic. II. Role of the North Atlantic Oscillation on *Calanus finmarchicus* and *C. helgolandicus*. *Marine Ecology Progress Series*, *134*, 11–118. https://doi.org/10.3354/meps134101.

Gaudy, R., Thibault-Botha, D. (2007). Metabolism of *Centropages* species in the Mediterranean Sea and the North Atlantic Ocean. *Progress in Oceanography*, *72*, 151–163. https://doi.org/10.1016/j.pocean.2007.01.005.

Gluchowska, M., Trudnowska, E., Goszczko, I., Kubiszyn, A. M., Blachowiak-Samolyk, K., Walczowski, W., Kwasniewski, S. (2017). Variations in the structural and functional diversity of zooplankton over vertical and horizontal environmental gradients en route to the Arctic Ocean through the Fram Strait. *PLoS ONE*, *12*(2). https://doi.org/10.1371/journal.pone.0171715.

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., Bruno, J. F., Casey, K. S., Ebert, C., Fox, H. E., Fujita, R., Heinemann, D., Lenihan, H. S., Madin, E. M. P., Perry, M. T., Selig, E. R., Spalding, M., Steneck, R., Watson, R. (2008). A Global Map of Human Impact on Marine Ecosystems. *Science*, *319*, 948–952. doi: 10.1126/science.1149345.

Hátún, H., Payne, M. R., Beaugrand, G., Reid, P. C., Sandø, A. B., Drange, H., Hansen, B., Bloch, D. (2009). Large bio-geographical shifts in the north-eastern Atlantic Ocean: From the subpolar gyre, via plankton, to blue whiting and pilot whales. *Progress in Oceanography, 80*, 149–162. https://doi.org/10.1016/j.pocean.2009.03.001.

Haury, L. R., McGowan, J. A., Wiebe, P. H. (1978). Patterns and process in the time-space scales of plankton distributions. *Spatial Pattern in Plankton Communities*, *3*, 277–327. https://doi.org/10.1017/CBO9781107415324.004.

Hays, G. C., Richardson, A. J., Robinson, C. (2005). Climate change and marine plankton. *Trends in Ecology and Evolution*, *20*(6), 337–344. doi: 10.1016/j.tree.2005.03.004.

Heinrich, A. K. (1962). The life histories of planktonic animals and seasonal cycles of plankton communities in the oceans. *Journal Du Conseil Permanent International Pour L´Exploration de La Mer*, *27*, 15–24.

Herveye, H. M., Richardson, A. J. (1998). Long-term increase in crustacean zooplankton abundance in the southern Benguela upwelling region (1951–1996): bottom-up or top-down control? *ICES Journal of Marine Science, 55,* 803–807. https://doi.org/10.1006/jmsc.1998.0387.

Hoegh-Guldberg, O., Bruno, J. F. (2010). The Impact of Climate Change on the World’s Marine Ecosystems. *Science*, *328*, 1523–1528. doi: 10.1126/science.1189930.

Ikeda, T. (1985). Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. *Marine Biology*, *85*, 1–11. https://doi.org/10.1007/BF00396409.

Iriarte, A., Aravena, G., Villate, F., Uriarte, I., Ibañez, B., Llope, M., Stenseth, N. C. (2010). Dissolved oxygen in contrasting estuaries of the Bay of Biscay: effects of temperature, river discharge and chlorophyll *a*. *Marine Ecology-Progress Series*, *418*, 57–71. https://doi.org/10.3354/meps08812.

Jamet, J.-L., Bogé, G., Richard, S., Geneys, C., Jamet, D. (2001). The zooplankton community in bays of Toulon area (northwest Mediterranean Sea, France). *Hydrobiologia*, *457*, 155–165. https://doi.org/10.1023/A:1012279417451.

Ji, R., Edwards, M., MacKas, D. L., Runge, J. A., Thomas, A. C. (2010). Marine plankton phenology and life history in a changing climate: Current research and future directions. *Journal of Plankton Research*, *32*(10), 1355–1368. https://doi.org/10.1093/plankt/fbq062.

Kane, J. (2007). Zooplankton abundance trends on Georges Bank , 1977 – 2004. *ICES Journal of Marine Science*, *64*(5), 909–919. https://doi.org/10.1093/icesjms/fsm066.

Kane, J. (2011). Inter-decadal variability of zooplankton abundance in the Middle Atlantic Bight. *Journal of Northwest Atlantic Fishery Science*, *43*, 81–92. https://doi.org/10.2960/J.v43.m674.

Kwasniewski, S., Gluchowska, M., Walkusz, W., Karnovsky, N. J., Jakubas, D., Wojczulanis-Jakubas, K., Harding, A. M. A., Goszczko, I., Cisek, M., Beszczynska-Möller, A., Walczowski, W., Weslawski, J. M., Stempniewicz L. (2012). Interannual changes in zooplankton on the West Spitsbergen Shelf in relation to hydrography and their consequences for the diet of planktivorous seabirds. *ICES Journal of Marine Science*, 69(5), 890–901. https://doi.org/10.1093/icesjms/fss076.

Lees, K., Pitois, S., Scott, C., Frid, C., MacKinson, S. (2006). Characterizing regime shifts in the marine environment. *Fish and Fisheries*, *7*, 104–127. https://doi.org/10.1111/j.1467-2979.2006.00215.x.

Lenz, J. (2000). Introduction. In *ICES Zooplankton Methodology Manual*. (1-32 pp.). Academic Press. https://doi.org/10.1016/B978-012327645-2/50007-4.

Llope, M., Anadón, R., Viesca, L., Quevedo, M., González-Quirós, R., Stenseth, N. C. (2006). Hydrography of the southern Bay of Biscay shelf-break region: Integrating the multiscale physical variability over the period 1993-2003. *Journal of Geophysical Research: Oceans*, *111*, 1–14. https://doi.org/10.1029/2005JC002963.

Longhurst, A.R. (1998). Ecological geography of the sea. *Academic Press, San Diego*. 398 pp.

Lorenzen, C. J. (1967) Determination of chlorophyll and pheo-pigments: spectrophotometric equations. *Limnology and Oceanography*, *12*, 343–346. https://doi.org/10.4319/lo.1967.12.2.0343.

Mackas, D. L., Beaugrand, G. (2010). Comparisons of zooplankton time series. *Journal of Marine Systems*, *79*, 286–304. https://doi.org/10.1016/j.jmarsys.2008.11.030.

Mackas, D. L., Greve, W., Edwards, M., Chiba, S., Tadokoro, K., Eloire, D., Mazzochi, M. G., Batten, S., Richardson, A. J., Johnson, C., Head, E., Conversi, A., Peluso, T. (2012). Changing zooplankton seasonality in a changing ocean: Comparing time series of zooplankton phenology. *Progress in Oceanography*, *97–100*, 31–62. https://doi.org/10.1016/j.pocean.2011.11.005.

Magurran, A. E., Baillie, S. R., Buckland, S. T., Dick, J. McP., Elston, D. A., Scott, E. M., Smith, R. I., Somerield, P. J., Watt, A. D. (2012). Long-term datasets in biodiversity research and monitoring: assessing change in ecological communities through time. *Trends in Ecology and Evolution*, *25*, 574–582. https://doi.org/10.1016/j.tree.2010.06.016.

McClanahan, T. R. (1988). Seasonality in East Africa's coastal waters. *Marine Ecology Progress Series*, *44*, 191–199. https://doi.org/10.3354/meps044191.

Medellín-Mora, J., Escribano, R., Schneider, W. (2016). Community response of zooplankton to oceanographic changes (2002-2012) in the central/southern upwelling system of Chile. *Progress in Oceanography*, *142*, 17–29. https://doi.org/10.1016/j.pocean.2016.01.005.

Molinero, J. C., Reygondeau, G., Bonnet, D. (2013). Climate variance influence on the non-stationary plankton dynamics. *Marine Environmental Research*, *89*, 91–96. https://doi.org/10.1016/j.marenvres.2013.04.006.

Möllmann, C., Müller-Karulis, B., Kornilovs, G., St John, M. A. (2008). Effects of climate and overfishing on zooplankton dynamics and ecosystem structure: regime shifts, trophic cascade, and feedback loops in a simple ecosystem. *ICES Journal of Marine Science*, *65*(3), 302–310. https://doi.org/10.1093/icesjms/fsm197.

Moore, G. W. K., Renfrew, I. A. (2012). Cold European winters: interplay between the NAO and the East Atlantic mode. *Atmospheric Science Letters*, *13*, 1–8. doi: 10.1002/asl.356.

Morse, R. E., Friedland, K. D., Tommasi, D., Stock, C., Nye, J. (2017). Distinct zooplankton regime shift patterns across ecoregions of the U.S. Northeast continental shelf Large Marine Ecosystem. *Journal of Marine Systems*, *165*, 77–91. https://doi.org/10.1016/j.jmarsys.2016.09.011.

National Oceanic and Atmospheric Administration (NOAA). https://www.esrl.noaa.gov/psd/gcos_wgsp/Timeseries/AMO/.

National Oceanic and Atmospheric Administration (NOAA). http://www.cpc.ncep.noaa.gov/data/teledoc/ea.shtml.

National Oceanic and Atmospheric Administration (NOAA). http://www.cpc.ncep.noaa.gov/data/teledoc/nao.shtml.

National Oceanic and Atmospheric Administration (NOAA). https://www.ncdc.noaa.gov/teleconnections.

Nye, J. A., Baker, M. R., Bell, R., Kenny, A., Kilbourne, K. H., Friedland, K. D., Martino, E., Stachura, M. M., Van Houtan, K. S., Wood, R. (2014). Ecosystem effects of the Atlantic Multidecadal Oscillation. *Journal of Marine Systems, 133,* 103–116. https://doi.org/10.1016/j.jmarsys.2013.02.006.

O’Brien, T. D., Wiebe, P. H., Falkenhaug, T. (2013). ICES Zooplankton Status Report 2010/2011. *ICES Cooperative Research Report. 318*. 208 pp. ISSN 1017-6195.

Omori, M., Ikeda, T. (1992). Methods in Marine Zooplankton Ecology. Malabar, USA: Krieger Publishing Company. ISBN 0-89464-653-2.

Pepin, P., Johnson, C. L., Harvey, M., Casault, B., Chassé, J., Colbourne, E. B., Galbraith, P. S., Hebert, D., Lazin, G., Maillet, G., Plourde, S., Starr, M. (2015). A multivariate evaluation of environmental effects on zooplankton community structure in the western North Atlantic. *Progress in Oceanography*, *134*, 197–220. https://doi.org/10.1016/j.pocean.2015.01.017.

Peters, R. H., Downing, J. A. (1984). Empirical analysis of zooplankton filtering and feeding rates. *Limnology and Oceanography*, *29*(4), 763–784. https://doi.org/10.4319/lo.1984.29.4.0763.

Piontkovski, S. A., O’Brien, T. D., Umani, S. F., Krupa, E. G., Stuge, T. S., Balymbetov, K. S., Grishaeva, O. V., Kasymov, A. G. (2006). Zooplankton and the North Atlantic oscillation: A basin-scale analysis. *Journal of Plankton Research*, *28*(11), 1039–1046. https://doi.org/10.1093/plankt/fbl037.

Rassoulzadegan, F., Sheldon, R. W. (1986). Predator-prey interactions of nanozooplankton and bacteria in an oligotrophic marine environment. *Limnology and Oceanography*, *31*(5), 1010–1021. https://doi.org/10.4319/lo.1986.31.5.1010.

Reid, P. C., Hari, R. E., Beaugrand, G., Livingstone, D. M., Marty, C., Straile, D., Bariwich, J., Goberville, E., Adrian, R., Aono, Y., Brown, R., Foster, J., Groisman, P., Héalouet, P., Hsu, H.-H., Kirby, R., Knight, J., Kraberg, A., Li, J., Lo, T.-T., Myneni, R. B., North, R. P., Pounds, J. A., Sparks, T., Stübi, R., Tian, Y., Wiltshire, K. H., Xiao, D., Zhu, Z. (2016). Global impacts of the 1980s regime shift. *Global Change Biology*, *22*(2), 682–703. https://doi.org/10.1111/gcb.13106.

Reygondeau, G., Beaugrand, G. (2011). Future climate-driven shifts in distribution of *Calanus finmarchicus*. *Global Change Biology*, *17*(2), 756–766. https://doi.org/10.1111/j.1365-2486.2010.02310.x.

Reygondeau, G., Molinero, J. C., Coombs, S., MacKenzie, B. R., Bonnet, D. (2015). Progressive changes in the Western English Channel foster a reorganization in the plankton food web. *Progress in Oceanography, 137,* 524–532. https://doi.org/10.1016/j.pocean.2015.04.025.

Richardson, A. J. (2008). In hot water: Zooplankton and climate change. *ICES Journal of Marine Science*, *65*(3), 279–295. https://doi.org/10.1093/icesjms/fsn028.

Roff, J. C., Middlebrook, K., Evans, F. (1988). Long-Term variability in north sea zooplankton off the northumberland coast: Productivity of small copepods and analysis of trophic interactions. *Journal of the Marine Biological Association of the United Kingdom*, *68*(1), 143–164. https://doi.org/10.1017/S0025315400050153.

Rombouts, I., Beaugrand, G., Ibañez, F., Gasparini, S., Chiba, S., Legendre, L. (2010). A multivariate approach to large-scale variation in marine planktonic copepod diversity and its environmental correlates. *Limnology and Oceanography*, *55*(5), 2219–2229. https://doi.org/10.4319/lo.2010.55.5.2219.

Sánchez-López, G., Hernández, A., Pla-Rabes, S., Trigo, R. M., Toro, M., Granados, I., Sáez, A., Masqué, P., Pueyo, J. J., Rubio-Inglés, M. J., Giralt, S. (2016). Climate reconstruction for the last two millennia in central Iberia: The role of East Atlantic (EA), North Atlantic

Oscillation (NAO) and their interplay over the Iberian Peninsula. *Quaternary Science Reviews*, *149*, 135–150. https://doi.org/10.1016/j.quascirev.2016.07.021.

Smith, S. L., Whitledge, T. E. (1977). The role of zooplankton in the regeneration of nitrogen in a coastal upwelling system off northwest Africa. *Deep-Sea Research*, *24*, 49–56. doi: 10.1248/cpb.32.452.

Spencer, M., Mieszkowska, N., Robinson, L. A., Simpson, S. D., Burrows, M. T., Birchenough, S. N. R., Capasso, E., Cleall-Harding, P., Crummy, J., Duck, C., Eloire, D., Frost, M., Hall, A. J., Hawkins, S. J., Johns, D. G., Sims, D. W., Smyth, T. J., Frid, C. L. J. (2012). Region-wide changes in marine ecosystem dynamics: State-space models to distinguish trends from step changes. *Global Change Biology*, *18*(4), 1270–1281. https://doi.org/10.1111/j.1365-2486.2011.02620.x.

Starr, M., Himmelman, J. H., Therriault, J. C. (1991). Coupling of nauplii release in barnacles with phytoplankton blooms: A parallel strategy to that of spawning in urchins and mussels. *Journal of Plankton Research*, *13*(3), 561–571. https://doi.org/10.1093/plankt/13.3.561.

Stenseth, N. C., Mysterud, A., Ottersen, G., Hurrell, J. W., Chan, K. S., Lima, M. (2002). Ecological effects of climate fluctuations. *Science*, *297*(5585), 1292–1296. doi: 10.1126/science.1071281.

Sugimoto, T., Tadokoro, K. (2003). Interannual–interdecadal variations in zooplankton biomass, chlorophyll concentration and physical environment in the subarctic Pacific and Bering Sea. *Fisheries Oceanography*, *6*(2), 74–93. https://doi.org/10.1046/j.1365-2419.1997.00031.x.

Tirelli, V., Mayzaud, P. (2005). Relationship between functional response and gut transit time in the calanoid copepod *Acartia clausi*: Role of food quantity and quality. *Journal of Plankton Research*, *27*(6), 557–568. https://doi.org/10.1093/plankt/fbi031.

Tiselius, P., Saiz, E., Kiørboe, T. (2013). Sensory capabilities and food capture of two small copepods, *Paracalanus parvus* and *Pseudocalanus* sp. *Limnology and Oceanography*, *58*(5), 1657–1666. https://doi.org/10.4319/lo.2013.58.5.1657.

Tomita, M., Shiga, N., Ikeda, T. (2003). Seasonal occurrence and vertical distribution of appendicularians in Toyama Bay , southern Japan Sea. *Journal of Plankton Research*, *25*(6), 579–589. https://doi.org/10.1093/plankt/25.6.579.

Uriarte, I., Villate, F. (2004). Effects of pollution on zooplankton abundance and distribution in two estuaries of the Basque coast (Bay of Biscay). *Marine Pollution Bulletin*, *49*, 220–228. https://doi.org/10.1016/j.marpolbul.2004.02.010.

Uriarte, I., Villate, F., Iriarte, A. (2016). Zooplankton recolonization of the inner estuary of Bilbao: influence of pollution abatement, climate and non-indigenous species. *Journal of Plankton Research*, *38*(3), 718–731. https://doi.org/10.1093/plankt/fbv060.

Vecchione, M. (1989). Zooplankton distribution in three estuarine bayous with different types of anthropogenic influence. *Estuaries*, *12*(3), 169–179. https://doi.org/10.2307/1351821.

Villate, F., Iriarte, A., Uriarte, I., Sanchez, I. (2017). Seasonal and interannual variability of mesozooplankton in two contrasting estuaries of the Bay of Biscay: Relationship to environmental factors. *Journal of Sea Research*, *130*, 189–203. https://doi.org/10.1016/j.seares.2017.05.002.

Weijerman, M., Lindeboom, H., Zuur, A. F. (2005). Regime shifts in marine ecosystems of the North Sea and Wadden Sea. *Marine Ecology Progress Series*, *298*, 21–39. https://doi.org/Doi 10.3354/Meps298021.

Woollings, T., Hannachi, A., Hoskins, B. (2010). Variability of the North Atlantic eddy-driven jet stream. *Quarterly Journal of the Royal Meteorological Society*, *136*, 856–868. doi:

10.1002/qj.625.

Worm, B., Hilborn, R., Baum, J. K., Branch, T. A., Collie, J. S., Costello, C., Gogarty, M. J., Fulton, E. A., Hutchings, J. A., Jennings, S., Jensen, O. P., Lotze, H. K., Mace, P. M., McClanahan, T. R., Minto, C., Palumbi, S. R., Parma, A. M., Ricard, D., Rosenberg, A. A., Watson, R., Zeller, D. (2009). Rebuilding Global Fisheries. *Science*, *325*(5940), 578–585. https://doi.org/10.1126/science.1173146.

Xiong, L. L., Yin, J. Q., Huang, L. M., Li, K. Z., Lian, S. M. (2012). Seasonal and spatial variations of cladocerans on the northwest continental shelf of the South China Sea. *Crustaceana*, *85*, 473–496. doi: 10.1163/156854012X636742.

Xu, J., Xu, Z., Gao, Q. (2016). Distribution of dominant zooplankton species along a latitudinal gradient in China sea during spring. *Journal of Ocean University of China*, *15*(3), 502–508. https://doi.org/10.1007/s11802-016-2871-6.

Chapter 1. *Analyzing the different scales of zooplankton variability*

Zooplankton abundance series (1999 – 2013) from the coastal sites of Bilbao 35 (B35), Urdaibai 35 (U35), Plymouth L4 (L4) and Stonehaven (SH), in the Northeast Atlantic were compared to assess differences in the magnitude of seasonal, interannual and residual scales of variability, and in patterns of seasonal and interannual variation in relation to latitudinal location and trophic status. Results showed highest seasonal variability at SH in coherence with its northernmost location, highest interannual variability at U35 associated to an atypical event identified in 2012 in the Bay of Biscay, and highest residual variability at U35 and B35 likely related to lower sampling frequency and higher natural and anthropogenic stress. Interannual zooplankton variations were not coherent across sites, suggesting the dominance of local influences over large scale environmental drivers. For most taxa the seasonal pattern showed coherent differences across sites, the northward delay of the annual peak being the most common feature. The between-site seasonal differences in spring-summer zooplankton taxa were related mainly to phytoplankton biomass, in turn, related to differences in latitude or anthropogenic nutrient enrichment. The northward delay in water cooling likely accounted for between-site seasonal differences in taxa that increase in the second half of the year.

Fanjul, A., *Villate, F., Uriarte, I., Iriarte, A., Atkinson, A., & Cook, K. (2017). Zooplankton variability at four monitoring sites of the Northeast Atlantic Shelves differing in latitude and trophic status. Journal of Plankton Research, 39(6), 891-909.*

1.1. Introduction

The abundance of zooplankton may be highly variable at time scales that span from minutes to decades in response to environmental drivers and stressors operating across a wide range of temporal scales (Haury *et al*., 1978). Relevant time scales of variance, ranging from days to years involve changes in growth, production, mortality and community function (Marine Zooplankton Colloquium 1, 1989). The seasonal cycle is a key scale because of the large physical and biotic variations (Mackas and Beaugrand, 2010; Mackas *et al.*, 2012), and the importance of phenological timing for predator-prey interactions (Sydeman and Bograd, 2009). For example, fish larvae survival and recruitment success is highly dependent on the availability of suitable zooplankton prey in synchrony with their seasonal spawning and development, according to the match-mismatch hypothesis (Cushing, 1990), and there is the potential for differential phenology shifts of predator and prey in response to environmental changes (Edwards and Richardson, 2004; Durant *et al.*, 2007).

In addition to phenological shifts, interannual variations in overall abundance of zooplankton are driven by year-to-year variations of the physical and nutritional environments, which also help to modulate the recruitment of fish populations (Liu *et al.*, 2014). Therefore it is important to determine the extent to which the seasonal and interannual variations differ from site to site in order to build an ecological classification of pelagic ecosystems on a geographical basis (Longhurst, 1998). At a large spatial scale, latitude-dependent differences in light and temperature are the main factors responsible for the largest changes in the plankton annual cycles. The general patterns for oceanic zooplankton are (i) a large amplitude single summer peak at high-latitudes, (ii) bimodal cycles with a spring bloom and a secondary peak in autumn at middle latitudes, and (iii) no clear seasonal patterns in low latitude tropical waters (Heinrich, 1962). In shallow shelf seas, however, local natural (e.g. river discharge, coastal upwelling) and anthropogenic (wastewater inputs) stressors may substantially modify the standard plankton cycles (e.g. Cloern, 1996; Jamet *et al.*, 2001; Ribera d´Alcalà *et al.*, 2004).

In the ICES area a large number of time series are available which have been obtained using comparable methodology (O´Brien *et al.*, 2013), but there have been few attempts to synthesise across multiple time series (Valdes *et al*., 2007; Bode *et al.*, 2012; Mackas *et al.*, 2012; Castellani *et al.*, 2016). Policy directives such as the Marine Strategy Framework Directive need to assess baseline envelopes of variability and its causes, and provide a broad scale geographical context for this variability.

Many zooplankton time series of the ICES area are from sites located within the Northeast

Atlantic Shelves Province (NECS), a biogeographical unit established by Longhurst (1998) for the continental shelves of western Europe that extends from northern Spain to the Faroe-Shetland Channel and the Norwegian Trench. However, this is a wide area that includes the North Sea, the Baltic Sea, the outer shelves off Britain, and the Bay of Biscay. In fact, Longhurst himself recognized that this Province can be subdivided in a way which is more sensitive to ecological differences. The classical biogeographic divisions established for the Eastern North Atlantic are also suitable to look at ecological differences within the studied area. For instance, the northern part of the North Sea is included in the Eastern Atlantic boreal region, while the English Channel and the Bay of Biscay belong to the Eastern Atlantic warm temperate region (Briggs and Bowen, 2012).

In this study we have selected four of these ICES sites covering most of the latitudinal gradient in NECS, from the northern North Sea (1 site) to the southern Bay of Biscay (2 sites) with the western English Channel as an equidistant central part (1 site). From the 2 sites of the southernmost zone, one of them differs from the rest of sites in the trophic status (established on the basis of chlorophyll *a* concentration criteria (see Molvær *et al*., 1997; Smith *et al*., 1999). The aim was to assess between-site differences in (i) the magnitude of the temporal components of zooplankton variability (i.e. interannual, seasonal and residual components, *sensu* Cloern and Jassby, 2010), and (ii) the patterns of interannual and seasonal variation. We have tried to contribute to define zooplankton scales and patterns of variability within the NECS in relation to differences in latitude, local features and anthropogenic nutrient enrichment.

1.2. Methods

1.2.1. Data treatment

Several zooplankton taxa were selected for the analysis: non-crustacean holoplankton (appendicularians, chaetognaths, siphonophores and doliolids), cladocerans and copepods (*Evadne*, *Podon*, *Acartia*, *Centropages*, *Temora*, *Oithona*, *Oncaea*, *Corycaeus*, the Calanidae family and the PCPC-calanus assemblage) and meroplankton (cirripede larvae, decapod larvae, gastropod larvae, bivalve larvae, polychaete larvae, fish eggs and larvae, bryozoan larvae, echinoderm larvae and hydromedusae). Then, the scales and patterns of variability for temperature, Chl *a*, total zooplankton and selected zooplankton taxa were extracted for each site by using the following multiplicative model described by Cloern and Jassby (2010):

$$\mathbf{c}_{ij} = C\, y_i\, m_j\, \mathcal{E}_{ij}$$

where $\mathbf{c}_{ij}$ is the value in year i (i=1…, N) and month j (j=1…, 12); C is the long-term mean of the series; y_i is the annual effect in the ith year; m_j is the seasonal (monthly) effect in the jth month;

and ε_{ij} is the residual. This method decomposes time series into (i) an annual component, herein named "interannual variability", where trends, shifts and events can be detected, (ii) a seasonal component or "seasonal variability", where a standard seasonal pattern can be identified, and (iii) a residual component, or "residual variability", associated to the event scale, which includes the variability that cannot be attributed to the average seasonal pattern or to fluctuations in the annual mean. In plankton time series, residual variability may reflect sampling uncertainty associated to low frequency temporal variability within months but it may also be affected by the year to year stability of the seasonal pattern both in terms of magnitude and phenological variations (Cloern and Jassby, 2010).

To assess the possible effect of this high frequency temporal variability on the residual variability, monthly anomalies on the time series were calculated as the difference between each single value and the series mean and divided by the standard deviation. These anomalies were calculated for five selected taxa that were abundant and showed a clear temporal segregation in the timing of the standard annual maximum at all sites (i.e. copepods, cirripede larvae, appendicularians, chaetognaths and siphonophores), as well as for total zooplankton abundance, Chl *a* concentration and water temperature. To show and compare seasonal variability between years, year vs. month diagrams of the anomalies were produced for each of the above-mentioned variables at each of the four study sites.

To make the calculations of interannual and seasonal variability of all selected taxa possible, the data gap for *Centropages* in 1999 at L4 was filled by assuming the same abundance data as in 2000, and the lack of data for doliolids in some years at SH was solved by adding in such years a value of 0.01 in the month of the annual maximum obtained from the years with presence of doliolids. In addition, an unusually high value of fish eggs at L4 in March 2000 was considered erroneous, and replaced by the mean value of the month obtained from the rest of years of the series.

Paired t-tests were performed to determine differences between sites in the interannual, seasonal and residual components of variability of zooplankton taxa, and differences between the three components of variability within each site. Spearman rank correlation analyses were performed to test the relationships between the year-to-year variations of total zooplankton abundance, Chl *a* concentration, water temperature and zooplankton taxa abundance at each site, and the between-site relationships of the year-to-year variations of each zooplankton taxa. Both types of analyses were performed using SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY).

Resemblance analyses were carried out by means of the Bray Curtis similarity index (Bray and Curtis, 1957), using the group average method, to measure the dissimilarity between all the selected zooplankton taxa, according to their patterns of variability at the four monitoring sites jointly. Dissimilarity was tested both for the interannual and the seasonal variability using the PRIMER v6 software package (Clarke and Warwick, 2001), and results were displayed in dendrograms.

1.3. Results

1.3.1. Scales of variability

Values of interannual, seasonal and residual variability for zooplankton taxa at the four sites are depicted as box plots in **Figure 1**. Interannual variability was the lowest and residual variability the highest at all sites, although the difference between seasonal and interannual variability at U35, and between residual and seasonal variability at SH were not significant (**Table 1**). The lowest interannual, seasonal and residual variability were obtained at L4, the highest interannual and residual variability at U35 and the highest seasonal variability at SH. Interannual variability was higher at U35 than at B35, L4 and SH, seasonal variability was higher at SH than at L4, and residual variability was higher at U35 and B35 than at L4 and SH (**Table 2**). Due to the fact that a single value was used as monthly estimate for B35 and U35 while within month values (usually 4) were considered replicates and averaged for L4 and SH, a reduction by a factor of 2 of the within-month standard deviation could be expected at L4 and SH.

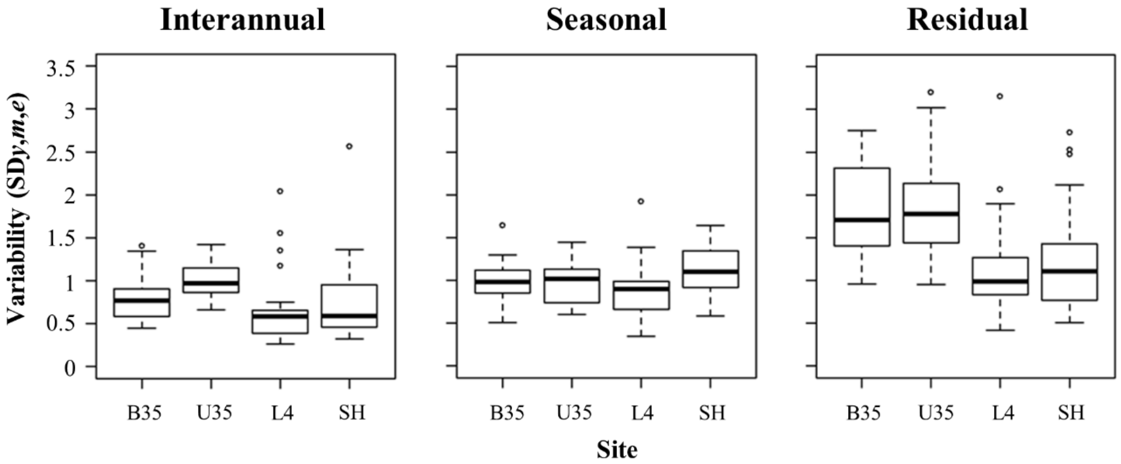

Figure 1. Box plot of data from interannual, seasonal and residual components of zooplankton taxa variability for Bilbao 35 (B35), Urdaibai 35 (U35), Plymouth L4 (L4) and Stonehaven (SH). Box represents the interquartile (IQ) range which contains the 50% of the records. Line across the box indicates the median. Whiskers extend to the highest and lowest values which are no greater than 1.5 times the IQ range. Circles indicate outliers with values between 1.5 and 3 times the IQ range. Note that the residual variability at L4 and SH is reduced by within month averaging (see methods).

Table 1. *p*-values obtained from paired t-tests for differences between the interannual (I), seasonal (S) and residual (R) components of zooplankton taxa at Bilbao 35 (B35), Urdaibai 35 (U35), Plymouth L4 (L4) and Stonehaven (SH). In bold statistically significant differences ($p < 0.05$).

	B35		U35		L4		SH	
	I	S	I	S	I	S	I	S
S	**0.002**	---	0.919	---	**0.044**	---	**0.006**	---
R	< **0.001**	< **0.001**	< **0.001**	< **0.001**	< **0.001**	**0.001**	< **0.001**	0.210

Table 2. *p*-values obtained from paired t-tests for differences between sites (Bilbao 35: B35, Urdaibai 35: U35, Plymouth L4: L4 and Stonehaven: SH) in the interannual, seasonal and residual components of zooplankton taxa. In bold statistically significant differences ($p < 0.05$).

	Interannual			Seasonal			Residual		
	U35	L4	SH	U35	L4	SH	U35	L4	SH
B35	< **0.001**	0.248	0.798	0.855	0.202	0.061	0.743	< **0.001**	< **0.001**
U35	---	< **0.001**	**0.023**	---	0.078	0.060	---	< **0.001**	< **0.001**
L4	---	---	0.220	---	---	**0.002**	---	---	0.399

Figure 2 shows that the between-year differences in the timing of the annual maximum were much lower for all taxa at SH, where the range of months within which the annual maximum occurred was of two months for siphonophores (September – October), three for chaetognaths (July – September) and cirripede larvae (March – May), four for appendicularians (May – august) and five for copepods (May – September). For the same taxa the range of months within which the annual maximum occurred varied from two (cirripede larvae: March – April) to eight (copepods: March – October) months at L4, from five (chaetognaths: late May – September) to eight (appendicularians and siphonophores: late March – October) at U35, and from six (chaetognaths: late May – October) to nine (copepods: late February – October) at B35.

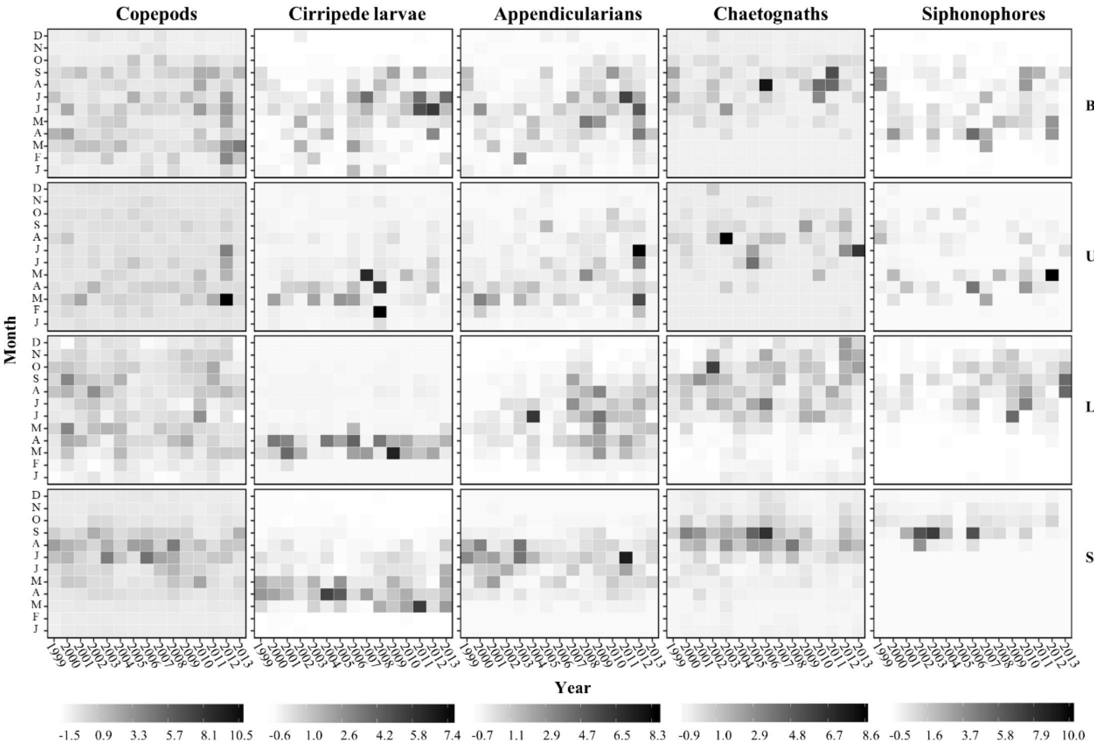

Figure 2. Year vs. month variations of abundance (expressed as anomalies) for copepods, cirripede larvae, appendicularians, chaetognaths and siphonophores at Bilbao 35 (B35), Urdaibai 35 (U35), Plymouth L4 (L4) and Stonehaven (SH).

As shown in **Figure 3**, the between-year differences in the timing of the annual maximum of total zooplankton abundance was also lowest at SH, with a range of five months (May – September), whereas the range was of six months at U35 (March – August), seven at L4 (March – September) and eight at B35 (February – September). The period within which Chl *a* showed annual maximum was of six months at SH and L4 (April – September), seven at B35 (late February – August) and nine at U35 (late February – October). The range for water temperature annual maxima was of two months at SH (August – September) and U35 (late July – August), and of three months at L4 (July – September) and B35 (late July – September).

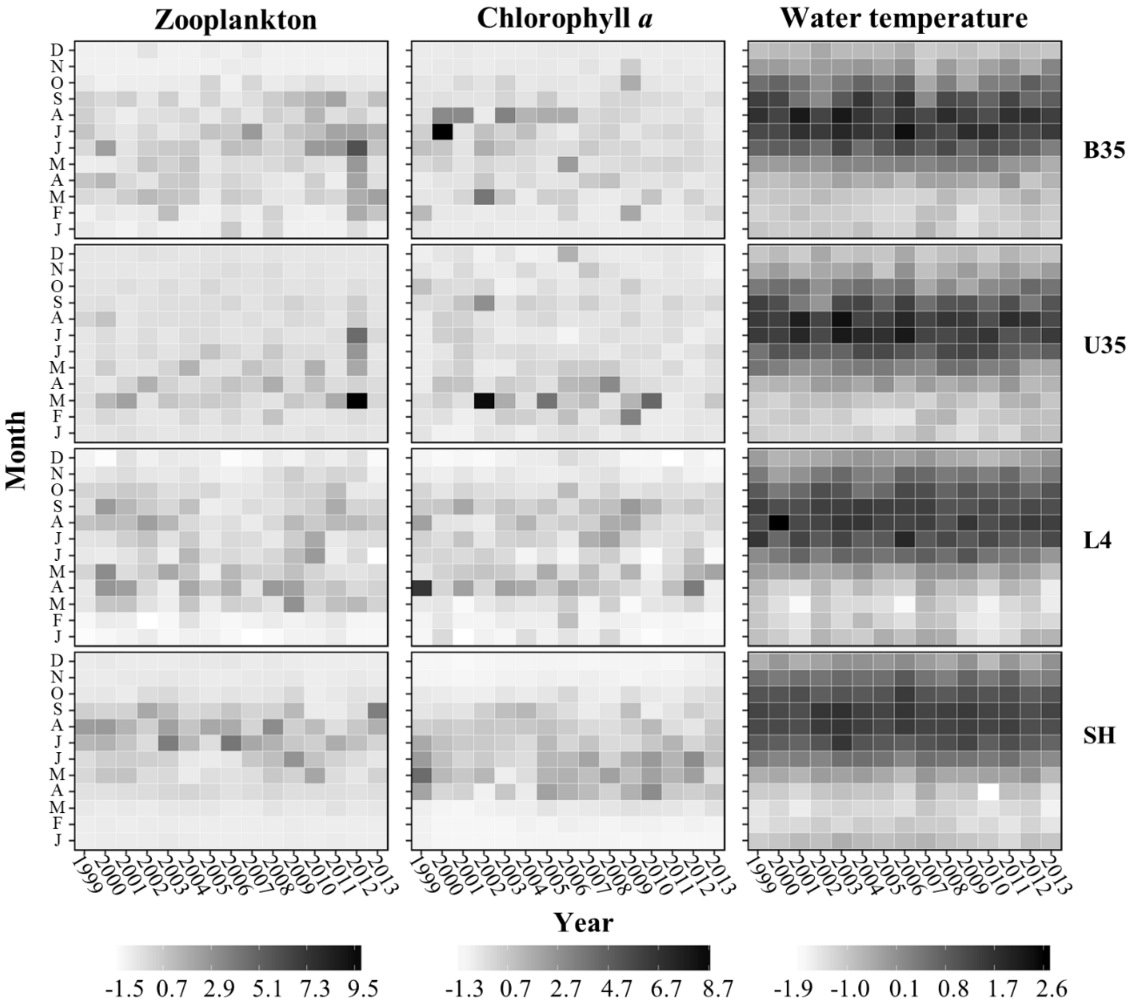

Figure 3. Year vs. month variations of total zooplankton abundance, chlorophyll *a* concentration and water temperature (expressed as anomalies) at Bilbao 35 (B35), Urdaibai 35 (U35), Plymouth L4 (L4) and Stonehaven (SH).

1.3.2. Interannual variations

1.3.2.1. Total zooplankton, Chlorophyll a and temperature

The annual mean values and the interannual variability (dimensionless) of total zooplankton abundance, Chl *a* concentration and temperature are shown in **Figure 4**. Zooplankton abundance fluctuated between 972 and 5097 ind. m^{-3} (all sites pooled), except in 2012 at B35 and U35, where values of 9116 and 12866 ind. m^{-3} where obtained respectively. Annual mean values of Chl *a* at U35, L4 and SH were similar and ranged between 0.49 and 1.81 µg L^{-1}, whereas at B35 they were higher than at the other sites ($p < 0.001$), with a maximum value of 4.76 µg L^{-1} in 2000 and a decrease over the study period. The warmest and the coldest years in the series differed between sites, although in all of them the warmest ones were recorded from 2003 to 2007 (2003 at SH, 2003 and 2006 with similar values at U35, 2006 at B35 and 2007 at L4) and the coldest ones in the second half of the series (2007 at B35 and U35, 2010 at L4 and 2013 at SH).

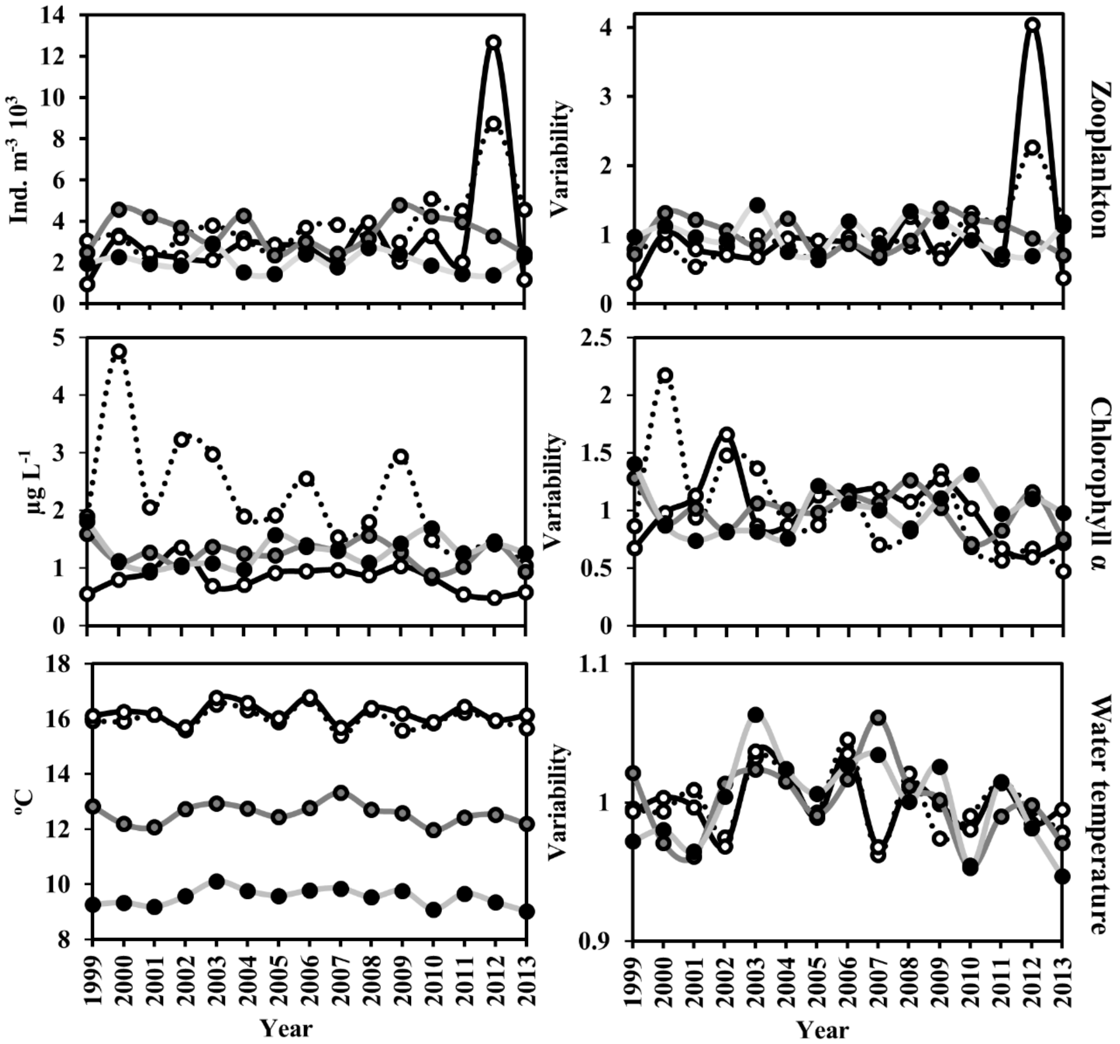

Figure 4. Annual mean raw values (left) and dimensionless year-to-year variability values (right) of total zooplankton abundance, chlorophyll *a* concentration and water temperature in Bilbao 35 (white circle, dotted black line), Urdaibai 35 (white circle, black line), Plymouth L4 (dark grey circle and line) and Stonehaven (black circle, light grey line).

Zooplankton abundance and Chl *a* were not correlated between sites, whereas water temperature correlated between B35 and U35 ($p< 0.001$), and between L4 and SH ($p= 0.001$). Within sites zooplankton abundance, Chl *a* concentration and water temperature were not correlated, except for the negative correlation ($p= 0.026$) between zooplankton and Chl *a* at B35 and the positive correlation ($p= 0.024$) between Chl *a* and temperature at L4.

1.3.2.2. Zooplankton taxa

There were no defined clusters of zooplankton taxa according to their interannual variations (**Figure 5**), and most zooplankton taxa showed irregular fluctuations unsynchronised between sites (**Figure 6**). The most noteworthy feature of the interannual variations of zooplankton taxa was the prominent peak of some holoplankton (i.e. copepods, PCPC-calanus, *Oithona*, *Acartia*, and appendicularians) and meroplankton (i.e. bivalve larvae and echinoderm larvae) taxa in 2012 at U35, and to a lesser extent at B35. The number of taxa that reached the highest abundance of the series in 2012 was 11 and 9 at U35 and B35, respectively, while only 4 taxa at L4 (in 2009 and 2011) and 5 at SH (in 2008) were found to reach the highest abundance in a same year of the series.

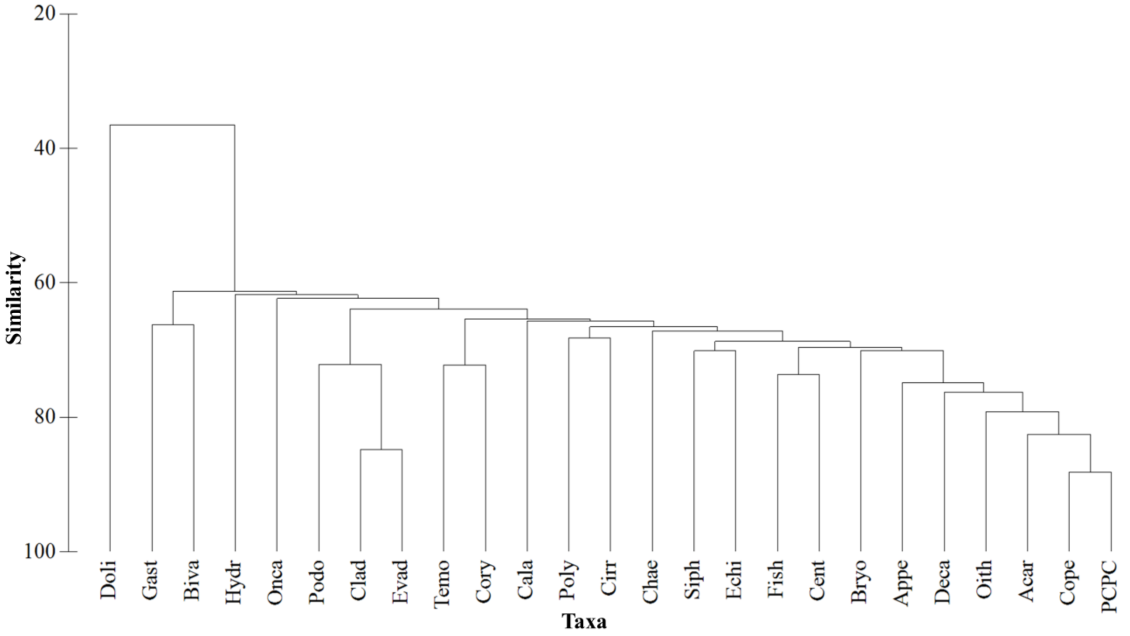

Figure 5. Group-averaged clustering from Bray-Curtis similarities of interannual variations of zooplankton taxa (pooled for the four sites: Bilbao 35, Urdaibai 35, Plymouth L4 and Stonehaven). Acar: *Acartia*, Appe: appendicularians, Biva: bivalve larvae, Bryo: bryozoans, Cala: Calanidae, Cent: *Centropages*, Chae: chaetognaths, Cirr: cirripede larvae, Clad: cladocerans, Cope: copepods, Cory: *Corycaeus*, Deca: decapod larvae, Doli: doliolids, Echi: echinoderm larvae, Evad: *Evadne*, Fish: fish eggs and larvae, Gast: gastropod larvae, Hydr: hydromedusae, Oith: *Oithona*, Onca: *Oncaea*, PCPC: PCPC-calanus, Podo: *Podon*, Poly: polychaete larvae, Siph: siphonophores, Temo: *Temora*.

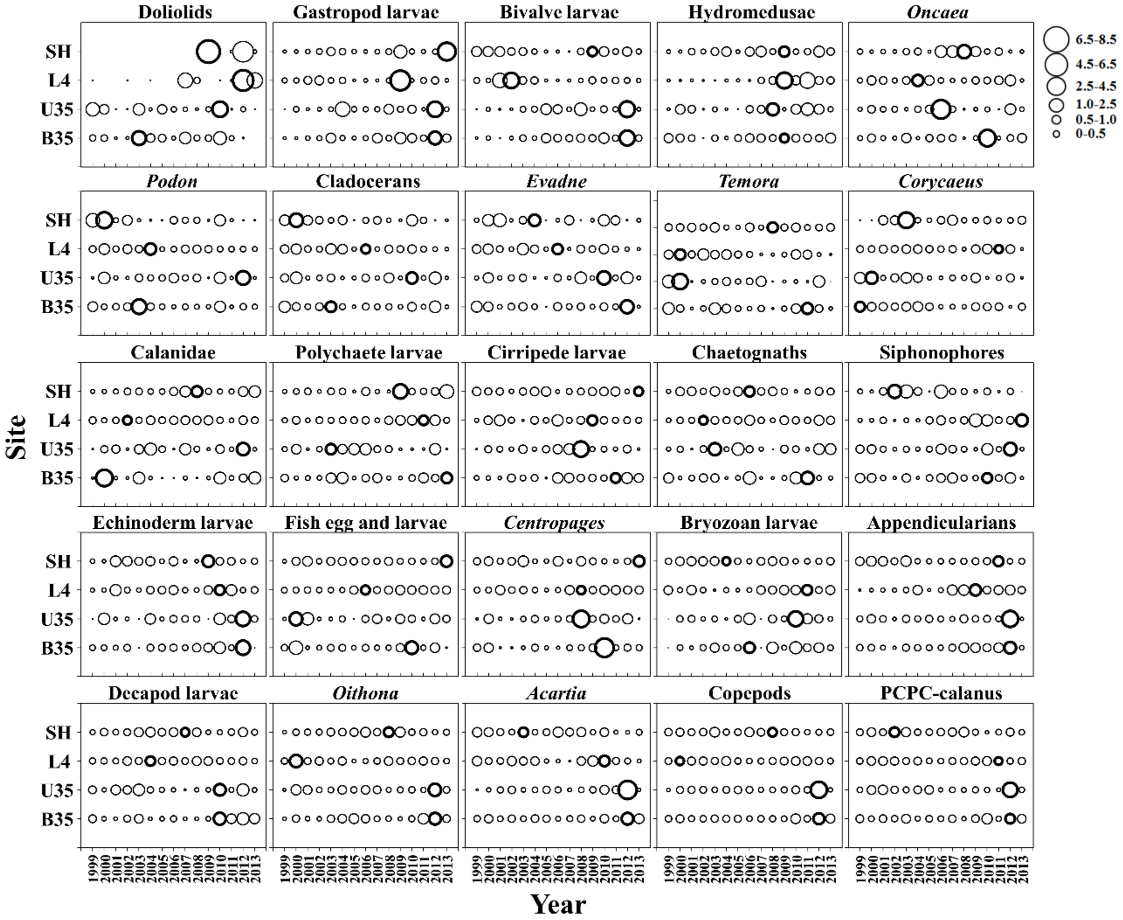

Figure 6. Interannual dimensionless variability of zooplankton taxa at Bilbao 35 (B35), Urdaibai 35 (U35), Plymouth L4 (L4) and Stonehaven (SH) from 1999 to 2013. Thickest bubbles indicate highest values.

None of the taxa showed interannual synchrony between the four sites. The number of taxa that correlated ($p < 0.05$) between sites was highest between B35 and U35, with 8 taxa (cladocerans, siphonophores, doliolids, bivalve larvae, bryozoan larvae, decapod larvae, *Evadne* and *Corycaeus*), and was lowest between B35 and SH and between U35 and L4, where only gastropod larvae and chaetognaths correlated, respectively. Between B35 and L4 only cladocerans, appendicularians and bivalve larvae (this last one negatively) showed significant correlation; between U35 and SH cirripede larvae and bryozoan larvae (this last one also negatively); and between L4 and SH cladocerans, bivalve larvae and echinoderm larvae. A few significant correlations were also found between interannual variations of zooplankton taxa and environmental variables, i.e. water temperature and Chl *a*, and such correlations were unrelated between sites.

1.3.3. *Seasonal patterns*

1.3.3.1. Total zooplankton, Chlorophyll a and temperature

The monthly mean values and the seasonal variability (dimensionless) of total zooplankton abundance, Chl *a* concentration and temperature are shown in **Figure 7**. At U35 the zooplankton maximum was in early spring (10494 ind. m^{-3} in late March), but the dimensionless values evidenced a bimodal cycle with a secondary peak in late summer. At B35 three peaks were observed in early spring (late March), early summer (maximum of 9657 ind. m^{-3} in late June) and early autumn (late September). At L4 a clear bimodal pattern with two similar peaks in spring (maximum of 5519 ind. m^{-3} in April) and summer (August) were observed. At SH, the seasonal pattern was unimodal, with a maximum of 5237 ind. m^{-3} in summer (July – August), although the stair-step shape suggests two consecutive periods for zooplankton increase in spring and summer.

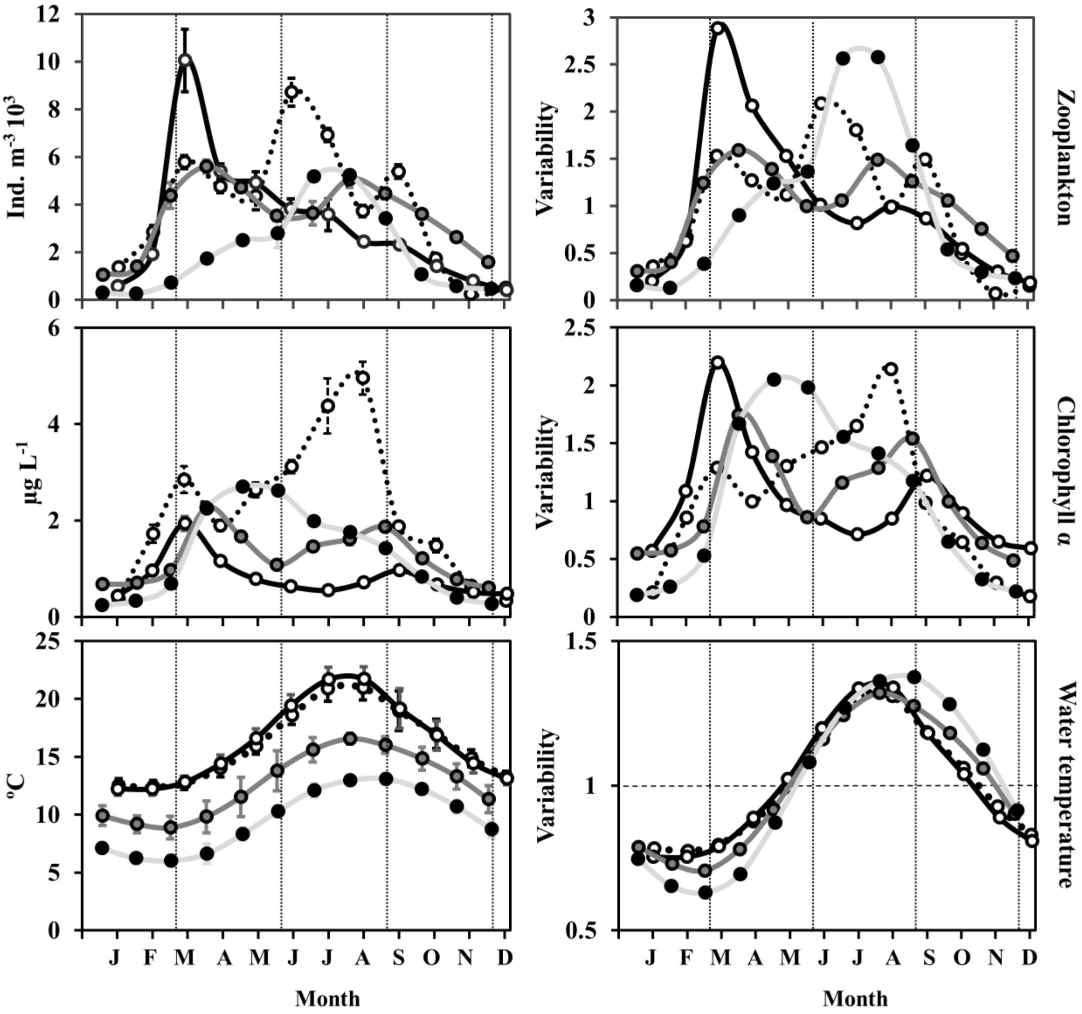

Figure 7. Monthly mean raw values (left) and dimensionless seasonal variability values (right) of total zooplankton abundance, chlorophyll *a* concentration and water temperature in Bilbao 35 (white circle, dotted black line), Urdaibai 35 (white circle, black line), Plymouth L4 (dark grey circle and line) and Stonehaven (black circle, light grey line). Dotted lines separate seasons.

Chl *a* concentration showed two peaks at B35 (a small one in early spring and the largest in summer), U35 (the major one in early spring and a secondary one in late summer) and L4 (in April and August with similar magnitudes). At SH, an extended single peak in late spring (May – June) was observed, but the stair-step shape of the decrease in August suggests masking of a secondary peak in summer. The monthly mean maximum Chl *a* was highest at B35 (4.95 µg L^{-1}) and lowest at U35, showing a small increase from U35 (1.94 µg L^{-1}) to L4 (2.26 µg L^{-1}), and to SH (2.70 µg L^{-1}).

Monthly mean values of water temperature ranged from around 12.4 ºC in January – February to around 21.0 ºC in August at B35 and U35, from 8.9 ºC in March to 15.6 ºC in August at L4, and from 6.0 ºC in March to 13.1 ºC in September at SH. The standard dimensionless variability evidenced that both warming and cooling occur earliest at B35 and U35 and latest at SH.

1.3.3.2. Zooplankton taxa

The clustering of zooplankton taxa (**Figure 8**) according to their patterns of seasonal variability (**Figure 9**) revealed five taxa assemblages with similarity levels between 60% and 80%. Similarity was highest between cladocerans, *Evadne*, *Podon*, appendicularians and *Acartia*, which showed a seasonal progression of annual maxima northwards, from U35 in late March, to L4 in May – July and to SH in July – August. At B35 they peaked in late May – late June, except *Acartia* (in late March). A delay of the annual maxima was also observed from spring – early summer at U35 and B35 to summer – late summer in echinoderm larvae, gastropod larvae and *Centropages*. Calanidae, *Temora*, decapod larvae, PCPC-calanus, copepods and *Oithona* were characterised in most cases by bimodal patterns, (or trimodal patterns at B35), in which the first peak was delayed from U35 and B35 (late February – April) to SH (May), while the last one generally occurred earlier at L4 and SH (July – September) than at U35 and B35 (late August – late October). The last peak was the annual maximum for a larger number of taxa at SH (Calanidae, *Temora*, decapod larvae, copepods and *Oithona*) and B35 (*Temora*, decapod larvae, PCPC-calanus and *Oithona*) than at U35 (*Temora*, PCPC-calanus), where the first peak was clearly the highest one for copepods and *Oithona*. For PCPC-calanus the importance of the first peak decreased from SH to B35. The annual maximum of polychaete larvae was delayed from U35 and B35 (late February) to L4 (June) and to SH (July). Siphonophores showed bimodal cycles at B35 and U35, with maxima in May, but unimodal cycles at L4 and SH, with maxima in September. Hydromedusae showed bimodal cycles at B35, U35 and SH, with maxima in April, but unimodal cycles at L4 with the maximum in July. Bryozoan larvae and fish eggs and larvae showed annual maxima or higher abundance earlier at L4 and SH (March – April) than at U35 and B35 (late April – late June), and cirripede larvae reached annual maxima in late March – April at SH, L4 and U35, but markedly later (late June) at B35. Doliolids, *Oncaea*, chaetognaths and *Corycaeus* reached annual maxima in the second half of the year at all sites, with the exception of *Oncaea* at SH. Doliolids and chaetognaths showed a marked seasonality with maxima in August – September at all sites, while *Oncaea* peaked from late September to November (except at SH) and *Corycaeus* peaked in late August at U35 and B35 and in October at L4 and SH.

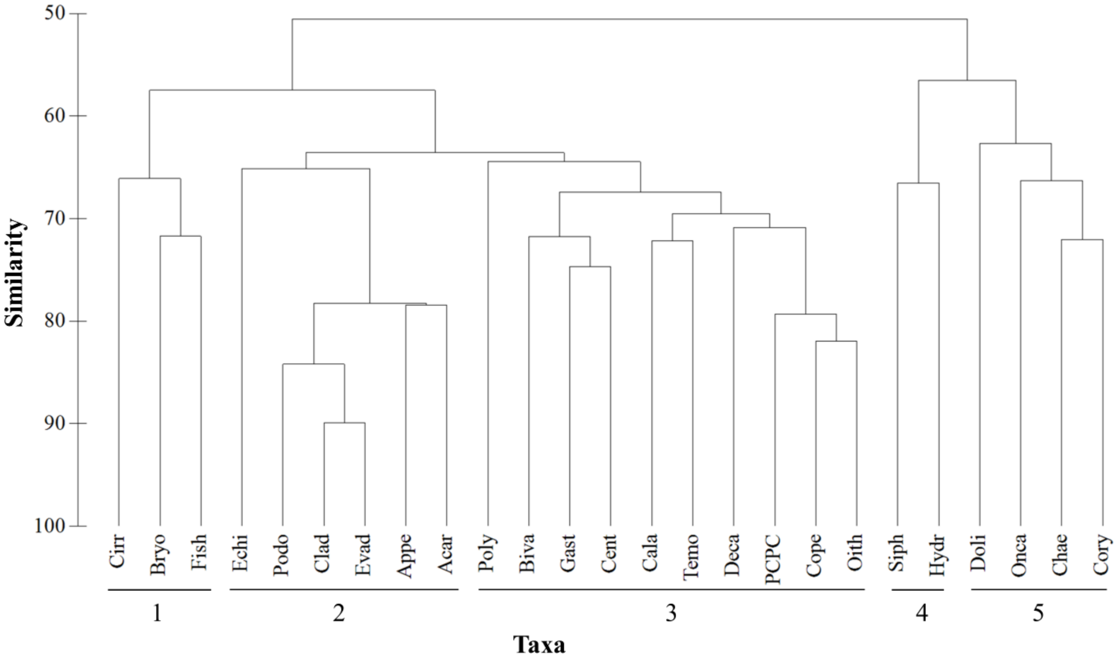

Figure 8. Group-averaged clustering from Bray-Curtis similarities of seasonal patterns of zooplankton taxa (pooled for the four sites: Bilbao 35, Urdaibai 35, Plymouth L4 and Stonehaven). Taxa abbreviations as in **Figure 5**.

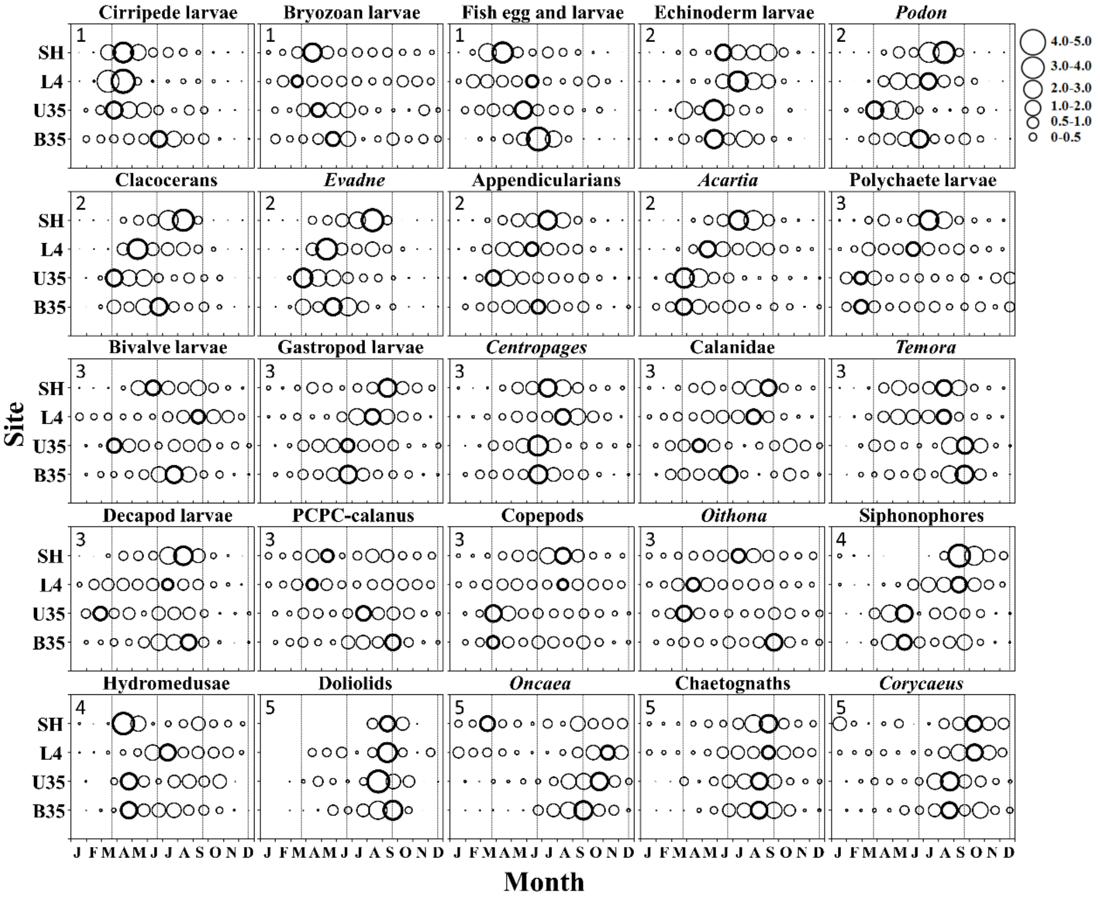

Figure 9. Seasonal dimensionless variability of zooplankton taxa at Bilbao 35 (B35), Urdaibai 35 (U35), Plymouth L4 (L4) and Stonehaven (SH) during the 1999 – 2013 period. Thickest bubbles indicate highest values. Dotted lines separate seasons.

Figure 10 shows the number of taxa that showed their annual maximum of abundance in a given month of the year. This distribution was skewed towards spring at U35 and L4 and towards autumn at B35 and SH, with maxima in early spring at U35, early summer at B35, midsummer at L4 and late summer at SH. The extent of the period within which holoplankton groups peaked along the year showed a clear reduction from U35 (6 months, from March to August) to L4 (5 months, from May to September) and to SH (3 months, from July to September), and it was longest (7 months, from March to September) at B35. Overall, meroplankton groups peaked earlier than holoplankton groups at all sites. The largest difference was observed at SH, with most meroplankton groups peaking in April – June and most holoplankton groups in August – September, and the smallest difference at U35 and B35, with most meroplankton and holoplankton groups peaking in the same season. Most cladoceran-copepod genera peaked in spring at U35 and in summer at SH, while at L4 the number of genera peaking in spring and summer was similar, and at B35 most of them peaked in summer – early autumn.

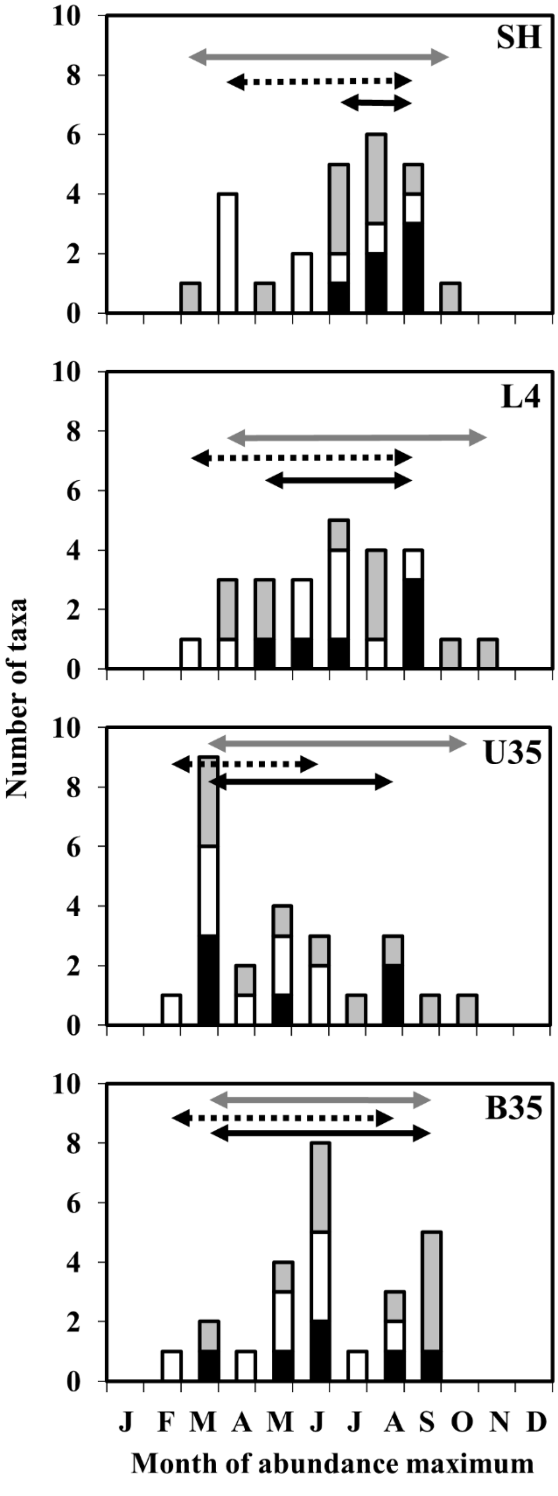

Figure 10. Histogram of the number of taxa that showed the standard annual maximum in each month at Bilbao 35 (B35), Urdaibai 35 (U35), Plymouth L4 (L4) and Stonehaven (SH). These are cumulative bars representing the number of holoplankton groups (black bars), meroplankton groups (white bars) and copepod-cladoceran genera (grey bars). Arrows indicate the period within which annual maxima of holoplankton groups (black line), meroplankton groups (pointed line) and copepod-cladoceran genera (grey bars) occur.

1.4. Discussion

1.4.1. Scales of variability

The dominance of the seasonal component over the interannual, and of the residual component over the former two, that we found for zooplankton taxa abundance at all sites under study, seems to be the most common feature for coastal plankton variability (e.g. Cloern and Jassby, 2010; Zingone *et al*., 2010; Bode *et al*., 2013). However, the magnitude of the scales of zooplankton variability showed no clear relationship with latitude or trophic status. Latitude appears as a key driver of the seasonal variability of phytoplankton biomass when a wide latitudinal range and many cases are considered (Cloern and Jassby, 2010). However, in our study only the fact that the highest seasonal variability of zooplankton taxa was obtained at the northernmost site (SH) fits this assumption. The higher values of the residual component at B35 and U35 might be due to some extent to the use of single measurements as estimators of monthly mean values, instead of the weekly values used for L4 and SH, but also to the combined effect of natural and anthropogenic local factors acting at time scales shorter than the seasonal cycle and high frequency temporal changes like those related to unusual events in single years or year-to-year shifts in phenology (Cloern and Jassby, 2010). The nutrient-rich estuarine plume at B35 and the strong tidal mixing and transport at U35 have a marked influence on phytoplankton biomass and dissolved oxygen dynamics at these sites (Villate *et al*., 2008; Iriarte *et al*., 2010; Villate *et al*., 2013; Iriarte *et al*., 2015), and might enhance residual zooplankton variability as compared to further offshore and deeper sites such as L4 and SH, which can be expected to be less affected by disturbances occurring close to the coast. Strong high frequency events like the unusual increase of some taxa in 2012 at U35, and to a lesser extent at B35, and the high variability in the timing of taxa annual maxima between years at B35, and to a lesser extent at U35, when compared to L4 and SH, very likely also contributed to increase the residual variability from L4 and SH to U35 and B35.

1.4.2. Interannual variations

Our results did not evidence the effect of strong atmospheric forcing that can lead to synchronous population fluctuations across wide areas (Goberville *et al.*, 2014; Kang and Ohman, 2014), since neither total zooplankton nor any taxa abundance correlated between all sites. The highest synchrony was observed between B35 and U35, likely due to their geographic proximity, as this enhances the probability of being affected by the same mesoscale shelf water oceanographic structures. The idea that the year-to-year changes in zooplankton might be primarily driven by a combination of forces that differ locally is reinforced by the few cases of synchrony between zooplankton taxa and temperature or phytoplankton biomass observed. Results also strengthen the hypothesis of meaningful differences within the NECS province established by Longhurst

(1998), which is also supported by other biogeographical classifications that locate our sites in a variety of units. For instance, the marine ecoregions defined for coastal and shelf areas by Spalding *et al*. (2016) separate the sites of the Bay of Biscay (Lusitanian province) from those located in the English Channel and North Sea (Northern European Seas province). In addition, although all the study sites are within the north European shelf latitudinally parallel to the North Atlantic Drift ecoregion of the Westerly winds' biome (Sutton *et al*., 2017), the SH site is near to the Northwest Atlantic Subarctic ecoregion of the Polar biome, whereas U35 and B35 are in the boundary with the Central North Atlantic ecoregion of the Trade wind biome. At SH water moves generally southerly and it is a mix of coastal and oceanic Atlantic waters, with an increase of the latter in late summer – early autumn; and L4 is affected by oceanic waters coming in with the dominant southwesterly winds (Falkenhaugh *et al*., 2013). In the narrow Basque shelf, the Eastern North Atlantic Central water is the main water mass and influences coastal water (U35 and B35) (Valencia *et al*., 2004).

The unusually high abundance of total zooplankton at U35 and B35 in 2012, mainly as a result of the marked increase of *Acartia*, PCPC-calanus, *Oithona*, appendicularians and bivalve larvae, corroborated the importance of local or region-specific physical processes driven by meteo-climatic conditions in modifying the range of interannual fluctuations of zooplankton abundance (Buttay *et al*., 2015). 2012 has been reported as a peculiar year in the southern Bay of Biscay, with atypical positive values of the upwelling index for February and March (Rodriguez *et al*., 2015) and exceptional changes in specific phytoplankton species related to climate anomalies (Díaz *et al*., 2013).

The concurrent increase of zooplankton abundance and decrease of phytoplankton biomass at the anthropogenically enriched site of B35 excludes the bottom-up control as a plausible cause of zooplankton increase, in contrast to findings for other systems (Steinberg *et al*., 2012). Environmental changes associated to the rehabilitation of the estuary of Bilbao might have had opposite effects on zooplankton and phytoplankton, since the phytoplankton biomass decline in the system during the period of study occurred concomitant to the decrease in anthropogenic nutrient loadings (Villate *et al*., 2013) as observed elsewhere too (Mozetič *et al*., 2010; Zingone *et al*., 2010).

1.4.3. Seasonal patterns

Seasonal patterns of many taxa and total zooplankton abundance, as well as phytoplankton biomass, seemed to be related to latitude. This was mainly evidenced by the clear delay of the early peak, and to a lesser extent by the advancement of the late peak, from the southernmost site (U35) to the northernmost one (SH), in agreement with the principle that spring processes tend to occur earlier and autumn processes later in the year with increasing temperature (Mackas and Beaugrand, 2010). However, taxa with a coincident seasonal pattern at all latitudes and taxa with a delay in the seasonal distribution at the southernmost sites were also found.

Coincident seasonal distributions at the three latitudes, such as those of cirripede larvae, chaetognaths and doliolids, could be attributable to an environmental stimulus that does not change within the latitude range we studied. No information on the species composition of cirripede larvae is available in our zooplankton series, but latitudinal differences in barnacle species distribution is supported by studies covering areas from Scotland to Portugal (Crisp *et al*., 1981; O'Riordan *et al*., 2004). The coincidence of a major early spawning peak of cirripedes at all sites, regardless of compositional differences, seems to be the result of a common response to the timing of phytoplankton increase from winter to spring (Starr *et al.*, 1991; Highfield *et al.*, 2010). Similarly, the coincidence of the seasonal distribution of chaetognaths, despite the dominance of different species such as *Parasagitta friderici* at U35 and B35, *Parasagitta setosa* at L4 and *Parasagitta elegans* at SH, could be attributable to them sharing the same diet, which consists mainly of small copepods (Falkenhaug, 1991; Gibbons and Stuart, 1994; Tönnesson and Tiselius, 2005) that peak in the warmest period. The coincidence of doliolid maxima at all sites in late summer agrees with the fact that doliolid development occurs at high temperature and is favoured by the stratification of the water column (Menard *et al*., 1997).

The delay of the annual maxima of bryozoan larvae and ichthyoplankton at the southernmost sites may be related to compositional differences associated to different environmental preferences. No information was available about bryozoan species composition, but the differences in fish species distribution between sites are well known and support the observed differences in ichthyoplankton seasonality. The most abundant fish larvae off the east coast of Scotland are those of sandeel, which are almost restricted to the first half of the year and usually peak in March, whereas in the western English Channel the larvae of whiting and a mixture of clupeids (mainly sprat and sardine) are more abundant, peaking from March to June (Edwards *et al*., 2011). In the inner Bay of Biscay fish larvae reach annual maxima around June and sardine and anchovy larvae are the most abundant ones, the anchovy ones being clearly associated to warmer conditions (d'Elbée *et al*., 2009).

The northward delay of the annual maxima across sites in cladocerans and their genera *Podon* and *Evadne* (mainly *Evadne nordmanni*), the copepod *Acartia* (almost exclusively *Acartia clausi*), and appendicularians was linked to the timing of the spring phytoplankton peak, but it may also reflect specific temperature optima. This was evident mainly for *A. clausi*, which peaked in late March, at temperatures near the annual minimum (12.4 ºC) at the southernmost sites, and later in the year, near the annual maximum (13 ºC), at the northernmost site.

Among taxa with bimodal cycles, or bimodal cycles that become unimodal at the northernmost site, latitudinal differences in timing and magnitude of peaks were related to compositional differences in some cases. For *Temora*, *Temora longicornis* was responsible for the first annual peak at all sites, whereas *Temora stylifera* was responsible for the second one at U35 and B35. Similarly, PCPC-calanus is dominated by the spring peaking species *Pseudocalanus elongatus* at SH (Bresnan *et al*., 2015) and by *Paracalanus parvus* at U35 and B35, where this species is responsible for the much higher value of the second annual peak. At L4, both species are similar in abundance, but *P. elongatus* peaks in spring and *P. parvus* in autumn (Eloire *et al*., 2010). *Oithona similis*, accounted for the early peak of the *Oithona* genus at U35 and the only peak of this genus at SH, whereas *O. nana* was not recorded at SH and L4 (Castellani *et al*., 2016), but it was the main responsible for the second peak of *Oithona* at U35 and B35. In contrast, *Oncaea* and *Corycaeus* showed bimodal cycles at SH and unimodal ones at U35. In agreement with previous reports by Eloire *et al.* (2010), our results showed a skewed distribution of *Corycaeus* and *Oncaea* towards autumn and winter at L4 and SH, which may be related to the later cooling of water as compared to U35 and B35.

A plot showing how many taxa had their annual maximum of abundance in each month of the year evidenced that the largest number of taxa had their peak abundances in early spring at the southernmost site (U35) but summer at the intermediate and northernmost sites (L4 and SH). This may be a response to differences in phytoplankton availability during spring – summer, since the availability decreases strongly in summer at U35 but it remains rather high at L4 and SH. At this last site, the occurrence of most meroplankton groups' maxima in spring and the later concentration of the annual maxima of all holoplankton groups and most cladoceran-copepod genera in the 3-month summer period coincide with changes in phytoplankton availability (higher in spring than in summer), but also with the succession of the late spring diatom bloom by the annual maximum of dinoflagellates in summer at SH (Bresnan *et al*., 2015). The wider seasonal distribution of the annual maxima for meroplankton groups than for holoplankton groups at all sites also suggests that the spawning behaviour of different benthic populations does not only depend on phytoplankton abundance, but also on phytoplankton composition or physical factors such as temperature (Starr *et al.*, 1992; Starr *et al.*, 1993; Highfield *et al.*, 2010).

The effect of the trophic status was mainly evidenced by the delay in the annual maximum of many taxa and the transformation of the bimodal cycles of total zooplankton and some taxa at the oligotrophic site into trimodal cycles at the mesotrophic site. Because no significant differences in temperature occur between U35 and B35, the modification of the phytoplankton biomass cycle and composition at the mesotrophic site by man-made eutrophication (Garmendia *et al.*, 2013) seems the main factor responsible for such differences. At U35, Chl *a* showed the classical summer drop related to nutrient-limitation, as in other nearby continental shelf areas of the southern Bay of Biscay (Stenseth *et al.*, 2006), whereas at B35 summer Chl *a* values exceeded those of spring. The seasonal delay of most holoplankton taxa at B35 revealed that the same species were able to reach higher densities later than at U35 due to the maintenance of high phytoplankton biomass until autumn. In *Oithona*, however, seasonal differences were mainly related to between-site differences in species dominance. The spring species *O. similis*, which may be limited by high (>20 °C) temperatures (Castellani *et al*., 2016), dominated at U35, whereas the summer – autumn species *O. nana*, which is associated to high temperature and Chl *a*, and to eutrophicated/polluted conditions (Arfi *et al*., 1981; Villate, 1991; Jamet *et al*., 2001), dominated at B35. In spite of their vicinity, the between-site differences in the seasonal patterns of phytoplankton and zooplankton at U35 and B35 were larger than those reported by Bresnan *et al*. (2015) between SH and Loch Ewe. The latter are also located around the same latitude but Loch Ewe is in the west Scottish coast and is more influenced by river discharges. In this case, although both phytoplankton and zooplankton showed earlier increases at Loch Ewe than at Stonehaven, the seasonal maxima occurred only 1 month earlier in spring at the former site for phytoplankton and in the same month in summer at both sites for zooplankton (Bresnan *et al*., 2015).

The comparison of our results with those obtained at L4 and sites of the Cantabrian coast (Valdes *et al*., 2007; Bode *et al*., 2012) near U35 and B35 in previous decades corroborates seasonal differences from the English Channel to the southern Bay of Biscay, but it also suggests that phenological changes could be occurring in some taxa. This is the case of *Centropages* (almost exclusively *C. typicus* at L4, U35 and B35), which in our study was found to have the standard annual maximum in August at L4 and in late June at U35 and B35, but in other studies where previous decades were considered, maxima were observed in September at L4 and July at the coastal site of Santander (around 100 Km from B35 and U35) (Bonnet *et al*., 2007). *C. typicus* is a typical temperate neritic-coastal species of the North Atlantic which responds to temperature increases and changes in the structure and timing of occurrence of phytoplankton (Beaugrand *et al*., 2007). The seasonal advance experienced by this species may be related to the warming of the northwest European shelf region (Smith *et al*., 2010). Similarly, the timing of the annual maximum in September observed in this study for Calanidae (mainly *C. helgolandicus*) at SH can be interpreted in the context of the replacement in the dominance of *Calanus finmarchicus* (subarctic spring peaking Calanidae) by *C. helgolandicus* (temperate species) in the North Sea from the late 80s as a result of warming, since temperature has been identified as the main environmental variable that has influenced the abundance of both species (Beaugrand *et al*., 2002, 2009; Bonnet *et al*., 2005; Helaouët and Beaugrand, 2007). However, the differences in the seasonal pattern of *C. helgolandicus* at U35 and B35 suggest a response of population dynamics to the trophic status that was not observed for *C. typicus*. Expanding our study in the future to include more updated information would be of much interest to follow the evolution of these and other zooplankton components in contrasting areas within the NECS province, and to be able to detect significant local effects.

1.5. Conclusions

The present study showed that in the four coastal sites of the Northeast Atlantic Shelves Province of the ICES area, during the 1999 – 2013 period, the magnitude of zooplankton interannual, seasonal and residual components of variability did not show clear relationship with the latitudinal gradient, and the interannual zooplankton variations were not coherent across sites, this suggesting the dominance of local forces over wider scale climatic drivers. Seasonal patterns, however, differed across sites in such a way that allowed to identify north-south trends. The most recurrent one was the delay of the early seasonal peak of many spring – summer taxa northwards, together with the earlier occurrence of the late peak in taxa showing bimodal cycles during the spring – summer period. In addition, taxa with coincident seasonal patterns at all sites, taxa peaking earlier with increasing latitude over the first half of the year or taxa peaking later with increasing latitude over the second half were also observed. Phenological differences in zooplankton from sites at the same latitude but with different trophic status allowed us to distinguish the effect of climatic variability from the effect of man-induced perturbations, which is one of the priorities stated by the Marine Strategy Framework Directive (MSFD). In addition, envelopes of zooplankton variability that can be used as reference baselines to detect anomalous years have been defined, and helped to establish that 2012 was an anomalous year in our southern Bay of Biscay sites.

1.6. References

Arfi, R., Champalbert, G., Patriti, G. (1981). Système planctonique et pollution urbaine: un aspect des populations zooplanctoniques. *Marine Biology*, *61*, 133–141. doi:10.1007/BF00386652.

Atkinson, A., Harmer, R. A., Widdicombe, C. E., McEvoy, A. J., Smyth, T. J., Cummings, D. G., Somerfield, P. J., Maud, J. L., McConville, K. (2015). Questioning the role of phenology shifts and trophic mismatching in a planktonic food web. *Progress in Oceanography*, *137*, 498–512. doi:10.1016/j.pocean.2015.04.023.

Beaugrand, G. (2009). Decadal changes in climate and ecosystems in the North Atlantic Ocean and adjacent seas. *Deep Sea Reearch. II*, *56*, 656–673. https://doi.org/10.1016/j.dsr2.2008.12.022.

Beaugrand, G., Reid, Ph. C., Ibañez, F., Lindley, J. A., Edwards. M. (2002). Reorganization of North Atlantic Marine Copepod Biodiversity and Climate. *Science*, *296*, 1692-1694.

Beaugrand, G., Lindley, J. A., Helaouet, P., Bonnet, D. (2007). Macroecological study of Centropages typicus in the North Atlantic Ocean. *Progress in Oceanography*, *72,* 259–272. https://doi.org/10.1016/j.pocean.2007.01.002.

Bode, A., Alvarez-Ossorio, M. T., Miranda, A., López-Urrutia, A., Valdés, L. (2012). Comparing copepod time-series in the north of Spain: Spatial autocorrelation of community composition. *Progress in Oceanography*, *97–100*, 108–119. doi:10.1016/j.pocean.2011.11.013.

Bode, A., Bueno, J., Lopez-Urrutia, A., Villate, F., Uriarte, I., Iriarte, A., Alvarez-Ossorio, M. T., Miranda, A., Casas, G., Dos Santos, A., Santos, A. M. P., Chicharo, M. A., Cruz, J., Ben-Hamadou, B., Chicharo, L. (2013). Zooplankton of the Bay of Biscay and western Iberian shelf. In O´Brien, T. D., Wiebe, P. H., Falkenhaug, T. (eds.), *ICES Zooplankton Status Report 2010/2011*. ICES Cooperative Research Report, 318. 208 pp. ISSN 1017-6195.

Bonnet, D., Richardson, A., Harris, R., Hirst, A., Beaugrand, G., Edwards, M., Ceballos, S., Diekman, R., Lopez-Urrutia, A., Valdes, L., Carlotti, F., Molinero, J. C., Weikert, H., Greve, W., Lucic, D., Albaina, A., Yahia, N. D., Umani, S. F., Miranda, A., Santos, A. dos, Cook, K., Robinson, S., Fernandez de Puelles, M. L. (2005). An overview of *Calanus helgolandicus* ecology in European waters. *Progress in Oceanography*, *65*, 1–53. doi:10.1016/j.pocean.2005.02.002.

Bonnet, D., Harris, R., Lopez-Urrutia, A., Halsband-Lenk, C., Greve, W., Valdes, L., Hirche, H. J., Engel, M., Alvarez-Ossorio, M. T., Wiltshire, K. (2007). Comparative seasonal dynamics of *Centropages typicus* at seven coastal monitoring stations in the North Sea, English Channel and Bay of Biscay. *Progress in Oceanography*, *72*, 233-248. doi:10.1016/j.pocean.2007.01.007.

Borja, Á., Dauer, D. M., Elliott, M., Simenstad, C. A. (2010). Medium- and long-term recovery of estuarine and coastal ecosystems: patterns, rates and restoration effectiveness. *Estuaries and Coasts*, *33*, 1249–1260. doi:10.1007/s12237-010-9347-5.

Bray, J. R., Curtis, J. T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecology Monographs*, *27*, 325–349. doi:10.2307/1942268.

Bresnan, E., Cook, K. B., Hughes, S. L., Hay, S. J., Smith, K., Walsham, P., Webster, L. (2015). Seasonality of the plankton community at an east and west coast monitoring site in Scottish waters. *Journal of Sea Research*, *105*, 16–29. doi:10.1016/j.seares.2015.06.009.

Briggs, J. C., Bowen, B. W. (2012). A realignment of marine biogeographic provinces with particular reference to fish distributions. *Journal of Biogeography*, *29*, 12–30. doi:10.1111/j.1365-2699.2011.02613.x.

Buttay, L., Miranda, A., Casas, G., González-Quirós, R., Nogueira, E., Rafael, G.-Q., Nogueira,

E. (2015). Long-term and seasonal zooplankton dynamics in the northwest Iberian shelf and its relationship with meteo-climatic and hydrographic variability. *Journal of Plankton Research*, *38*, 106–121. doi:10.1093/plankt/fbv100.

Castellani, C., Licandro, P., Fileman, E., di Capua, I., Mazzocchi, M. G. (2016). *Oithona similis* likes it cool: evidence from two long-term time series. *Journal of Plankton Research, 38*, 703–717. doi:10.1093/plankt/fbv104.

Clarke, K. R., Warwick, R. M. (2001). Change in marine communities: an approach to statistical analysis and interpretation, 2nd ed. PRIMER-E, Plymouth.

Cloern, J. E. (1996) Phytoplankton bloom dynamics in coastal ecosystems: A review with some general lessons from sustained investigation of San Francisco Bay, California. *Reviews of Geophysics.*, *34*, 127. doi:10.1029/96RG00986.

Cloern, J. E., Jassby, A. D. (2010). Patterns and scales of phytoplankton variability in estuarine-coastal ecosystems. *Estuaries and Coasts*, *33*, 230–241. doi:10.1007/s12237-009-9195-3.

Crisp, D. J., Southward, A., Southward, E. C. (1981). On the distribution of the intertidal barnacles *Chthamalus stellatus*, *Chthamalus montagui* and *Euraphia depressa*. *Journal of the Marine Biological Association of the United Kingdom*, *61*, 359–380. doi:10.1017/S0025315400047007.

Cushing, D. H. (1990). Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Advances in Marine Biology*, *26*, 249–293. doi:10.1016/S0065-2881(08)60202-3.

Díaz, P. A., Reguera, B., Ruiz-Villarreal, M., Pazos, Y., Velo-Suárez, L., Berger, H., Sourisseau, M. (2013). Climate variability and oceanographic settings associated with interannual variability in the initiation of *Dinophysis acuminata* blooms. *Marine Drugs*, *11*, 2964–2981. doi:10.3390/md11082964.

Durant, J. M., Hjermann, D., Ottersen, G., Stenseth, N. C. (2007). Climate and the match or mismatch between predator requirements and resource availability. *Climate Research*, *33*, 271–283. doi:10.3354/cr033271.

d’Elbée, J., Castège, I., Hémery, G., Lalanne, Y., Mouchès, C., Pautrizel, F., D’Amico, F. (2009). Variation and temporal patterns in the composition of the surface ichthyoplankton in the southern Bay of Biscay (W. Atlantic). *Continental Shelf Research*, *29*, 1136–1144. doi: 10.1016/j.csr.2008.12.023.

Edwards, M., Helaouët, P., Halliday, N. C., Beaugrand, G., Fox, C., Johns, D. G., Licandro, P., Lynam, C., Pitois, S., Stevens, D., Coombs, S., Fonseca, L. (2011). Fish larvae atlas of the NE Atlantic. Results from the Continuous Plankton Recorder survey 1948-2005. *Sir Alister Hardy Foundation for Ocean Science*. 22 p. Plymouth, U.K. ISBN: 978- 0-9566301-2-7.

Edwards, M., Richardson, A. J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature*, *430*, 881–884. doi:10.1038/nature02808.

Eloire, D., Somerfield, P. J., Conway, D. V. P., Halsband-Lenk, C., Harris, R., Bonnet, D. (2010). Temporal variability and community composition of zooplankton at station L4 in the Western Channel: 20 years of sampling. *Journal of Plankton Research*, *32*, 657–679. doi:10.1093/plankt/fbq009.

Falkenhaug, T. (1991). Prey composition and feeding rate of *Sagitta elegans* var. *arctica* (Chaetognatha) in the Barents Sea in early summer. *Polar Research*, *10*, 487–506. doi:10.1111/j.1751-8369.1991.tb00668.x.

Falkenhaugh, T., Omli, L., Boersma, M., Renz, J., Cook, K. Atkinson, A., Fileman, E., Widdicombe, C., Harmer, R., McEvoy, A., Harris, R., Smyth, T., Hay, S. (2013). Zooplankton of the North Sea and English Channel. In O´Brien, T. D., Wiebe, P. H., Falkenhaug, T. (eds.), *ICES Zooplankton Status Report 2010/2011*. ICES Cooperative

Research Report, 318. 208 pp. ISSN 1017-6195.

Garmendia, M., Borja, Á., Franco, J., Revilla, M. (2013). Phytoplankton composition indicators for the assessment of eutrophication in marine waters: Present state and challenges within the European directives. *Marine Pollution Buletin.*, *66*, 7–16. doi:10.1016/j.marpolbul.2012.10.005.

Gibbons, M. J., Stuart, V. (1994). Feeding and vertical migration of the chaetognath *Sagitta friderici* (Ritter-Zahony, 1911) in the southern Benguela during spring 1987, with notes on seasonal variability of feeding ecology. *South African Journal of Marine Science*, *14*, 361–372. doi:10.2989/025776194784286888.

Goberville, E., Beaugrand, G., Edwards, M. (2014). Synchronous response of marine plankton ecosystems to climate in the Northeast Atlantic and the North Sea. *Journal of Marine Systems,* 129, 189–202. doi:10.1016/j.jmarsys.2013.05.008.

Haury, L. R., McGowan, J. A., Wiebe, P. H. (1978). Patterns and processes in the time-space scales of plankton distributions. In Steele, J. H. (ed.), *Spatial pattern in plankton communities*. Springer US, Boston, MA, pp.277–327. doi:10.1007/978-1-4899-2195-6_12.

Helaouët, P., Beaugrand, G. (2007). Macroecology of *Calanus finmarchicus* and *C. helgolandicus* in the North Atlantic Ocean and adjacent seas. *Marine Ecology Progress Series*, *345*, 147–175.

Heinrich, A. K. (1962). The life histories of plankton animals and seasonal cycles of plankton communities in the oceans. *ICES Journal of Marine Science*, *27*, 15–24. doi:10.1093/icesjms/27.1.15.

Highfield, J. M., Eloire, D., Conway, D. V. P., Lindeque, P. K., Attrill, M. J., Somerfield, P. J. (2010). Seasonal dynamics of meroplankton assemblages at station L4. *Journal of Plankton Research*, *32*, 681–691. doi:10.1093/plankt/fbp139.

Iriarte, A., Aravena, G., Villate, F., Uriarte, I., Ibáñez, B., Llope, M., Stenseth, N. C. (2010). Dissolved oxygen in contrasting estuaries of the Bay of Biscay: Effects of temperature, river discharge and chlorophyll *a*. *Marine Ecology Progress Series*, *418*, 57–71. doi:10.3354/meps08812.

Iriarte, A., Villate, F., Uriarte, I., Alberdi, L., Intxausti, L. (2015). Dissolved oxygen in a temperate estuary: the influence of hydro-climatic factors and eutrophication at seasonal and inter-annual time scales. *Estuaries and Coasts*, *38*, 1000–1015. doi:10.1007/s12237-014-9870-x.

Jamet, J. L., Bogé, G., Richard, S., Geneys, C., Jamet, D. (2001). The zooplankton community in bays of Toulon area (northwest Mediterranean Sea, France). *Hydrobiologia*, *457*, 155–165. doi:10.1023/A:1012279417451.

Kang, Y. S., Ohman, M. D. (2014). Comparison of long-term trends of zooplankton from two marine ecosystems across the North Pacific: Northeastern Asian marginal sea and Southern California current system. *California cooperative ocean fisheries investigations reports*, *55*, 169–182.

Litt, E. J., Hardman-Mountford, N. J., Blackford, J. C., Mitchelson-Jacob, G., Goodman, A., Moore, G. E., Cummings, D. G., Butenschon, M. (2010). Biological control of $p\mathrm{CO}_2$ at station L4 in the Western English Channel over 3 years. *Journal of Plankton Research*, *32*, 621–629. https://doi.org/10.1093/plankt/fbp133.

Liu, H., Fogarty, M. J., Hare, J. A., Hsieh, C.-hao, Glaser, S. M., Ye, H., Deyle, E., Sugihara, G. (2014). Modeling dynamic interactions and coherence between marine zooplankton and fishes linked to environmental variability. *Journal of Marine Systems*, *131*, 120–129. doi:10.1016/j.jmarsys.2013.12.003.

Longhurst, A. R. (1998). Ecological geography of the sea, 1st ed. Academic Press, San Diego.

398 pp.

Lorenzen, C. J. (1967). Determination of chlorophyll and pheo-pigments: spectrophotometric equations. *Limnology and Oceanography, 12,* 343-346. https://doi.org/10.4319/lo.1967.12.2.0343.

Mackas, D. L., Beaugrand, G. (2010). Comparisons of zooplankton time series. *Journal of Marine Systems*, *79*, 286–304. doi:10.1016/j.jmarsys.2008.11.030.

Mackas, D. L., Greve, W., Edwards, M., Chiba, S., Tadokoro, K., Eloire, D., Mazzocchi, M. G., Batten, S., Richardson, A. J., Johnson, C., Head, E., Conversi, A., Peluso, T. (2012). Changing zooplankton seasonality in a changing ocean: Comparing time series of zooplankton phenology. *Progress in Oceanography, 97–100,* 31–62. doi:10.1016/j.pocean.2011.11.005.

Marine Zooplankton Colloquium 1 (1989). Future marine zooplankton research - a perspective. *Marine Ecology Progress Series, 55,* 197–206. doi:10.3354/meps222297.

Menard, F., Fromentin, J. M., Goy, J., Dallot, S. (1997). Temporal fluctuations of doliolid abundance in the bay of Villefranche-sur-Mer (Northwestern Mediterranean Sea) from 1967 to 1990. *Oceanologica*. *Acta*, *20,* 733–742.

Molvær, J., Knutzen, J., Magnusson, J., Rygg, B., Skei J., Sørensen, J (1997). Klassifisering av miljøkvalitet i fjorder og kystfarvann. Veiledning. Classification of environmental quality in fjords and coastal waters. A guide. Norwegian Pollution Control Authority. TA no. TA-1467/1997. 36 pp. ISBN 82-7655-367-2.

Mozetič, P., Solidoro, C., Cossarini, G., Socal, G., Precali, R., Francé, J., Bianchi, F., De Vittor, C., Smodlaka, N., Fonda Umani, S. (2010). Recent trends towards oligotrophication of the northern adriatic: Evidence from chlorophyll *a* time series. *Estuaries and Coasts*, *33*, 362–375. doi:10.1007/s12237-009-9191-7.

O'Brien, T. D., Wiebe, P. H. and Falkenhaug, T. (2013). ICES Zooplankton Status Report 2010/2011. *ICES CooperativeResearch Report*, *318*. 208 pp. ISSN 1017-6195.

O'Riordan, R. M., Arenas, F., Arrontes, J., Castro, J. J., Cruz, T., Delany, J., Martínez, B., Fernandez, C., Hawkins, S. J., McGrath, D., Myers, A. A., Oliveros, J., Pannacciulli, F. G., Power, A. M., Relini, G., Rico, J. M., Silva, T. (2004). Spatial variation in the recruitment of the intertidal barnacles *Chthamalus montagui* Southward and *Chthamalus stellatus* (Poli) (Crustacea: Cirripedia) over an European scale. J*ournal of Experimental Marine Biology and Ecology*, *304*, 243–264. doi:10.1016/j.jembe.2003.12.005.

Rees, A. P., Hope, S. B., Widdicombe, C. E., Dixon, J. L., Woodward, E. M. S., Fitzsimons, M. F. (2009). Alkaline phosphatase activity in the western English Channel: Elevations induced by high summertime rainfall. *Estuarine, Coastal and Shelf Science, 81,* 569–574. doi:10.1016/j.ecss.2008.12.005.

Ribera d´Alcalà, M., Conversano, F., Corato, F., Licandro, P., Mangoni, O. (2004). Seasonal patterns in plankton communities in a pluriannual time series at a coastal Mediterranean site (Gulf of Naples): an attempt to discern recurrences and trends. *Scientia Marina, 68,* 65–83. doi:10.3989/scimar.2004.68s165.

Rodriguez, J. M., Cabrero, A., Gago, J., Guevara-Fletcher, C., Herrero, M., de Rojas, A. H., Garcia, A., Laiz-Carrion, R., Vergara, A. R., Alvarez, P., Piñeiro, C., Saborido-Rey, F. (2015). Vertical distribution and migration of fish larvae in the NW Iberian upwelling system during the winter mixing period: Implications for cross-shelf distribution. *Fisheries Oceanography*, *24*, 274–290. doi:10.1111/fog.12107.

Smith, V. H., Tilman, G. D., Nekola, J. C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution, 100,* 179–196. doi:10.1016/S0269-7491(99)00091-3.

Smith, T. J., Fishwick, J. R., Al-Moosawi, L., Cummings, D. J. Harris, C., Kitidis, V., Rees, A., Martinez-Vicente, V., Woodward, E. M. S. (2010). A broad spatio-temporal view of the Western English Channel observatory. *Journal of Plankton Research, 32*, 585–601. doi: 10.1093/plankt/fbp128.

Southward, A. J., Langmead, O., Hardman-Mountford, N. J., Aiken, J., Boalch, G. T., Dando, P. R., Genner, M. J., Joint, I., Kendall, M. A., Halliday, N. C., Harris, R. P., Leaper, R., Mieszkowska, N., Pingree, R. D., Richardson, A. J., Sims, D. W., Smith, T., Walne, A. W., Hawkins, S. J. (2004). Long-term oceanographic and ecological research in the western English Channel. *Advances in Marine Biology, 47,* 1–105. doi:10.1016/S0065-2881(04)47001-1.

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., Halpern, B. S., Jorge, M. A., Lombana, A., Lourie, S. A., Martin, K. D., McManus, E., Molnar, J., Recchia, Ch. A., Robertson, J. (2007). Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. *BioScience*, *57,* 573–583. doi:10.1641/B570707.

Starr, M., Himmelman, J. H., Therriault, J. C. (1991). Coupling of nauplii release in barnacles with phytoplankton blooms: A parallel strategy to that of spawning in urchins and mussels. *Journal of Plankton Research, 13,* 561–571. doi:10.1093/plankt/13.3.561

Starr, M., Himmelman, J. H., Therriault, J. C. (1992). Isolation and properties of a substance from the diatom *Phaeodactylum tricornutum* which induces spawning in the sea urchin *Strongylocentrotus droebachiensis*. *Marine Ecology Progress Series*, *79*, 275–287.

Starr, M., Himmelman, J. H., Therriault, J. C. (1993). Environmental control of green sea urchin, *Strongylocentrotus droebachiensis*, spawning in the St Lawrence estuary. *Canadian Journal of Fisheries and Aquatic Sciences*, *50,* 894–901. https://doi.org/10.1139/f93-103.

Steinberg, D. K., Lomas, M. W., Cope, J. S. (2012). Long-term increase in mesozooplankton biomass in the Sargasso Sea: Linkage to climate and implications for food web dynamics and biogeochemical cycling. *Global Biogeochemichal Cycles*, *26*, GB1004. doi:10.1029/2010GB004026.

Stenseth, N. C., Llope, M., Anadón, R., Ciannelli, L., Chan, K.-S., Hjermann, D. Ø., Bagøien, E., Ottersen, G. (2006). Seasonal plankton dynamics along a cross-shelf gradient. *Proceedings of the Royal Society B*, *273,* 2831–2838. doi:10.1098/rspb.2006.3658.

Sutton, T. T., Clark, M. R., Dunn, D. C., Halpin, P. N., Rogers, A. D., Guinotte, J., Bogrand, S. J., Angel, M. V., Perez, J. A. A., Wishner, K., Haedrich, R. L., Lindsay, D. J., Drazen, J. C., Vereshchaka, A., Piatkowski, U., Morato, T., Blachowiak-Samolyk, K., Robison, B. H., Gjerde, K. M., Pierrot-Bults, A., Bernal, P., Reygondeau, G., Heino, M. (2017). A global biogeographic classification of the mesopelagic zone. *Deep Sea Research Part I,* in press. doi.org/10.1016/j.dsr.2017.05.006.

Sydeman, W. J., Bograd, S. J. (2009). Marine ecosystems, climate and phenology: introduction. *Marine Ecology Progress Series, 393,* 185-188. doi: 10.3354/meps08382.

Tönnesson, K., Tiselius, P. (2005). Diet of the chaetognaths *Sagitta setosa* and *S. elegans* in relation to prey abundance and vertical distribution. *Marine Ecology Progress Series, 289,* 177–190. doi:10.3354/meps289177.

Valdés, L., López-Urrutia, A., Cabal, J., Alvarez-Ossorio, M., Bode, A., Miranda, A., Cabanas, M., Huskin, I., Anadón, R., Alvarez-Marqués, F., Llope, M., Rodríguez, N. (2007). A decade of sampling in the Bay of Biscay: What are the zooplankton time series telling us? *Progress in Oceanography*, *74,* 98–114. doi:10.1016/j.pocean.2007.04.016.

Valencia, V., Franco, J., Borja, A., Fontán, A. (2004). Hydrography of the southeastern Bay of Biscay. In Borja, A., Collins, M. (eds.), *Oceanography and marine environment of the Basque Country*. Elsevier Oceanography Series, *70,* 616 pp.

Villate, F. (1991). Annual cycle of zooplankton community in the Abra Harbour (Bay of Biscay): Abundance, composition and size spectra. *Journal of Plankton Research*, *13*, 691–706. doi:10.1093/plankt/13.4.691.

Villate, F., Aravena, G., Iriarte, A., Uriarte, I. (2008). Axial variability in the relationship of chlorophyll *a* with climatic factors and the North Atlantic oscillation in a Basque coast estuary, Bay of Biscay (1997 – 2006). *Journal of Plankton Research*, *30*, 1041–1049. doi: 10.1093/plankt/fbn056.

Villate, F., Iriarte, A., Uriarte, I., Intxausti, L., de la Sota, A. (2013). Dissolved oxygen in the rehabilitation phase of an estuary: Influence of sewage pollution abatement and hydro-climatic factors. *Marine Pollution Bulletin*, *70*, 234–246.

Zingone, A., Phlips, E. J., Harrison, P. J. (2010). Multiscale variability of twenty-two coastal phytoplankton time series: A global scale comparison. *Estuaries and Coasts*, *33*, 224–229. doi:10.1007/s12237-009-9261-x.

Chapter 2. *Zooplankton seasonality and the influence of environmental factors*

Zooplankton seasonality and its environmental drivers were studied at four coastal sites within the Northeast Atlantic Shelves Province (Bilbao35 (B35) and Urdaibai35 (U35) in the Bay of Biscay, Plymouth L4 (L4) in the English Channel and Stonehaven (SH) in the North Sea) using time series spanning 1999 – 2013. Seasonal community patterns were extracted at the level of broad zooplankton groups and copepod and cladoceran genera using redundancy analysis. Temperature was generally the environmental factor that explained most of the taxa seasonal variations at the four sites. However, between-site differences related to latitude and trophic status (i.e. from oligotrophic to mesotrophic) were observed in the seasonality of zooplankton community, mainly in the pattern of taxa that peaked in spring – summer as opposed to late autumn – winter zooplankton, which were linked primarily to differences in the seasonal pattern of phytoplankton. The percentage of taxa variations explained by environmental factors increased with latitude and trophic status likely related to the increase in the co-variation of temperature and chlorophyll a, as well as in the increase in regularity of the seasonal patterns of both temperature and chlorophyll a from south to north, and of chlorophyll a with trophic status. Cladocerans and cirripede larvae at B35 and U35, echinoderm larvae at L4 and decapod larvae at SH made the highest contribution to shape the main mode of seasonal pattern of zooplankton community, which showed a seasonal delay with latitude, as well as with the increase in trophic status.

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2.1 Introduction

Zooplankton comprise a key component of marine pelagic ecosystems, forming a major link between primary producers and upper trophic level consumers (Dam, 2013). In the marine environment the seasonal cycle of zooplankton abundance and composition, together with those of key environmental variables, are among the strongest contributors to total temporal variance (Mackas *et al*., 2012). Furthermore, the seasonality of zooplankton may have profound implications for the coupling or decoupling of trophic interactions (e.g. match-mismatch hypothesis, Cushing, 1990). Seasonal variations of zooplankton have been reported in the literature (e.g. Colebrook, 1984; Longhurst, 1998), but better knowledge of zooplankton seasonality is needed to understand how climate change impacts on phenology shifts (Ji *et al*., 2010), particularly in coastal areas where there is greater variability (Ribera d'Alcalà *et al*., 2004). In addition to describing temporal patterns of variation, knowledge of the influence of environmental factors in marine plankton is also essential to understand ecological properties of pelagic ecosystems and their potential responses to a changing environment (Pepin *et al*., 2015).

Given the relevance for the entire food web dynamics, zooplankton seasonality is also important in the definition of marine eco-geographical units (Longhurst, 1998). Longhurst (1998) divided the sea into biomes, each one containing one or several provinces. The Northeast Atlantic Shelves Province (NECS) extends from Cape Finisterre (NW Spain) to the edge of the Faroe Shetland channel in the north and as far east as the Baltic Sea, and is one of the largest continental shelf regions. Seasonal patterns of zooplankton have been studied in different areas within the NECS, e.g. North Sea (e.g. Greve *et al*., 2004; Van Ginderdeuren *et al*., 2014; Bresnan *et al*., 2015), English Channel (e.g. Eloire *et al*., 2010; Highfield *et al*., 2010) and Bay of Biscay (e.g. Huskin *et al*., 2006; Stenseth *et al*., 2006; Valdes *et al*., 2007). However, fewer attempts have been made to compare zooplankton community seasonal dynamics and their drivers between these different shelf areas. For example, using CPR data Beaugrand *et al*. (2000) compared zooplankton seasonality between the English Channel and the Bay of Biscay and Mackas *et al*. (2012) made comparisons between the North Sea (using a combination of CPR data and Helgoland Roads time-series data) and the English Channel (using Plymouth L4 time series data). However, to the best of our knowledge, comparative studies on coastal zooplankton community seasonal dynamics and their environmental drivers from these three areas (North Sea, English Channel and Bay of Biscay) using the same methodology for data analysis have not been conducted so far. Moreover, most studies of zooplankton seasonality have dealt with the seasonal timing of the abundance or biomass of individual taxa. The seasonal variation of individual zooplankton taxa, at four study sites in the NECS, located in the Bay of Biscay, the English Channel and the North Sea, have been examined by our group in a previous work (Chapter 1, this work), identifying different types of seasonal patterns within the main components of zooplankton communities. However, a need to extend the analysis studying the influence of environmental drivers on the seasonal patterns at the community level was identified. Multivariate ordination methods are very useful for this purpose, as they help to understand the drivers of seasonality based on a more holistic and synthetic approach (Walker and Jackson, 2011).

The aim of the present work was to compare zooplankton community seasonal patterns and their environmental drivers at four coastal sites in the NECS, located in the Bay of Biscay (Urdaibai 35 and Bilbao 35), the English Channel (Plymouth L4) and the North Sea (Stonehaven), for which comparable time-series exist, thus covering almost the entire latitudinal range within this province. In addition, the two sites located in the Bay of Biscay are at the same latitude but differ in their trophic status (Iriarte *et al*., 2010). Therefore, time-series from these four sites allowed us to explore the influence of latitude and anthropogenic nutrient enrichment on the seasonal dynamics of zooplankton community in the NECS. This is in line with the objectives of policy directives such as the Marine Strategy Framework Directive that seek to better understand the dynamics of coastal communities over broad geographical areas, identifying the effect of human activities.

2.2 Methods

2.2.1 Data preparation

Zooplankton data were grouped and analysed at: (i) the herein termed *Zooplankton Group* level (ZG), which included six holoplankton categories (copepods, cladocerans, appendicularians, chaetognaths, siphonophores and doliolids) and nine meroplankton categories (cirripede larvae, decapod larvae, gastropod larvae, bivalve larvae, polychaete larvae, fish eggs and larvae, bryozoan larvae, echinoderm larvae and hydromedusae) and (ii) the *Copepod and Cladoceran Genera* level (CCGen), consisting of genera or genera-assemblages (exceptionally family) of cladocerans and copepods: *Evadne* and *Podon* genera for the cladocerans and *Acartia*, *Centropages*, *Temora*, *Oithona*, *Oncaea*, *Corycaeus* genera, the “PCPC-calanus” genera assemblage (this includes *Paracalanus*, *Clausocalanus*, *Pseudocalanus* and *Ctenocalanus*), and the family Calanidae for the copepods. Zooplankton was expressed in units of density (individuals m^{-3}) and prior to Redundancy Analyses (RDA), the zooplankton density data were transformed using log (x + 1) (ter Braak and Šmilauer, 2002).

2.2.2 Statistical analyses

Multivariate ordination methods were used to model the relationship between zooplankton community structure and explanatory variables using Canoco v. 4.55 (ter Braak and Šmilauer, 2002). Depending on whether the relationships between taxa and environmental variables are unimodal or linear, the use of Canonical Correspondence Analysis (CCA) or Redundancy Analysis (RDA), respectively, is advised (ter Braak and Šmilauer, 2002). To elucidate this, as a first step, Detrended Correspondence Analyses were performed, as recommended by ter Braak and Šmilauer (2002). Since the length of the longest gradient was in all cases < 2, we opted for conducting RDAs. Separate RDA analyses were performed for each of the four sites (B35, U35, L4 and SH) and each of the two taxonomic levels (ZG and CCGen) tested.

In order to extract the seasonal pattern of the zooplankton community, as well as the contribution of the different taxa to this seasonal variability, partial RDAs in which months were used as categorical explanatory variables and years as categorical covariables (thereby removing the effect of years) were performed.

In order to test the relationship between zooplankton community seasonal variations and environmental variables, preliminary partial RDAs were carried out using as explanatory variables the relevant water environment variables routinely monitored at all sites, namely, water temperature (WT), Chl *a* and salinity (Sal). Values of these variables are surface ones at L4 and SH and, although zooplankton samples were collected from surface down to 45 or 50 m, they are a valid proxy of the environmental variability that drives the seasonal variations of zooplankton. In order to obtain the final models, these partial RDAs were re-done, but using as explanatory variables only those that, in the preliminary partial RDAs, significantly explained some of the zooplankton data variation (conditional effects, with forward selection of variables). The rest of environmental factors (i.e. the non-significant ones) were included as supplementary variables, thus not influencing the analyses.

In all RDAs Monte Carlo tests were performed with 499 permutations under reduced model (ter Braak and Šmilauer, 2002). The permutations were unrestricted and the blocks defined by the covariables.

In order to test more specifically the relationship between environmental factors and the seasonal patterns represented along the two main ordination axes obtained in RDAs, Spearman’s rank-order correlations were carried out between the sample scores and environmental variables, separately for each ordination axis. Additionally, in order to test for differences between the sites in the seasonal patterns obtained for each of the two main axes, between-site Spearman rank-order correlations of the month scores along each axis were carried out.

Finally, between-year correlation analyses were performed for temperature, Chl *a* and total zooplankton abundance (Pearson correlation for the former and Spearman rank-order correlation for the two latter) to assess differences between sites in the degree of year-to-year regularity of the annual cycles of those variables. These differences were determined from the frequency distribution of the correlation values for each variable at each site. These correlations were thus performed to examine how well correlated were the seasonal patterns of different years within the time series for each variable, and can be taken as indicators of the regularity or recurrence of the seasonal cycle over the time series at each site. A lower regularity would mean that the seasonal cycle shows more variations between the different years. All correlations were carried out using SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY). The use of the parametric Pearson test or the nonparametric Spearman rank-order test was decided after testing for normality (Shapiro-Wilk test) and homoscedasticity (Levene test).

2.3 Results

2.3.1 Seasonal patterns of zooplankton community and taxa contribution

The result of multivariate ordination analysis is the ordination of data along axes. The first and second axes represent the main (dominant) and second main (less dominant) modes of variability (in the present case, seasonal variability of zooplankton community), respectively. The RDA month scores along axis 1 revealed differences in the main seasonal mode of zooplankton variation from U35 to L4 and to SH, which were more evident for CCGen than for ZG (**Figure 1**).

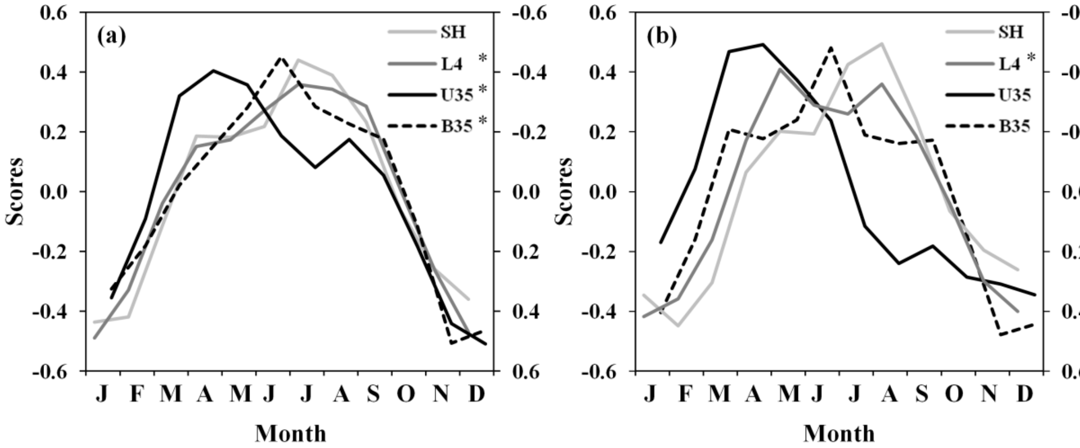

Figure 1. Month scores on axis 1 obtained from RDAs using months as explanatory variables and years as covariables for (a) Zooplankton Groups and (b) Copepod and Cladoceran Genera.

For ZG the main differences were found between U35 and both L4 and SH. The community which was most different from that of late autumn – winter occurred in spring (March – May) at U35, and in summer (July – August) at L4 and SH. For CCGen, the differences between the patterns of the three sites were much clearer: the most differentiated community from that of late autumn – winter was found in early spring (March – April) at U35 and in summer (July – August) at SH, whilst at L4 similar levels of differentiation were found in spring (May) and summer (August). Accordingly, month scores along axis 1 from U35 showed no correlation with those obtained for L4 or SH for any of the taxonomic levels tested (**Table 1**).

Table 1. Between-site correlation (Spearman rank correlation coefficients with *p*-values in parentheses) of the month scores on axis 1 for Zooplankton groups (ZG) and Copepod and Cladoceran genera (CCGen).

		U35	L4	SH
ZG	B35	**0.734 (0.007)**	**0.867 (<0.001)**	**0.832 (0.001)**
	U35	-	0.559 (0.059)	0.538 (0.071)
	L4	-	-	**0.986 (<0.001)**
CCGen	B35	**0.755 (0.005)**	**0.748 (0.005)**	0.475 (0.118)
	U35	-	0.329 (0.297)	-0.007 (0.983)
	L4	-	-	**0.874 (<0.001)**

For both levels of zooplankton grouping U35 and SH showed the strongest contrast in timing of maximum scores from spring (U35) to late summer (SH). In addition, the rather similar magnitude of the two peaks obtained for CCGen at L4 depicts an intermediate situation between those at U35 and SH. At the mesotrophic B35 site the major differences from the late autumn – winter community were found in early summer (June) for both taxonomic levels tested, but the distribution of the month scores showed two more subtle secondary peaks in early spring (March) and early autumn (September) for CCGen. Month scores along axis 1 from B35 showed weaker correlation with those from U35 than with those from L4 and SH for ZG, but no correlation with those from SH was observed for CCGen (**Table 1**).

Regarding the contribution of zooplankton taxa to the main mode of seasonal variation (axis 1) in each site (**Figure 2**), it is clear that cladocerans made a high contribution to the seasonal pattern of ZG at B35, U35 and L4, but a lower one at SH. They showed peaks in spring – early summer at B35, U35 and L4, but in late summer at SH (**Figure 1S** shown as **Supplementary material**). Cirripede larvae also made a high contribution at the lowest latitude sites (B35 and U35), but their contribution ranked lower at L4 and SH. Cirripedes showed maxima in spring at U35, L4 and SH, and in summer at B35 (**Figure 1S** shown as Supplementary material). In contrast, decapod larvae were the group that contributed most at the highest latitude site (SH), where they showed a well-defined seasonal pattern with maxima in summer (**Figure 1S**), but their contribution decreased with decreasing latitude, particularly at U35 and B35, where they showed rather similar abundances in spring and summer. Another meroplankton group, the echinoderm larvae, was the taxon with the highest contribution at L4, where they peaked in July (**Figure 1S**), but their relevance decreased at the other sites, particularly at the lowest latitude (B35 and U35), where they peaked earlier in the year. Appendicularians ranked high at all latitudes in oligotrophic sites (U35, L4 and SH), where they showed a latitudinal delay in their maximum densities from late winter to early summer, but not at the mesotrophic site (B35), where they peaked in late spring (**Figure 1S**).

Regarding the main mode of variability (axis 1) for CCGen, *Podon* and *Evadne* were among the three taxa with the highest contribution at B35, U35 and L4, but not at SH (**Figure 2b**). The occurrence of the annual maximum densities of the latter two genera showed a delay from late winter to summer from U35 to L4 and to SH, whereas peaks were observed in spring at B35 (**Figure 2S** shown as **Supplementary material**). *Acartia* showed the highest contribution at SH and U35, where they peaked in summer and early spring respectively, and *Temora* showed high contributions only at SH and L4, where they reached similar abundance peaks in spring and summer (**Figure 2S**).

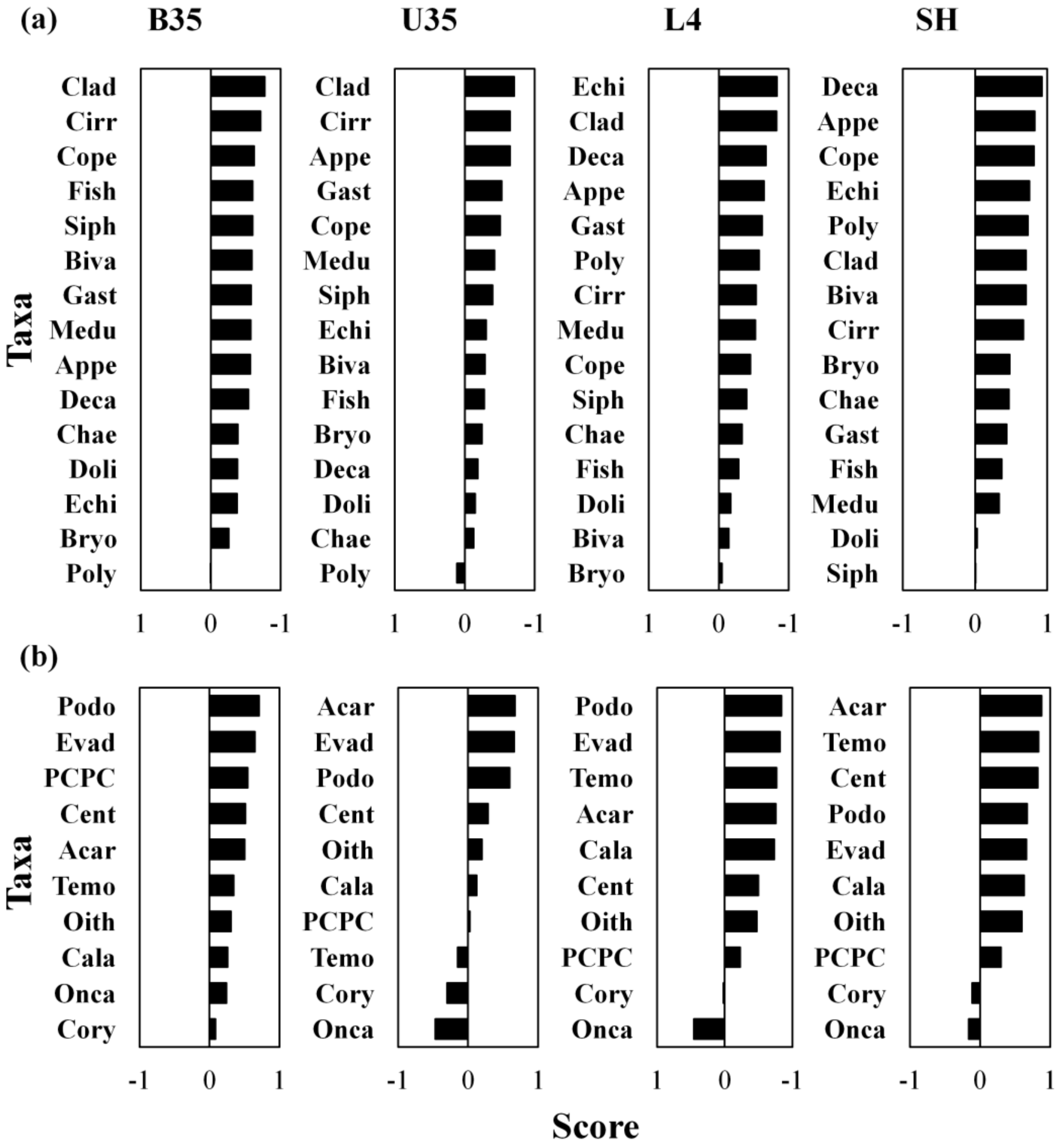

Figure 2. Taxa scores on axis 1 obtained from RDAs using months as explanatory variables and years as covariables for (a) zooplankton groups and (b) copepod and cladoceran genera. Acar: *Acartia*, Appe: appendicularians, Biva: bivalve larvae, Bryo: bryozoans, Cala: Calanidae, Cent: *Centropages*, Chae: chaetognaths, Cirr: cirripede larvae, Clad: cladocerans, Cope: copepods, Cory: *Corycaeus*, Deca: decapod larvae, Doli: doliolids, Echi: echinoderm larvae, Evad: Evadne, Fish: fish eggs and larvae, Gast: gastropod larvae, Hydr: hydromedusae, Oith: *Oithona*, Onca: *Oncaea*, PCPC: PCPC-calanus, Podo: *Podon*, Poly: polychaete larvae, Siph: siphonophores, Temo: *Temora*.

The scores of months along axis 2 showed that the second seasonal mode of zooplankton variation had a higher between-site similarity for ZG than for CCGen (**Figure 3**). The ZG responsible for this mode of variability showed the largest differences between late winter – early spring (February – April) and late summer – early autumn (August – October). L4 was an exception to this, where this second period extended through the entire second half of the year. Significant correlations of the month scores along axis 2 between all stations were found (**Table 2**). For CCGen the distribution of month scores was rather similar at all sites in the second half of the year (peaks in September-October) but not in the first half. Month scores along axis 2 only showed significant correlations between U35 and B35 and between L4 and SH (**Table 2**).

Table 2. Between-site correlation (Spearman rank correlation coefficients with *p*-values in parentheses) of the month scores on axis 2 for Zooplankton groups (ZG) and Copepod and Cladoceran genera (CCGen).

		U35	L4	SH
ZG	B35	**0.916 (<0.001)**	**0.874(<0.001)**	**0.902 (<0.001)**
	U35	-	**0.720 (0.008)**	**0.741 (0.006)**
	L4	-	-	**0.916 (<0.001)**
CCGen	B35	**0.615 (0.033)**	0.510 (0.090)	0.063 (0.846)
	U35	-	0.364 (0.245)	0.315 (0.319)
	L4	-	-	**0.755 (0.005)**

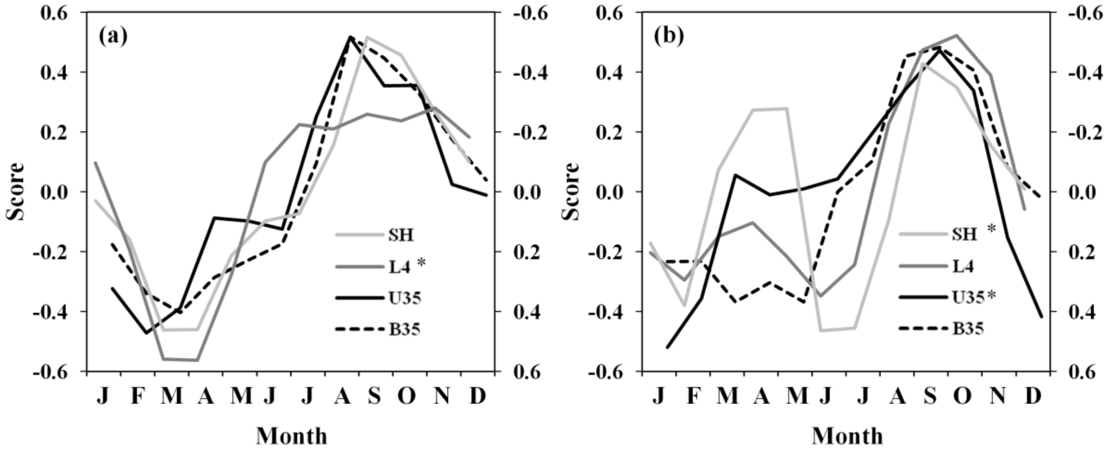

Figure 3. Month scores on axis 2 obtained from RDAs using months as explanatory variables and years as covariables for (a) Zooplankton Groups and (b) Copepod and Cladoceran Genera.

As shown in **Figure 4a**, for ZG, doliolids at the lowest latitude sites (B35 and U35) and siphonophores at L4 and SH contributed most to this secondary seasonal pattern, together with chaetognaths at all sites. In contrast to the abovementioned groups, cirripede larvae and fish eggs and larvae, together with polychaete larvae at U35, also showed high contributions at all sites. Chaetognaths and doliolids at all sites and siphonophores at L4 and SH were groups characterized by peaks in late summer – early autumn, whilst cirripede larvae and fish eggs and larvae showed early annual peaks (February – April) at all latitudes in oligotrophic sites (SH, L4 and U35) and polychaete larvae had winter maxima at U35 (**Figure 1S**). For copepod and cladoceran genera there were clear between-site differences in the contribution ranking of genera, but *Oncaea*, *Corycaeus* and PCPC-calanus at all sites, *Temora* at the lowest latitude sites (B35 and U35), *Centropages* at L4 and Calanidae at SH showed high contributions. Maximum densities in *Oncaea* and *Corycaeus* or high densities in PCPC-calanus were generally observed late in the year. The annual peak of *Temora* was later at B35 and U35 than at L4 and SH; that of *Centropages* was later at L4 than at the other sites, and that of Calanidae at SH than at the other sites (**Figure 2S**).

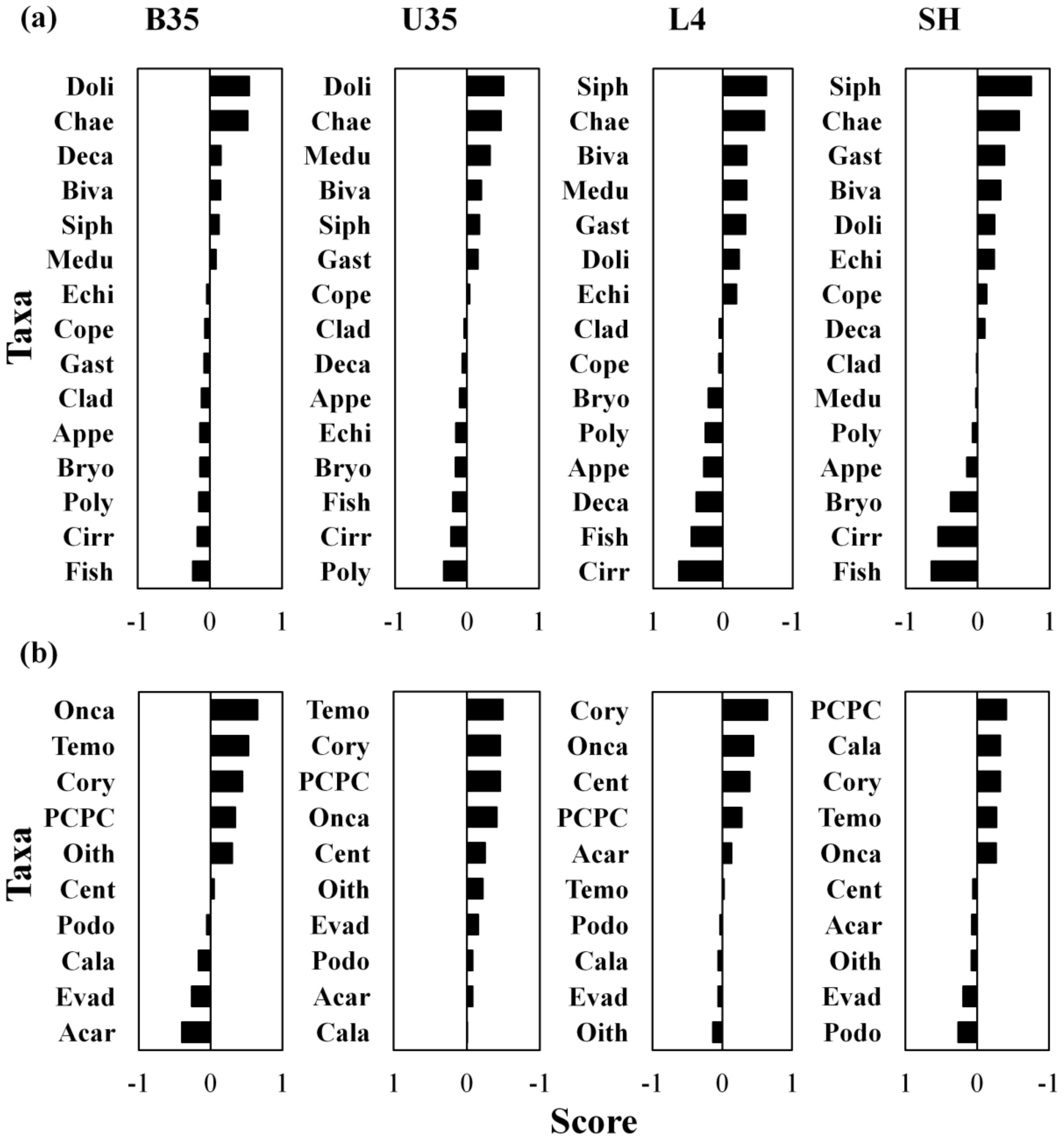

Figure 4. Taxa scores on axis 2 obtained from RDAs using months as explanatory variables and years as covariables for (a) zooplankton groups and (b) copepod and cladoceran genera. Abbreviations as in **Figure 2**.

2.3.2 Relationship between environmental factors and zooplankton seasonality

Results of the partial RDAs of zooplankton data with environmental variables as explanatory variables showed that there was a clear increase from U35 to L4 and to SH in the percentage of seasonal variation of zooplankton data explained by environmental variables at both taxonomic levels tested (**Figure 5**). It was also higher at the mesotrophic B35 site both for ZG and CCGen than at the oligotrophic U35 site.

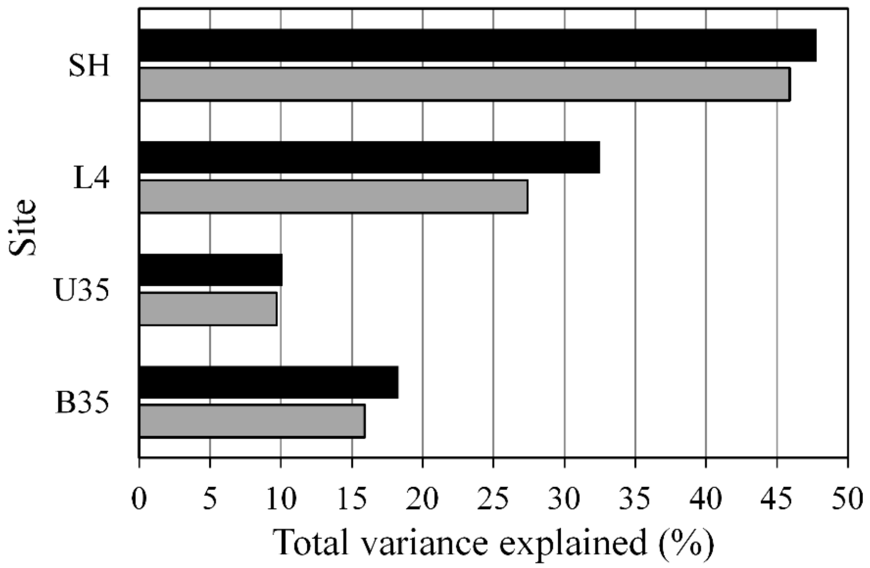

Figure 5. Percentage of total zooplankton variance explained by environmental factors at the level of Zooplankton Groups (black bars) and Copepod and Cladoceran Genera (grey bars).

Examination of the conditional effects of environmental variables on the seasonal zooplankton taxa variations (**Table 3**) showed that WT and Chl *a* were selected by the model for both taxonomic levels tested at all sites, but the largest percentage of variation was explained by WT at B35, U35 and L4, and by Chl *a* at SH. The percentage of variation explained both by WT and Chl *a* increased from south to north from U35 to SH. At the mesotrophic B35 site, WT explained a higher percentage of variance than at U35.

Table 3. Conditional effects of environmental variables for Zooplankton groups (ZG) and Copepod and Cladoceran genera (CCGen). Variables with significant effects in bold. Sal: salinity, WT: water temperature, Chl *a*: concentration of chlorophyll *a*.

	ZG				CCGen			
	Variable	LambdaA	F	*p*	Variable	LambdaA	F	*p*
B35	**WT**	**0.17**	**36.58**	**0.001**	**WT**	**0.14**	**29.25**	**0.001**
	Chl *a*	**0.01**	**2.90**	**0.023**	**Chl *a***	**0.01**	**2.43**	**0.041**
	Sal	0.00	0.62	0.710	Sal	0.01	1.83	0.096
U35	**WT**	**0.06**	**13.06**	**0.001**	**WT**	**0.07**	**13.40**	**0.001**
	Chl *a*	**0.04**	**6.48**	**0.002**	**Chl *a***	**0.02**	**4.86**	**0.004**
	Sal	0.00	1.34	0.185	Sal	0.01	1.09	0.318
L4	**WT**	**0.23**	**59.09**	**0.001**	**WT**	**0.18**	**42.66**	**0.001**
	Chl *a*	**0.08**	**23.22**	**0.001**	**Chl *a***	**0.09**	**23.81**	**0.001**
	Sal	**0.01**	**2.84**	**0.033**	Sal	0.00	1.63	0.169
SH	**Chl *a***	**0.31**	**79.61**	**0.001**	**Chl *a***	**0.28**	**71.77**	**0.001**
	WT	**0.17**	**59.95**	**0.001**	**WT**	**0.18**	**62.33**	**0.001**
	Sal	0.00	0.74	0.610	Sal	0.00	0.62	0.649

However, the relationship between environmental variables and each of the seasonal patterns represented by ordination along axis 1 and axis 2, respectively, was examined through the analysis of the correlations between environmental variables and sample scores along each ordination axis (**Tables 4** and **5**). This analysis showed that Chl *a* was the factor with the highest correlation with the main mode of zooplankton community seasonal variability (sample scores along axis 1) at all stations and taxonomic levels tested (exception was at L4 where very similar correlation coefficients were obtained for Chl *a* and WT). This is in accordance with the fact that the seasonal pattern of Chl *a* (**Figure 6**) showed quite a good agreement with that of monthly scores along axis 1 (**Figure 2**). Conversely, WT was the environmental variable that showed the highest correlation with the second seasonal mode of zooplankton community variability (axis 2) and in most cases no significant correlation with Chl *a* was observed (**Table 4**).

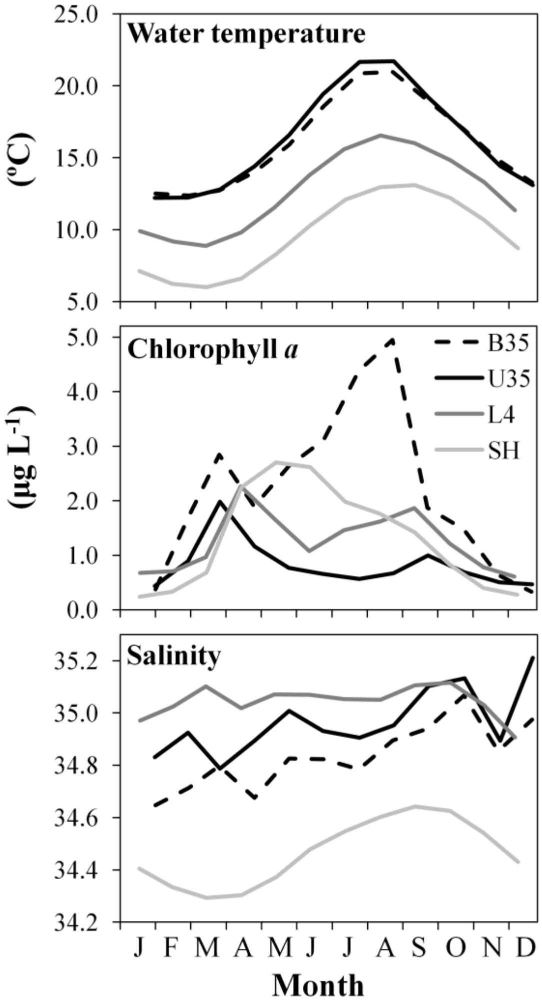

Figure 6. Seasonal variation of monthly means of water temperature, Chl *a* and salinity at B35 (dashed), U35 (black), L4 (dark grey) and SH (light grey).

Table 4. Correlations between environmental factors and sample scores on axis 1 of Zooplankton groups (ZG) and Copepod and Cladoceran genera (CCGen). Significant correlation coefficients in bold (** $p< 0.01$; * $p< 0.05$). Sal: salinity, WT: water temperature, Chl *a*: concentration of chlorophyll *a*.

	ZG				CCGen			
	B35	U35	L4	SH	B35	U35	L4	SH
Sal	0.041	0.070	**-0.164***	**0.247****	-0.011	-0.066	**-0.160***	**0.333****
WT	**-0.567****	**-0.313****	**-0.606****	**0.533****	**0.390****	-0.124	**-0.521****	**0.661****
Chl *a*	**-0.585****	**-0.359****	**-0.600****	**0.839****	**0.537****	**0.265****	**-0.582****	**0.774****

Table 5. Correlations between environmental factors and sample scores on axis 2 of Zooplankton groups (ZG) and Copepod and Cladoceran genera (CCGen). Significant correlation coefficients in bold (** $p< 0.01$; * $p< 0.05$). Sal: salinity, WT: water temperature, Chl *a*: concentration of chlorophyll *a*.

	ZG				CCGen			
	B35	U35	L4	SH	B35	U35	L4	SH
Sal	0.101	-0.065	-0.018	**0.566****	0.122	0.080	**0.184***	-0.079
WT	**0.529****	**0.609****	**-0.672****	**0.734****	**0.614****	**-0.525****	**0.445****	-0.131
Chl *a*	-0.055	-0.087	-0.010	**-0.159***	0.059	**-0.206****	0.101	-0.017

As shown in **Figures 7** and **8**, at B35 most zooplankton groups, and many copepod genera were strongly related to WT. In general, the number of zooplankton groups with high correlation with WT decreased with latitude from U35 to SH, and the relation of copepod and cladoceran genera with WT or Chl *a* also decreased with latitude from U35 to SH. At U35 a group of genera related to WT (*Corycaeus*, *Oncaea*, *Centropages*, *Temora*) was clearly distinguished from a group of genera related to Chl *a* (*Evadne*, *Podon*, *Acartia*). At SH, however, most genera showed similar relationships with WT and Chl *a*. In general, chaetognaths, doliolids and siphonophores were the zooplankton groups with the highest relationship with WT, and cirripede larvae and appendicularians the most highly related ones to Chl *a* at all sites.

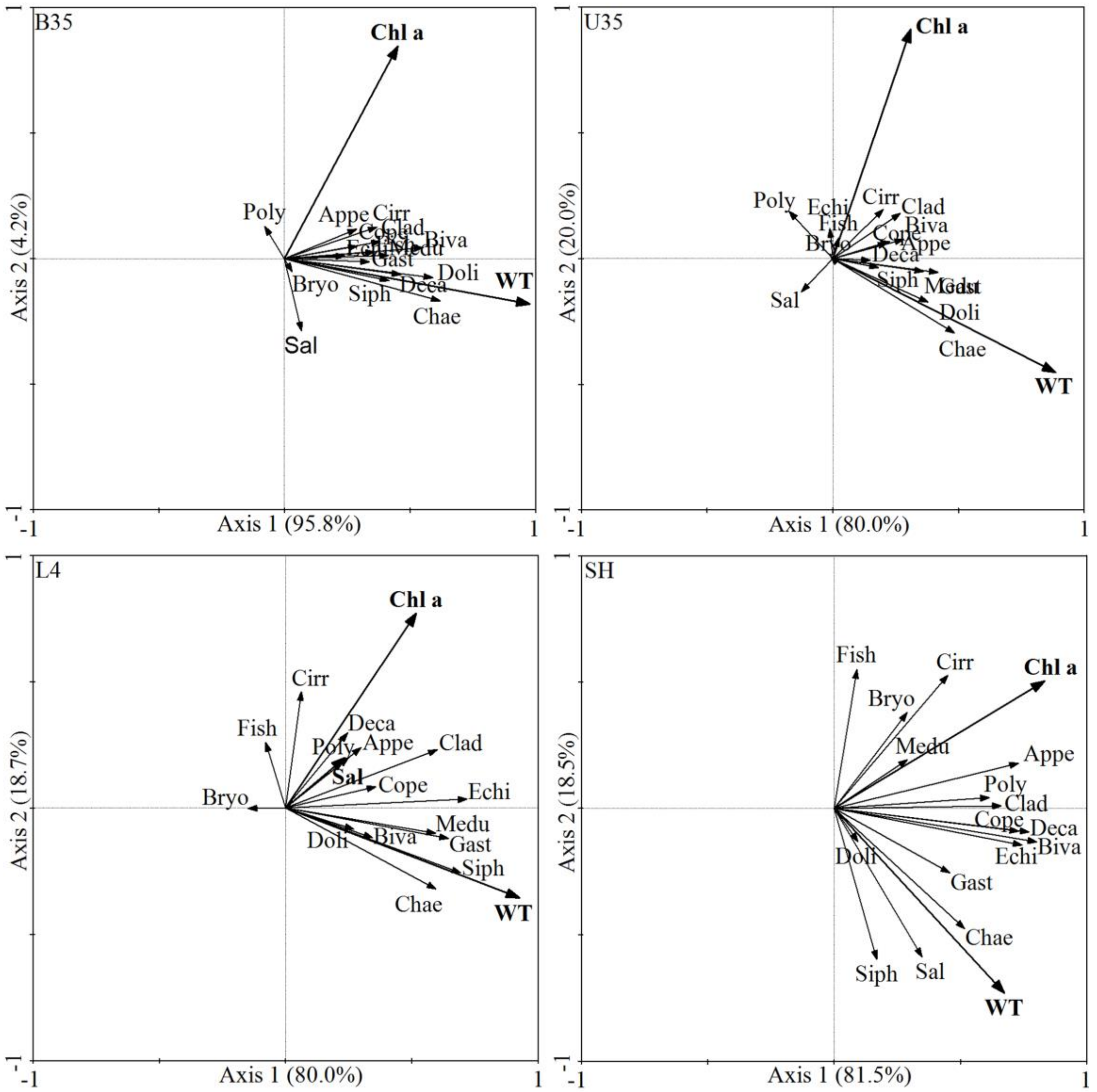

Figure 7. RDA biplot of Zooplankton Group taxa (thin black arrows) and environmental variables (thick black arrows for variables with significant conditional effects and grey arrows for variables with non-significant conditional effects). In parentheses the percentage of taxa-environment relationship explained by each axis. Taxa and environmental variable abbreviations as in **Figure 2** and **Table 3**, respectively.

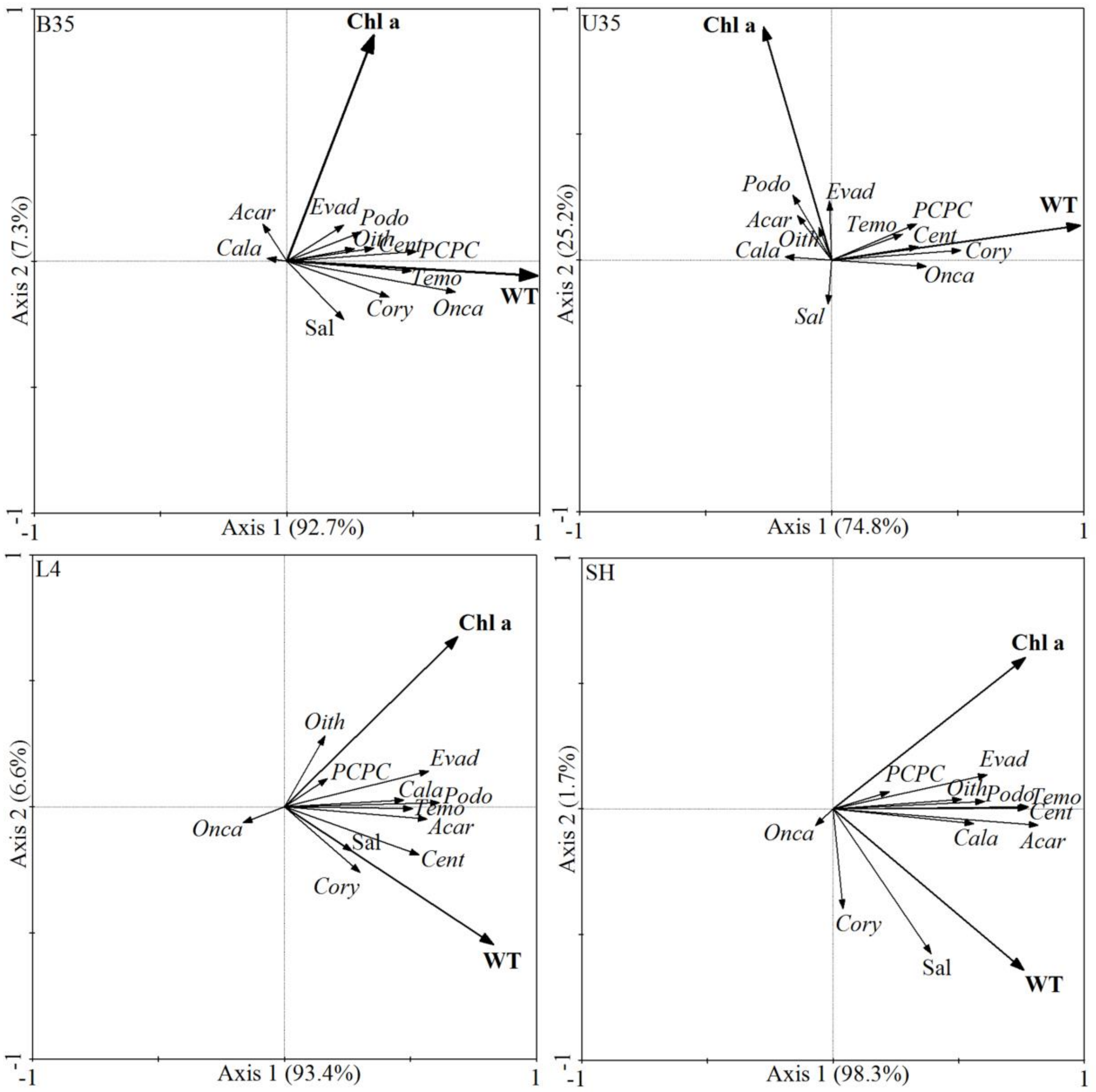

Figure 8. RDA biplot of Copepod and Cladoceran Genera (thin black arrows) and environmental variables (thick black arrows for variables with significant conditional effects and grey arrows for variables with non-significant conditional effects). In parentheses the percentage of taxa-environment relationship explained by each axis. Taxa and environmental variable abbreviations as in **Figure 2** and **Table 3**, respectively.

2.3.3 Year-to-year regularity in the seasonal patterns of water temperature, chlorophyll a and total zooplankton density

Figure 9 shows that the between-year correlation increased from B35 and U35 to L4 and to SH for WT, Chl *a* and total zooplankton abundance. This can be taken as an indication of an increase in the regularity of the annual cycle from U35 to L4 and to SH. Higher regularity would mean that the seasonal cycle varies less between years. Regularity was also higher at B35 than at U35 for Chl *a* and total zooplankton abundance.

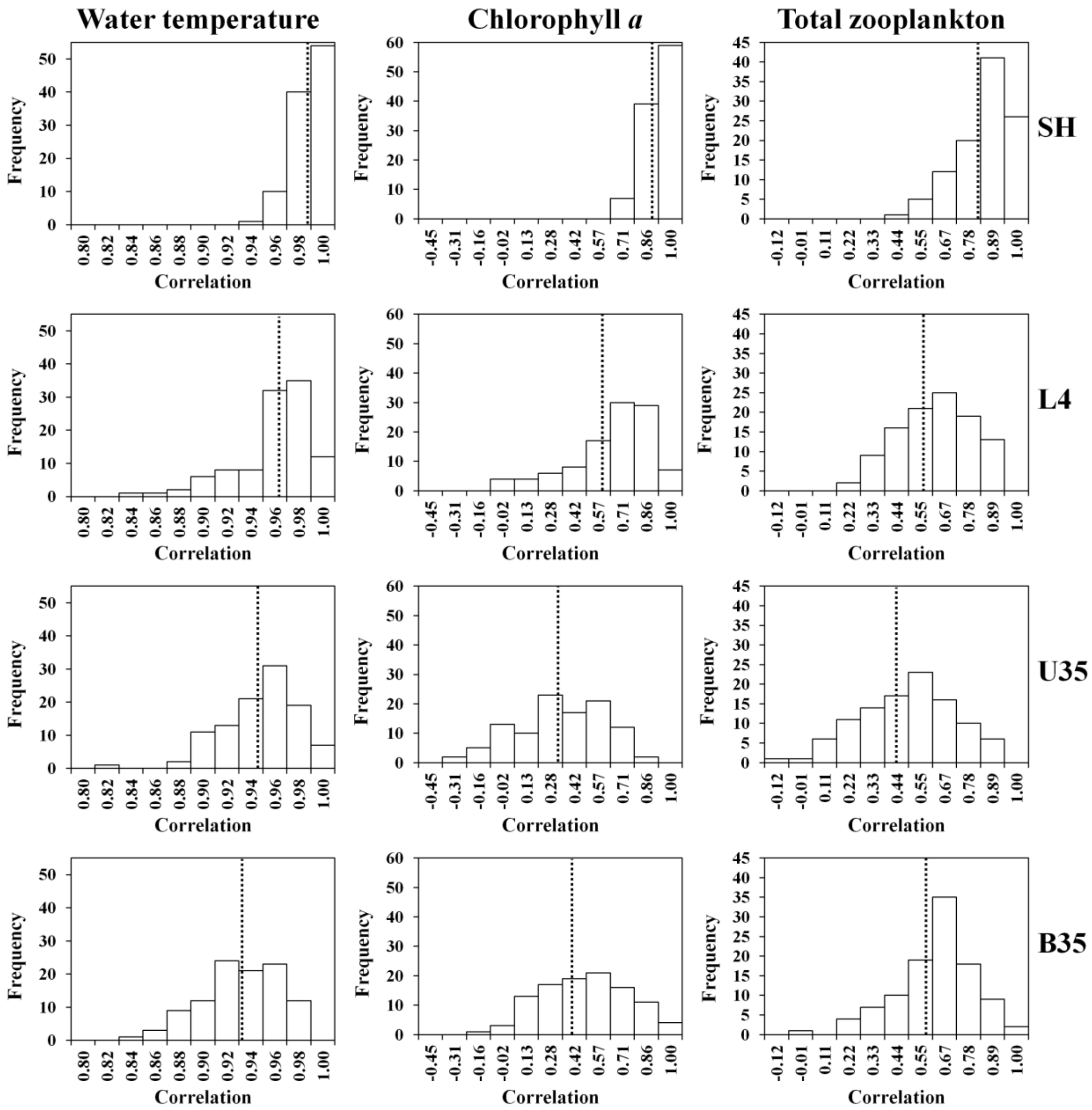

Figure 9. Histograms of between-year correlations of annual cycles of temperature, chlorophyll *a* and total zooplankton abundance. The vertical dotted line shows the mean value of the distribution.

Zooplankton abundance and Chl *a* were not correlated between sites, whereas water temperature correlated between B35 and U35 ($p< 0.001$), and between L4 and SH ($p= 0.001$). Within sites zooplankton abundance, Chl *a* concentration and water temperature were not correlated, except for the negative correlation ($p= 0.026$) between zooplankton and Chl *a* at B35 and the positive correlation ($p= 0.024$) between Chl *a* and temperature at L4.

2.4 Discussion

2.4.1 Seasonal patterns of zooplankton community

In general, our data evidenced differences between sites in the seasonal pattern that were chiefly related to the main mode of community variability (axis 1), which represented the pattern driven by taxa that peaked in the central part of the year (spring – summer) as opposed to late autumn – early winter zooplankton. On the contrary, no marked between-site differences were observed related to the second main mode of seasonal variability (axis 2) for ZG or for taxa that peaked latest in the second half of the year for CCGen. Within the oligotrophic sites a delay with latitude was apparent, since, in the southern Bay of Biscay (U35) the seasonal pattern represented by the main mode of variability of ZG was mainly accounted for by taxa that peaked in spring, whilst at the western English Channel (L4) and North Sea (SH) sites it was mainly accounted for by taxa that peaked in summer. Between-site differences along the gradient from south to north were clearer at the CCGen level than at the ZG level, likely due to the fact that genera reflect better a seasonal succession of species that is masked at the group level. For instance, *Acartia* is almost exclusively *Acartia clausi* at all four sites, *Temora* is dominated by *Temora longicornis* at SH and L4 and by *Temora stylifera* at U35 and B35, and PCPC-calanus is dominated by *Pseudocalanus elongatus* at SH, *Paracalanus parvus* at B35 and U35 and the two species in similar densities at L4 (Chapter 1, this work). The difference in the seasonal pattern of the zooplankton community we observed between U35 and L4 agrees well with that reported by Beaugrand *et al*. (2000) who studied an area from the Northeastern English Channel down to the southern Bay of Biscay. Similarly, Bot *et al*. (1996) observed a seasonal delay in the maxima of copepods from south to north in Northwest European shelves. This south to north gradient in the seasonal timing agrees with the general view that, for spring and summer zooplankton, the development, reproduction, and onset/termination of seasonal dormancy all shift earlier in the year where the environment is warmer (Mackas and Beaugrand, 2010; Beaugrand *et al*., 2014).

2.4.2 Environmental drivers of zooplankton community seasonality

Water temperature generally explained the highest zooplankton taxa seasonal variability, as shown by RDA analyses. This agrees with the role of temperature as a primary structuring factor of the seasonality of zooplankton (Mackas *et al*., 2012), since it controls their rates of egg development, feeding, production, respiration and other metabolic processes (Peters and Downing, 1984; Ambler *et al*., 1985; Ikeda, 1985) and, indirectly, it can also control their food availability (Mackas *et al*., 2012). Despite the key role of temperature, the correlations between environmental factors and sample scores on axis 1 and axis 2 performed separately, showed clearly that the sample scores on axis 1, unlike those on axis 2, were generally more strongly related to Chl *a* concentration than to temperature. Therefore, since axis 1 best represented the between-site differences in the seasonal variation of the zooplankton community, we can say that it was mainly the timing of phytoplankton availability which could account for the major between-site seasonal variations of zooplankton community. The facts that (i) the phytoplankton spring bloom is delayed from U35 to L4 and to SH, and (ii) delays in phytoplankton blooms with latitude may be a general pattern within the latitudinal range at which our stations are located in the east Atlantic shelf waters (Martinez *et al*., 2011; Racault *et al*., 2012), support the view that the main differences in the seasonal zooplankton community seasonal pattern between U35, L4 and SH may be the consequence of a latitudinal effect driven by latitudinal differences in the availability of phytoplankton throughout the spring-summer period.

In addition to differences between the oligotrophic sites, our data also showed a delay in the timing of the peak in the zooplankton seasonal pattern conformed by month scores along axis 1 from the oligotrophic U35 to the mesotrophic B35. These differences in zooplankton community seasonal pattern may be related to differences between these two sites in the level of anthropogenic nutrient enrichment and in hydrographical features. The presence of an estuarine plume at B35 (Ferrer *et al*., 2009) results in higher nutrient concentrations at B35 than at U35 (Iriarte *et al*., 1997; Villate *et al*., 2013). It also causes B35 to have a more estuarine/enclosed coastal ecosystem type of phytoplankton seasonal cycle (*sensu* Cebrián and Valiela, 1999), with high phytoplankton biomass in spring, but higher ones in summer. In contrast U35, where tidal flushing is high, is characterized by the typical temperate shelf water bimodal seasonal pattern found in the southern Bay of Biscay (Stenseth *et al*., 2006) with spring (main) and autumn (secondary) peaks, and low summer phytoplankton biomass (Iriarte *et al*., 2010; Villate *et al*., 2017). Another important finding was that for the oligotrophic sites (U35, L4, and SH), the proportion of zooplankton taxa variations explained by environmental factors increased from U35 to L4 and to SH. There was a higher seasonal covariation of water temperature and Chl *a* at SH, and this can contribute to a more similar correlation of most zooplankton taxa with these two factors. Conversely, as stated above, as we move from SH towards L4 and U35 the spring phytoplankton bloom occurs earlier in the year, and therefore, the effect of temperature and phytoplankton biomass becomes less additive. Another very influential factor can be the increase in the year-to-year regularity of the seasonal patterns of temperature and Chl *a* from U35 to SH. This causes the variation pattern of seasonal zooplankton also to be most similar between years at the northernmost site. An increase in the proportion of zooplankton community seasonal variations explained by environmental factors from the oligotrophic U35 to the mesotrophic B35 was also observed. In this case, the higher covariation between water temperature and Chl *a* could also be the most plausible explanation. In fact, the conditional effect of Chl *a* was low or not significant at B35, despite the marginal effect being significant (data not shown). It could also be affected by a higher regularity in the seasonal pattern of Chl *a* concentration. Differences in the regularity and predictability of the seasonal pattern of zooplankton can have important implications for predator-prey interactions (Atkinson *et al*., 2015). A more regular, predictable seasonal pattern of zooplankton that we have observed northwards or under mesotrophic conditions (understanding by more regular that the seasonal cycle varies less from year to year) can increase the trophic match probability between fish larvae and their zooplankton prey (Ji *et al*., 2010, Mackas *et al*., 2012), and therefore, enhance the feeding success probability of the former, according to the match-mismatch hypothesis (Cushing, 1990; Fortier *et al*., 1995).

Our analysis focused on the role of environmental factors on the seasonal distribution of zooplankton, but predation controls can also be important. For instance, work at L4 on phenology of successive planktonic trophic levels reveals high inter-annual variability in timings of both predators and prey (Atkinson *et al*., 2015). The effects of temperature and food availability on zooplankton seasonality are also influenced strongly by simultaneous and strong top-down effects that modify the timing and amplitude of abundance peaks (Maud *et al*., 2015).

2.4.3 Contribution of individual taxa to shape seasonal zooplankton community patterns and the relationship between individual taxa and environmental drivers

The differences in the main mode of seasonal variability pattern were accompanied by differences in the zooplankton taxa that contributed most to shape each pattern. At the southern Bay of Biscay sites, cladocerans made the highest contribution. Cladocerans can consume components of the microbial food web (Katechakis and Stibor, 2004), but they feed mainly on phytoplankton (Brown *et al*., 1997). In accordance, the timing of their seasonal peak differed from U35 to B35 (maxima in early spring and early summer, respectively) in relation to differences in the seasonal pattern of Chl *a*. At the English Channel and North Sea sites, meroplankton groups, i.e. echinoderm larvae and decapod larvae respectively, contributed most to shape the seasonal pattern. During the present study period, echinoderm larvae have been shown to peak in July at L4, and decapod larvae in August at SH. Other works have also shown both echinoderm and decapod larvae maxima in summer in the northern North Sea (Lindley and Kirby, 2007) and western English Channel (Highfield *et al*., 2010). It is noteworthy that both of these meroplankton groups are known to have increased their abundances in the North Sea especially since the mid-1980s, likely due to seawater warming (Kirby *et al*., 2008). Meroplankton was also important at U35 and B35, since cirripede larvae, a group that peaked in early spring at U35 but in early summer at B35, ranked high in the contribution to the main mode of seasonal variability at these sites. The relevance of meroplankton groups at all sites underscores the influence of benthic communities on the seasonality of pelagic ones, and provides support for the idea that benthic-pelagic coupling has a prominent role in coastal environments (Griffith *et al*., 2017).

The second main mode of variability of ZG showed a high degree of coincidence between sites because it was mainly accounted for by taxa that peaked later in the year at all sites and correlated strongly with WT (i.e. chaetognaths at all sites, doliolids at B35 and U35 and siphonophores at L4 and SH), in contrast to taxa that showed highest densities earlier in the year and correlated mainly with Chl *a* (i.e. cirripede larvae and appendicularians at most sites). Highfield *et al*. (2010) also found cirripede larvae to be related to the timing of Chl *a* at L4 and so did Korn and Kulikova (1995) in Avacha Inlet (eastern Kamchatka coast in North Pacific). Pelagic cirripede nauplius larvae are filter-feeders that feed on phytoplankton (Moyse, 1963) and Chl *a* concentration greatly influences the release of larvae by barnacles (Starr *et al*., 1991). This seasonal relation of cirripede larvae with phytoplankton biomass is a standard pattern that responds to seasonal averages for multiannual periods, but timing leads or lags for individual years have been reported at L4 (Atkinson *et al*., 2015). Within appendicularians the availability of food (phytoplankton) has been found to be the most limiting factor for *Oikopleura dioica* in coastal waters (Tomita *et al*., 2003). We have no information on the species composition of appendicularians at SH, but at L4 (López-Urrutia *et al*., 2005), U35 and B35 (Chapter 1, this work) *Oikopleura* was the dominant genus and *O. dioica* the most abundant one among the *Oikopleura* identified to species level. Among the zooplankton groups that best correlated with temperature, however, we have two groups of predators, the siphonophores and the chaetognaths. Within the siphonophores, *Muggiaea* spp. were most abundant at the four stations (see Chapter 1), and they are known to reproduce rapidly when temperature and prey densities are elevated (Blackett *et al*., 2014). In fact, peak periods of siphonophores coincided with high copepod densities or followed copepod peaks at our four study sites. Regarding chaetognaths, despite the dominance of different species at the different sites we studied (*Parasagitta friderici*, *Parasagitta setosa*, *Parasagitta elegans*), peak chaetognath densities were observed in late summer at all stations, at the time of highest densities of small copepods, i.e. their main prey (Falkenhaug, 1991; Tönnesson and Tiselius, 2005). The annual development of chaetognaths at the Abra Bay, where B35 is located, was found to be associated to the abundance of copepod nauplii for juvenile stages and postnaupliar cyclopoids and small calanoids for largest individuals (Villate, 1991). The high correlation of doliolids with temperature, however, may not be related to the timing of maximum food availability; instead, it may be linked to more stratified conditions being favourable for them (Menard *et al*., 1997).

At the finer taxonomic level among the copepods and cladocerans, various copepod genera appeared well correlated with temperature (*Corycaeus, Oncaea, Temora, Centropages*), but only *Corycaeus* (*Ditrichocorycaeus*) seemed to be consistently correlated with temperature across sites (less well correlated at SH). This may be because a single common species, *D. anglicus*, dominates this genus at B35, U35 and L4, a species considered to be a temperate warm water indicator (Bonnet and Frid, 2004), whereas larger differences in species composition between sites were observed within *Temora* and *Centropages* genera, for instance.

2.4.4 Coherence of coastal zooplankton seasonality within marine biogeographic units

In general, our results reinforce the view that coastal zooplankton community seasonal dynamics within the Northeast Atlantic Shelves Province (Longhurst, 1998) show geographical variations (Beaugrand *et al*., 2000; McGinty *et al*., 2011). Furthermore, the sites under study are located in two different provinces, i.e. the Northern European Seas province (SH and L4) and the Lusitanian province (U35 and B35) according to the classification by Spalding *et al*. (2007) and in three different Large Marine Ecosystems (LMEs) according to the classification by Sherman *et al*. (2004): the Iberian Coastal (U35 and B35), the Celtic-Biscay shelf (L4) and the North Sea (SH). However, the seasonal pattern of zooplankton community at U35 is a bimodal cycle similar to that described by Beaugrand *et al*. (2000) for the southern part of the Celtic Sea and the oceanic region of the Bay of Biscay, although the spring peak occurs earlier in the year at U35. Moreover, the seasonal variability of zooplankton abundance at U35 does not seem to conform to those observed at other coastal sites located on the north-western Iberian Peninsula (e.g. Vigo and A Coruña stations), where zooplankton maxima occur in summer/early autumn due to the influence of upwelling processes (Bode *et al*., 2013). Upwelling events show decreasing intensity easterly along the Cantabrian shelf, and the weakness of upwelling processes in the Basque coast (innermost Cantabrian shelf) precludes breaking of the stratification up to the surface layers (Valencia and Franco, 2004). This fact explains the decline of phytoplankton and zooplankton after the spring maxima at coastal sites that are poorly fertilized by river inputs, as is the case of U35. Therefore, a lack of homogeneity in terms of zooplankton community seasonality within the Iberian Coast LME is also evident. The division of the marine environment into coherent biogeographic units entails much complexity because of the many influencing variables (topographical, hydrographical, climatic, ecological etc.), and it is an even harder task for the more variable coastal areas where local processes usually interfere more with broader scale drivers to affect ecological phenomena. The present work provides helpful information to better delineate the boundaries between meaningful biogeographic units in the marine environment and baseline phenological data that can be useful to detect significant departures over time.

2.5 Conclusions

The use of multivariate ordination methods has allowed us to examine the seasonality of zooplankton at the community level, thus obtaining a more synthetic view than when dealing with seasonal patterns of individual taxa. The main mode of seasonal variability was due mainly to taxa that peaked in the central part of the year (spring – summer) and reached minima in late autumn – winter, and it was the mode of variability that showed the largest between-site differences. These differences consisted mainly in a seasonal delay (from spring to summer) with latitude as well as with the trophic status in the occurrence of the community that contrasted most with the late autumn – winter community. These delays, in turn, were primarily related to between-site differences in the seasonal pattern of phytoplankton biomass. Meroplankton taxa played a key role in shaping this main seasonal mode of variability of the zooplankton community, which highlights the influence of benthic dynamics on the pelagic ones. Furthermore, between-site differences were observed also in the percentage variance of zooplankton explained by Chl *a*, temperature and salinity, and in the regularity of the seasonal cycle of total zooplankton density, which increased with latitude and trophic status. This may be related to a higher co-variation of water temperature and chlorophyll *a* with the increase in latitude and trophic status, as well as to the increase in the regularity of the seasonal patterns of both temperature and chlorophyll *a* from south to north, and of chlorophyll *a* with trophic status. It is hypothesized that differences in the regularity, and therefore predictability, of the seasonal cycle of zooplankton can have profound implications for the trophic interactions with their predators (e.g. match-mismatch hypothesis). The present data have reinforced the view that within biogeographical units such as the Northeast Atlantic Province coastal zooplankton seasonality is not uniform, suggesting that in coastal ecosystems local processes interfere more with large scale ones than in oceanic waters.

2.6 References

Ambler, J.W. (1985). Seasonal factors affecting egg production and viability of eggs of Acartia tonsa Dana from East Lagoon, Galveston, Texas. *Estuarine, Coastal and Shelf Science*, *20,* 743–760. doi: 10.1016/0272-7714(85)90030-7.

Atkinson, A., Harmer, R. A., Widdicombe, C. E., McEvoy, A. J., Smyth, T. J., Cummings, D. G., Somerfield, P. J., Maud, J. L., McConville, K. (2015). Questioning the role of phenology shifts and trophic mismatching in a planktonic food web. *Progress in Oceanography*, *137*, 498–512. doi:10.1016/j.pocean.2015.04.023.

Beaugrand, G., Ibañez, F., Reid, P.C. (2000). Spatial, seasonal and long-term fluctuations of plankton in relation to hydroclimatic features in the English Channel, Celtic Sea and Bay of Biscay. *Marine Ecology Progress Series*, *200*, 93–102. doi: 10.3354/meps200093.

Beaugrand, G., Goberville, E., Luczak, C., Kirby, R.R. (2014). Marine biological shifts and climate. *Proceedings of the Royal Society B*, *281*, 20133350. doi: 10.1098/rspb.2013.3350.

Blackett, M., Licandro, P., Coombs, S.H., Lucas, C.H. (2014). Long-term variability of the siphonophores Muggiaea atlantica and M. kochi in the Western English Channel. *Progress in Oceanography*, *128*, 1–14. doi: 10.1016/j.pocean.2014.07.004.

Bode, A., Bueno, J., Lopez-Urrutia, A., Villate, F., Uriarte, I., Iriarte, A., Alvarez-Ossorio, M. T., Miranda, A., Casas, G., Dos Santos, A., Santos, A. M. P., Chicharo, M. A., Cruz, J., Ben-Hamadou, B., Chicharo, L. (2013). Zooplankton of the Bay of Biscay and western Iberian shelf. In O´Brien, T. D., Wiebe, P. H., Falkenhaug, T. (eds.), *ICES Zooplankton Status Report 2010/2011*. ICES Cooperative Research Report, 318. 208pp.

Bonnet, D., Frid, C. (2004). Seven copepod species considered as indicators of water-mass influence and changes: Results from a Northumberland coastal station. *ICES Journal of Marine Science*, *61*, 485–491. doi: 10.1016/j.icesjms.2004.03.005.

Bot, P.V.M., van Raaphorst, W., Batten, S.R., Laane W.P.M., Philippart, K., Radach, G., Frohse, A., Schultz, H., van den Eynde, D., Colijn, F. (1996). Comparison of changes in the annual variability of the seasonal cycles of chlorophyll, nutrients and zooplankton at eight locations on the northwest European continental shelf (1960–1994). *Deutsche Hydrografische Zeitschrift*, *48*, 349–364. doi: 10.1007/BF02799378.

Bresnan, E., Cook, K. B., Hughes, S. L., Hay, S. J., Smith, K., Walsham, P., Webster, L. (2015). Seasonality of the plankton community at an east and west coast monitoring site in Scottish waters. *Journal of Sea Research*, *105*, 16–29. doi:10.1016/j.seares.2015.06.009.

Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A. (1997). Nutritional properties of microalgae for mariculture. *Aquaculture*, *151*, 315–331. doi: 10.1016/S0044-8486(96)01501-3.

Cebrián, J., Valiela, I. (1999). Seasonal patterns in phytoplankton biomass in coastal ecosystems. *Jorunal of Plankton Research*, *21*, 429–444. doi: /10.1093/plankt/21.3.429.

Colebrook, J.M. (1984). Continuous Plankton Record: relationship between species of phytoplankton and zooplankton in the seasonal cycle. *Marine Biology*, *83*, 313–323. doi: 10.1007/BF00397464.

Cushing, D. H. (1990). Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Advances in Marine Biology*, *26*, 249–293. doi:10.1016/S0065-2881(08)60202-3.

Dam, H.G. (2013). Evolutionary adaptation of marine zooplankton to global change. *Annual Review of Marine Science*, *5*, 349–70. doi: 10.1146/annurev-marine-121211-172229.

Eloire, D., Somerfield, P. J., Conway, D. V. P., Halsband-Lenk, C., Harris, R. and Bonnet, D.

(2010). Temporal variability and community composition of zooplankton at station L4 in the Western Channel: 20 years of sampling. *Journal of Plankton Research*, *32*, 657–679. doi:10.1093/plankt/fbq009.

Falkenhaug, T. (1991). Prey composition and feeding rate of *Sagitta elegans* var. *arctica* (Chaetognatha) in the Barents Sea in early summer. *Polar Research*, *10*, 487–506. doi:10.1111/j.1751-8369.1991.tb00668.x.

Ferrer, L., Fontán, A., Mader, J., Chust, G., González, M., Valencia, V., Uriarte, A., Collins, M.B. (2009). Low-salinity plumes in the oceanic region of the Basque Country. *Continental Shelf Research*, *29*, 970–984. doi: 10.1016/j.csr.2008.12.014.

Fortier, L., Ponton, D., Gilbert, M. (1995). The match/mismatch hypothesis and the feeding success of fish larvae in ice-covered southeastern Hudson Bay. *Marine Ecology Progress Series*, *120*, 11–27. doi: 10.3354/meps120011.

Greve, W., Reiners, F., Nast, J., Hoffmann, S. (2004). Helgoland Roads meso- and macrozooplankton time-series 1974 to 2004: lessons from 30 years of single spot, high frequency sampling at the only off-shore island of the North Sea. H*elgoland Marine Research*, *58*, 274–288. doi: 10.1007/s10152-004-0191-5.

Griffith, J.R., Kadin, M., Nascimento, F.J. A., Tamelander, T., Tornroos, A., Bonaglia, S., Bonsdorff, E., Bruchert, V., Gardmark, A., Jarnstrom, M., Kotta, J., Lindegren, M., Nordström, M.C., Norkko, A., Olsson, J., Weigel, B., Žydelis, R., Blenckner, T., Niiranen, S., Winder, M. (2017). The importance of benthic-pelagic coupling for marine ecosystem functioning in a changing world. *Global Change Biology*, *23*, 2179–2196. doi:10.1111/gcb.13642.

Highfield, J. M., Eloire, D., Conway, D. V. P., Lindeque, P. K., Attrill, M. J. and Somerfield, P. J. (2010). Seasonal dynamics of meroplankton assemblages at station L4. *Journal of Plankton Research*, *32*, 681–691. doi:10.1093/plankt/fbp139.

Huskin, I., López, E., Viesca, L., Anadón, R. (2006). Seasonal variation of mesozooplankton distribution and copepod grazing in the Central Cantabrian Sea (Southern Bay of Biscay). *Scientia Marina*, *70*, 119–130. https://doi.org/10.3989/scimar.2006.70s1119.

Ikeda, T. (1985). Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. *Marine Biology*, *85*, 1–11. doi: 10.1007/BF00396409.

Iriarte, A., De Madariaga, I., Diez-Garagarza, F., Revilla, M., Orive, E. (1997). Primary plankton production, respiration and nitrification in a shallow temperate estuary during summer. *Journal of Experimental Marine Biology and Ecology*, *208*, 127–151. doi: 10.1016/S0022-0981(96)02672-X.

Iriarte, A., Aravena, G., Villate, F., Uriarte, I., Ibáñez, B., Llope, M. and Stenseth, N. C. (2010). Dissolved oxygen in contrasting estuaries of the Bay of Biscay: Effects of temperature, river discharge and chlorophyll *a*. *Marine Ecology Progress Series*, *418*, 57–71. doi:10.3354/meps08812.

Ji, R., Edwards, M., Mackas, D.L., Runge, J.A., Thomas, A.C. (2010). Marine plankton phenology and life history in a changing climate: Current research and future directions. *Journal of Plankton Research*, *32*, 1355–1368. doi: 10.1093/plankt/fbq062.

Katechakis, A., Stibor, H., (2004). Feeding selectivities of the marine cladocerans *Penilia avirostris, Podon intermedius* and *Evadne nordmanni*. *Marine Biology*, *145*, 529–539. doi: 10.1007/s00227-004-1347-1.

Kirby, R.R., Beaugrand, G., Lindley, J.A. (2008). Climate-induced effects on the meroplankton and the benthic-pelagic ecology of the North Sea. *Limnology and Oceanography*, *53*, 1805–1815. doi: 10.4319/lo.2008.53.5.1805.

Korn, O.M., Kulikova, V.A., (1995). Seasonal species composition and distribution of barnacle larvae in Avacha Inlet (Kamchatka). *Journal of Plankton Research*, *17*, 221-234. doi: 10.1093/plankt/17.2.221.

Lindley, J.A., Kirby, R.R., (2007). Long-term changes in the meroplankton of the North Sea. International Council for the Exploration of the Sea, CM 2007(A:16). ICES, Copenhagen 10 pp.

Litt, E. J., Hardman-Mountford, N. J., Blackford, J. C., Mitchelson-Jacob, G., Goodman, A., Moore, G. E., Cummings, D. G., Butenschon, M. (2010). Biological control of $p\mathrm{CO}_2$ at station L4 in the Western English Channel over 3 years. *Journal of Plankton Research*, *32*, 621-629. https://doi.org/10.1093/plankt/fbp133.

Longhurst, A. R. (1998). Ecological geography of the sea, 1st ed. Academic Press, San Diego. 398 pp.

López-Urrutia, A., Harris, R., Acuña, J., Båamstedt, U., Fyhnm, H.J., Flood. P., Gasser, B., Gorsky, G., Irigoien, X., Martinussen, M., (2005). A comparison of appendicularian seasonal cycles in four contrasting European coastal environments. In Gorsky G., Youngbluth, M., (eds.), Response of marine ecosystems to global change: Ecological impact of appendicularians, Contemporary Publishing International, Aditions Scientifiques GB. 255–276 pp.

Lorenzen, C. J. (1967). Determination of chlorophyll and phaeopigments: spectrophotometric equations. *Limnology and Oceanography*, *12*, 343–346. doi: 10.4319/lo.1967.12.2.0343.

Mackas, D. L., Beaugrand, G. (2010). Comparisons of zooplankton time series. *Journal of Marine Systems*, *79*, 286–304. doi:10.1016/j.jmarsys.2008.11.030.

Mackas, D. L., Greve, W., Edwards, M., Chiba, S., Tadokoro, K., Eloire, D., Mazzocchi, M. G., Batten, S., Richardson, A. J., Johnson, C., Head, E., Conversi, A., Peluso, T. (2012). Changing zooplankton seasonality in a changing ocean: Comparing time series of zooplankton phenology. *Progress in Oceanography*, *97–100*, 31–62. doi:10.1016/j.pocean.2011.11.005.

Martinez, E., Antoine, D., D'Ortenzio, F., de Boyer Montegut, C. (2011). Phytoplankton spring and fall blooms in the North Atlantic in the 1980s and 2000s, *Journal of Geophysical Research*, *116*, C11029, doi: 10.1029/2010JC006836.

Maud, J.L., Atkinson, A., Hirst, A.G., Lindeque, P.K., Widdicombe, C.E., Harmer, R.A., McEvoy, A.J., Cummings, D.G. (2015). How does *Calanus helgolandicus* maintain its population in a variable environment? Analysis of a 25-year time series from the English Channel. *Progress in Oceanography*, 137B: 513–523 doi: 10.1016/j.pocean.2015.04.028.

McGinty, N., Power, A.M., Johnson, M.P. (2011). Variation among Northeast Atlantic regions in the responses of zooplankton to climate change: not all areas follow the same path. J*ournal of Experimental Marine Biology and Ecology*, *400*, 120–131. doi: 10.1016/j.jembe.2011.02.013.

Menard, F., Fromentin, J. M., Goy, J. and Dallot, S. (1997). Temporal fluctuations of doliolid abundance in the bay of Villefranche-sur-Mer (Northwestern Mediterranean Sea) from 1967 to 1990. *Oceanologica. Acta*, *20*, 733–742.

Moyse, J. (1963). A comparison of the value of various flagellates and diatoms as food for barnacle larvae. *Journal du Conseil permanent international pour l'Exploration de la Mer*, *28*, 175–187. doi: 10.1093/icesjms/28.2.175.

Molvær, J., Knutzen, J., Magnusson, J., Rygg, B., Skei J., Sørensen, J (1997). Klassifisering av miljøkvalitet i fjorder og kystfarvann. Veiledning. Classification of environmental quality in fjords and coastal waters. A guide. Norwegian Pollution Control Authority. TA no. TA-1467/1997, 36 pp. ISBN 82-7655-367-2.

Olivar, M.P., Emelianov, M., Villate, F., Uriarte, I., Maynou, F., Álvarez I., Morote, E. (2010).

The role of oceanographic conditions and plankton availability in larval fish assemblages off the Catalan coast (NW Mediterranean). *Fisheries and Oceanography*, *19*, 209–229. doi:10.1111/j.1365-2419.2010.00538.x.

Pepin P., Johnson C.L., Harvey M., Casault B., Chassé J., Colbourne E.B., Galbraith P.S., Hebert D., Lazin G., Maillet G., Plourde S., Starr, M. (2015). A multivariate evaluation of environmental effects on zooplankton community structure in the western North Atlantic. *Progress in Oceanography*, *134*, 197–220. doi: 10.1016/j.pocean.2015.01.017.

Peters, R.H., Downing, J.A. (1984). Empirical analysis of zooplankton filtering and feeding rates. *Limnology and Oceanography*, *29*, 763–784. doi: 10.4319/lo.1984.29.4.0763.

Rees, A. P., Hope, S. B., Widdicombe, C. E., Dixon, J. L., Woodward, E. M. S. and Fitzsimons, M. F. (2009). Alkaline phosphatase activity in the western English Channel: Elevations induced by high summertime rainfall. *Estuarine, Coastal and Shelf Science*, *81*, 569–574. doi:10.1016/j.ecss.2008.12.005.

Racault, M.-F., Le Quéré, C., Buitenhuis, E., Sathyendranath, S., Platt, T. (2012). Phytoplankton phenology in the global ocean. *Ecological Indicators*, *14*, 152–163. doi: 10.1016/j.ecolind.2011.07.010.

Ribera d´Alcalà, M., Conversano, F., Corato, F., Licandro, P., Mangoni, O. (2004). Seasonal patterns in plankton communities in a pluriannual time series at a coastal Mediterranean site (Gulf of Naples): an attempt to discern recurrences and trends. *Scientia Marina, 68,* 65–83. doi:10.3989/scimar.2004.68s165.

Sherman, K., Celone, P., Adams, S. (2004). NOAA Fisheries Service´s Large Marine Ecosystems Program: Status Repport, NOAA Technical Memorandum NMFS-NE-183. NOAA, NEFSC.

Smith, V. H., Tilman, G. D. and Nekola, J. C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution*, *100*, 179–196. doi:10.1016/S0269-7491(99)00091-3.

Southward, A. J., Langmead, O., Hardman-Mountford, N. J., Aiken, J., Boalch, G. T., Dando, P. R., Genner, M. J., Joint, I., Kendall, M. A., Halliday, N. C., Harris, R. P., Leaper, R., Mieszkowska, N., Pingree, R. D., Richardson, A. J., Sims, D. W., Smith, T., Walne, A. W., Hawkins, S. J. (2004). Long-term oceanographic and ecological research in the western English Channel. *Advances in Marine Biology*, *47*, 1–105. doi:10.1016/S0065-2881(04)47001-1.

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., Halpern, B. S., Jorge, M. A., Lombana, A., Lourie, S. A., Martin, K. D., McManus, E., Molnar, J., Recchia, Ch. A., Robertson, J. (2007). Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. *BioScience*, *57*, 573–583. doi:10.1641/B570707.

Starr, M., Himmelman, J. H. and Therriault, J. C. (1991). Coupling of nauplii release in barnacles with phytoplankton blooms: A parallel strategy to that of spawning in urchins and mussels. *Journal of Plankton Research, 13,* 561–571. doi:10.1093/plankt/13.3.561.

Stenseth, N. C., Llope, M., Anadón, R., Ciannelli, L., Chan, K.-S., Hjermann, D. Ø., Bagøien, E. and Ottersen, G. (2006). Seasonal plankton dynamics along a cross-shelf gradient. *Proceedings of the Royal Society B, 273,* 2831–2838. doi:10.1098/rspb.2006.3658.

ter Braak, C.J.F., Šmilauer, P. (2002). CANOCO Reference manual and CanoDraw for Windows User’s guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power. 500pp. Ithaca, NY, USA.

Tomita, M., Shiga, N., Ikeda, T. (2003). Seasonal occurrence and vertical distribution of appendicularians in Toyama Bay, southern Japan Sea. *Journal of Plankton Research*, *25*, 579–589. doi: 10.1093/plankt/25.6.579.

Tönnesson, K., Tiselius, P. (2005). Diet of the chaetognaths *Sagitta setosa* and *S. elegans* in relation to prey abundance and vertical distribution. *Marine Ecology Progress Series, 289*, 177–190. doi:10.3354/meps289177.

Valdés, L., López-Urrutia, A., Cabal, J., Alvarez-Ossorio, M., Bode, A., Miranda, A., Cabanas, M., Huskin, I., Anadón, R., Alvarez-Marqués, F., Llope, M., Rodríguez, N. (2007). A decade of sampling in the Bay of Biscay: What are the zooplankton time series telling us? *Progress in Oceanography, 74*, 98–114. doi:10.1016/j.pocean.2007.04.016.

Valencia, V., Franco, J., Borja, A., Fontán, A. (2004). Hydrography of the southeastern Bay of Biscay. In Borja, A., Collins, M. (eds.), *Oceanography and marine environment of the Basque Country*. Elsevier Oceanography Series, *70*, 616 pp.

Van Ginderdeuren, K., Van Hoey, G., Vincx, M., Hostens, K. (2014). The mesozooplankton community of the Belgian shelf (North Sea). *Journal of Sea Research*, *85*, 48–58. doi: 10.1016/j.seares.2013.10.003.

Villate, F. (1991). Annual cycle of zooplankton community in the Abra Harbour (Bay of Biscay): Abundance, composition and size spectra. *Journal of Plankton Research, 13*, 691–706. doi:10.1093/plankt/13.4.691.

Villate, F., Iriarte, A., Uriarte, I., Intxausti, L., de la Sota, A. (2013). Dissolved oxygen in the rehabilitation phase of an estuary: Influence of sewage pollution abatement and hydro-climatic factors. *Marine Pollution Bulletin, 70*, 234–246.

Villate, F., Iriarte, A., Uriarte, I., Sanchez, I. (2017). Seasonal and interannual variability of mesozooplankton in two contrasting estuaries of the Bay of Biscay: relationship to environmental factors. *Journal of Sea Research, 130*, 189–203. doi: 10.1016/j.seares.2017.05.002.

Walker, S.C., Jackson, D.A., (2011). Random-effects ordination: describing and predicting multivariate correlations and co-occurrences. *Ecological Monographs*, *81*, 635–663. doi: 10.1890/11-0886.1.

Chapter 3. *Patterns and environmental drivers of zooplankton phenology changes*

Zooplankton have been suggested to be changing their phenology as a response to global warming. In this work we aimed to explore mesozooplankton phenological (timing of seasonal peak) variations during the 1999 – 2013 period from four coastal sites in the south-eastern Bay of Biscay (B35 and U35), the English Channel (L4) and northern North Sea (SH) and assess the influence of climatic indices and environmental factors such as water temperature, salinity and chlorophyll a. Overall, zooplankton phenological variability was higher at the southern, nearshore sites (B35 and U35) than at the northern more offshore sites (L4 and SH). Very few zooplankton taxa showed significant progressive advance or delay in the seasonal timing throughout the entire period of study, but overall opposite tendencies in phenology changes were detected between the southern sites (later occurrences with time) and the northernmost sites (earlier occurrences with time). At each site, taxa clusters (composition differing between sites) with similar interannual phenology patterns were found, which were generally composed of taxa that peaked in the same season. Climatic indices (mainly EA and AMO at B35 and U35; EA and NAO at SH) or water temperature (L4) showed a significant correlation with the phenological variations of most of the clusters of synchronous taxa, but in many cases, these appeared to be correlated also with phytoplankton biomass availability. In the case of the genus *Acartia*, which was dominated by the species *Acartia clausi* at all four sites, opposite tendencies of interannual variation of the seasonal timing at the southernmost and northernmost sites were observed.

***Fanjul*, A.**, *Iriarte, A., Villate, F., Uriarte, I., Atkinson, A., & Cook, K. (2018). Patterns and environmental drivers of zooplankton phenology changes.*

3.1. Introduction

Zooplankton constitute the main connection for energy transfer from primary producers to upper trophic level consumers in marine ecosystems (Richardson, 2008). The study of changes in their phenology (timing of annually recurring life cycle events) is of prime interest, since these changes can cause a decoupling in prey and predator interactions (match-mismatch hypothesis, Cushing, 1990). Zooplankton phenological changes can, therefore, affect the productivity of higher trophic level organisms, including commercially important fish (Beaugrand *et al*., 2010), although the adverse ecosystem effects of trophic mismatch may vary with system type (Atkinson *et al*., 2015). The study of changes in the phenology of zooplankton is nowadays receiving much attention (Thackeray *et al*., 2012; Usov *et al*., 2013; Marques *et al*., 2014; Atkinson *et al*., 2015; Post, 2017) because it is one of the potential consequences of global climate change (IPCC, 2015). It is acknowledged that, in general, in a warming climate spring processes tend to occur earlier and autumn processes shift later (Richardson, 2008; Atkinson *et al*., 2015).

Marine planktonic communities are believed to be quite sensitive to climatic changes (Edwards and Richardson, 2008) and phenological advances in zooplankton taxa associated to warming have been reported both for freshwater (Adrian *et al*., 2006; Thackery *et al*., 2012) and marine (Greve *et al*., 2001, 2005; Edwards and Richardson, 2004; Molinero *et al*., 2005; Conversi *et al*., 2009; Mackas *et al*., 2012; Usov *et al*., 2013; Atkinson *et al*., 2015; Reygondeau *et al*., 2015) ecosystems. These works, which cover different time-periods, have shown slightly different results, though. For example, Edwards and Richardson (2004) found that zooplankton taxa from the central North Sea that peak from May to August had almost all significantly advanced in their seasonality in response to warming from 1958 to 2002. Atkinson *et al*. (2015) only observed a weak tendency for earlier occurrences of spring zooplankton taxa and later ones for autumn taxa during warm years in the western English Channel during the period 1988 – 2012. Usov *et al*. (2013) concluded that in the White Sea the cold water *Calanus glacialis* showed an advance in its reproduction from 1961 to 2010 due to warming and the associated longer phytoplankton bloom, but warm water copepods had not changed their seasonality significantly.

It is noteworthy that, despite the relevance of the topic, there are still too few comparative cross-regional studies (e.g. Mackas *et al*., 2012), thus lacking a comprehensive view of zooplankton phenological changes. As a consequence, there are many key questions that have not been fully resolved yet. In this regard, it would be interesting to know if zooplankton are giving the same (synchronized) or different phenological responses across regions and which are the factors (e.g. latitude) that are responsible for the differential response (if this was the case). Likewise, it would be essential to know if most zooplankton taxa or just a few very sensitive taxa are the ones responding to climatic variations through phenological adjustments, and if there is consistency between sites. Another critical question would be if zooplankton phenological variations are governed by the same factors across regions and if the phenological response to a given environmental factor is the same at different sites.

The present study aims to contribute to answering to these questions by assessing the phenological changes of zooplankton and their driving forces across Northeast Atlantic shelf waters. For this purpose, zooplankton time series from four ICES monitoring sites (O'Brien *et al.*, 2013) located along a latitudinal gradient (Stonehaven (North Sea), Plymouth L4 (Western English Channel) and Urdaibai 35 and Bilbao 35 (Bay of Biscay) have been analyzed using the same time-window (1999 – 2013) and taxonomic resolution level.

3.2. Methods

3.2.1. Data preparation

For the analysis of the relationships between environmental variables (water temperature, chlorophyll *a* and salinity), climatic indices (NAO, EA and AMO) and phenological indices, both annual and seasonal (winter: January, February and March; spring: April, May and June; summer: July, August and September; autumn: October, November and December) means were used for the former.

3.2.2. Data analysis

3.2.2.1. Interannual trends in environmental variables and relationships between them

Annual mean values for each of the environmental variables (Chl *a*, WT, Sal and the indices AMO, NAO and EA) were calculated and tested for interannual trends using Spearman rank correlation analyses. Principal components analysis (PCA) was performed to identify correlations between environmental variables using *prcomp* function in R (v.2.3 – 4). Environmental variables were centred and scaled. Plots were created using *factoextra* package.

3.2.2.2. Phenological index

The timing of the seasonal peak also known as "central tendency" or " T index", which has been widely utilized for zooplankton (Chivers *et al.*, 2017; McGinty *et al.*, 2011; Conversi *et al.*, 2009; Edwards & Richardson, 2004), was used as phenological index. This index was calculated for every taxa and environmental variable using the following equation:

$$T = \frac{\sum_{i=1}^{i=12} D_i \cdot X_i}{\sum_{i=1}^{i=12} X_i}$$

where **T** is the day of the year of the seasonal peak, *i* is the sampling month, D_i is the day of the year of sampling (1–365) and X_i is the abundance at the corresponding month. For further analysis, T anomalies for each taxon were calculated as the T value of each year minus the mean value for the fifteen-year study period. At L4 and SH, the seasonal cycles of *Corycaeus* and *Oncaea* were frequently prolonged over to the following year (see Chapter 1). For those months in the following year, to obtain D, a value of 365 was added to the sampling day of the year, and those months were then removed from the calculations of sampling days of the subsequent year.

3.2.2.3. Differences between sites in T index anomalies

Analyses of similarity were carried out with the ANOSIM function (499 permutations; *vegan* (v.2.3 – 4) R package) to test whether differences in T index anomalies of zooplankton taxa between sites (B35, U35, L4 and SH) were statistically significant. In addition, we tested if there were differences between north (N; L4 and SH) and south (S; B35 and U35) sites.

3.2.2.4. Linear trends of T index

Spearman's rank correlation analyses between T index anomalies and years were used to test for significant linear trends in the T index of zooplankton taxa and environmental variables.

3.2.2.5. Coherence between the T index patterns of different zooplankton taxa at each site

For each cluster of the zooplankton taxa with similar interannual patterns in their T index anomalies, identified from the resemblance analysis, β diversity matrices were calculated using their T anomalies. ADONIS (bio-env method of *vegan* (v.2.3 – 4) R package; 499 permutations), which is a variance analysis using distance matrices, was used to test which environmental variables were significantly correlated to the T index anomalies of zooplankton taxa from each cluster. The combination of variables that explained the largest percentage of the T index variability was then selected.

Additionally, Spearman rank correlation analyses were performed between individual taxa T indices and environmental variables.

3.3. Results

3.3.1. Interannual variations of environmental variables

Interannual patterns of environmental variables plus their annual standard deviation are plotted in **Figures 1** and **2**. LOESS fitting to those values was plotted in order to show the path of variation between years. No linear trend of increase in water temperature was observed at any of the sites under study. At B35 mean annual Chl *a* showed a trend of decrease (r= 0.732; *p*= 0.002) due particularly to the decrease in summer Chl *a* (r= 0.721; *p*= 0.002). At both, B35 and U35 an increase in salinity was detected, in summer at B35 (r= 0.657; *p*= 0.008) and in winter (r= 0.665; *p*= 0.007) and with mean annual values (r= 0.518; *p*= 0.048) at U35. At SH Chl *a* showed significant trend of increase in autumn (r= 0.564; *p*= 0.028) and winter (r= 0.614; *p*= 0.015). At L4 the environmental factors tested showed no significant linear trends of variation.

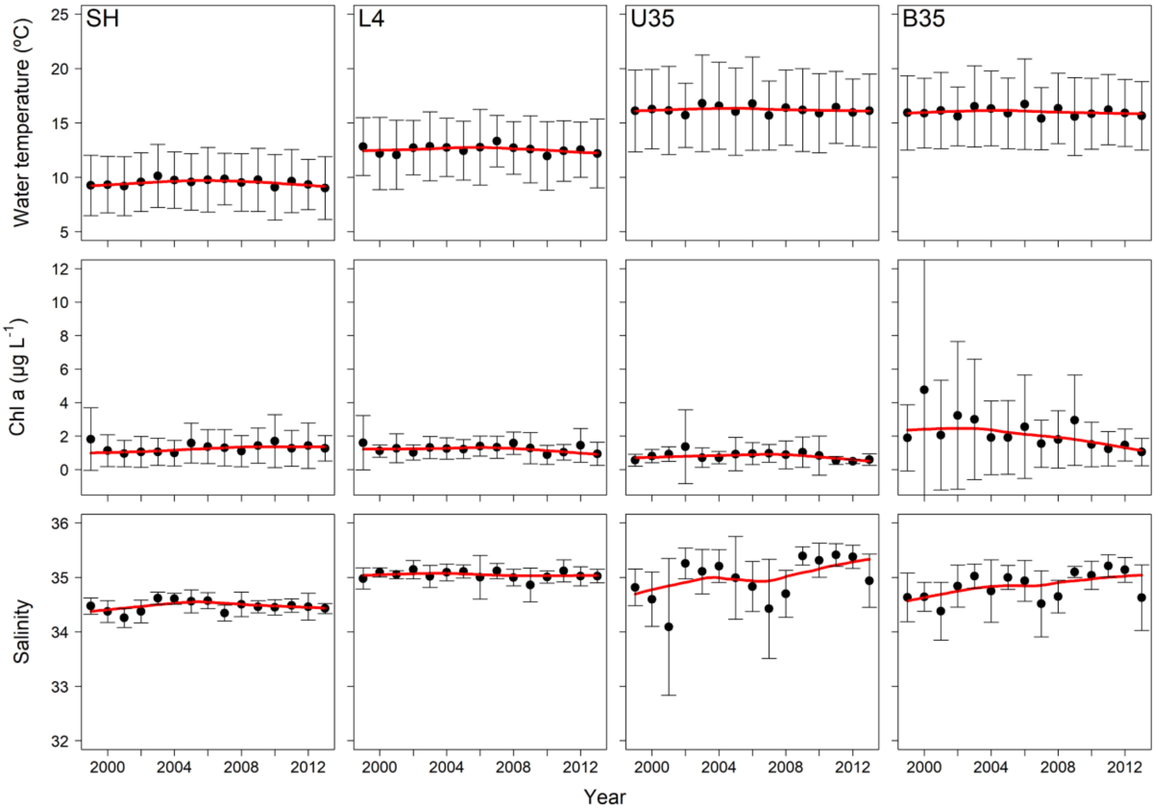

Figure 1. Annual mean ± standard deviation of water temperature, chlorophyll *a* and salinity. LOESS (thick red line) was fitted to the annual mean values to show the path of interannual change.

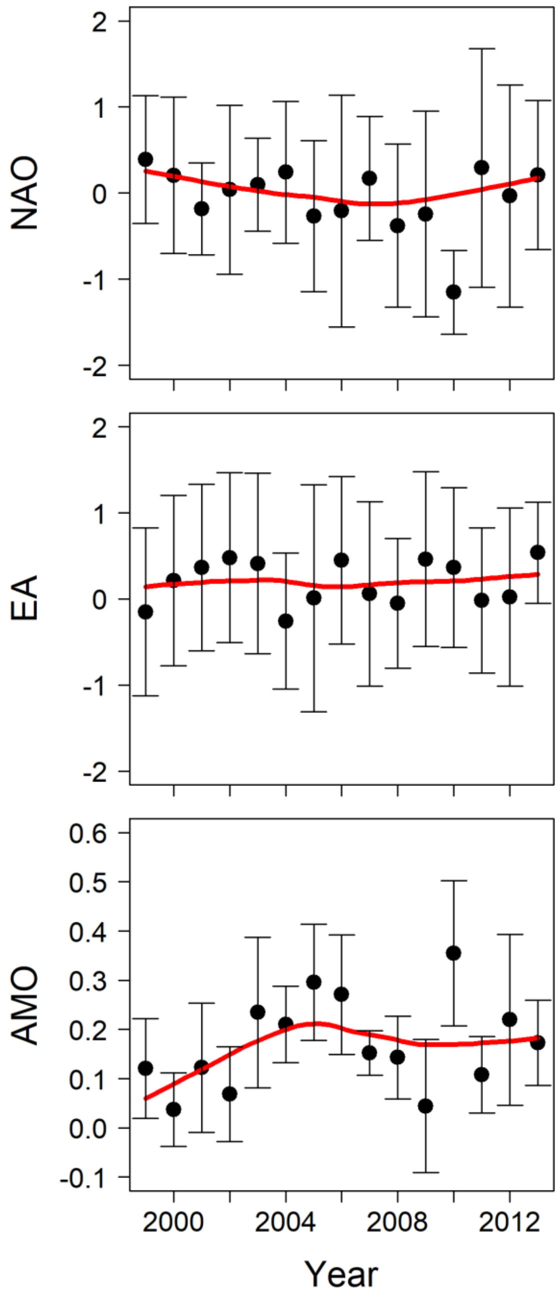

Figure 2. Annual mean ± standard deviation of NAO, EA and AMO indices. LOESS (thick red line) was fitted to the mean annual values to show the path of interannual change.

3.3.2. Magnitude of the interannual variability of zooplankton T index between sites

The magnitude of the interannual variability of the T index was compared between sites for broad taxonomic groups of non-copepods and non-cladoceran holoplankton and meroplankton and the main cladoceran and copepod genera (**Figure 3**). Overall, interannual variability decreased with latitude from B35 and U35 (the highest variability being at U35) to SH for broad taxonomic groups of non-copepods and non-cladoceran holoplankton and meroplankton. For the main copepod and cladoceran genera between site differences were not so clear, especially for cladocerans which comprise only a few genera.

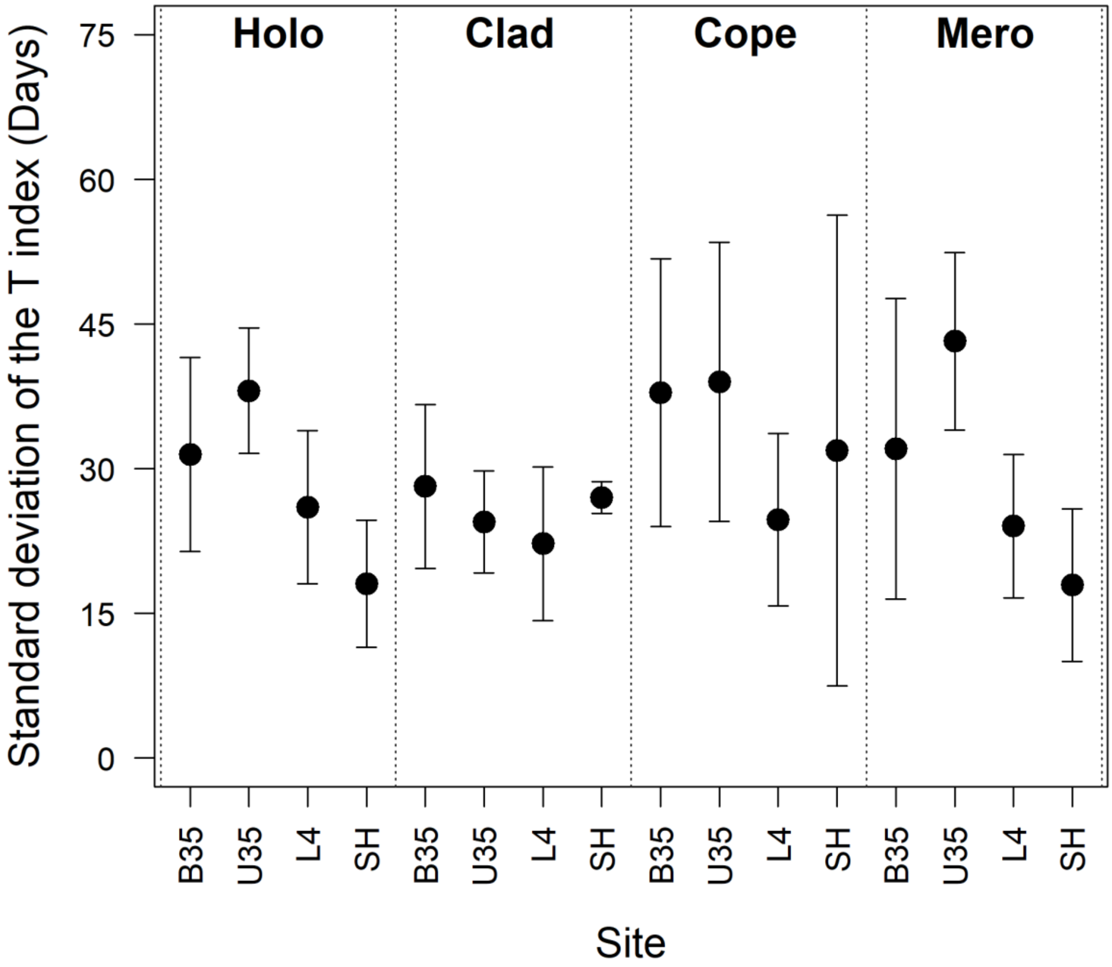

Figure 3. Standard deviation of the T index (mean ± standard deviation) for each of the following zooplankton taxa groups: non-copepod and non-cladoceran holoplankton (Holo); main cladoceran genera (Clad); main copepod genera (Cope) and meroplankton (Mero) at each of the sites under study.

3.3.3. Patterns of interannual variation of zooplankton T index and their environmental drivers

According to the ANOSIM test (**Table 1**) there were no significant differences in zooplankton taxa T index anomalies between the two southernmost sites (U35 and B35) nor between the two northernmost sites (L4 and SH). However, there were significant differences (*p*= 0.001) between the southernmost sites (U35 – B35) and the northernmost ones (L4 – SH) and also between U35 and L4 (*p*= 0.024) and U35 and SH (*p*= 0.02).

Table 1. ANOSIM test results for differences between sites in zooplankton T index.

Sites	Test statistic	*p*
All sites	0,009	0,294
B35 vs U35	-0,180	0,965
B35 vs L4	0,018	0,252
B35 vs SH	0,024	0,196
U35 vs L4	0,059	**0,024**
U35 vs SH	0,062	**0,020**
L4 vs SH	0,010	0,387
B35 & U35 vs L4 & SH	0,083	**0,001**

Only few taxa showed a significant unidirectional (linear) trend of variation in their T index. At B35 chaetognaths (r= 0.686, *p*= 0.005)) and decapod larvae (r= 0.643, *p*= 0.01) showed a significant increase in the T index along the study period. Both taxa peaked in summer and their T index was negatively correlated to summer Chl *a* (r= -0.554, *p*= 0.032 for chaetognaths; r= -0.761, *p*= 0.001 for decapod larvae). At U35, the seasonal peak of two spring taxa showed a significant tendency for a delay during the study period, i.e. *Podon* (r= 0.55, *p*= 0.034) and appendicularians (r= 0.621, *p*= 0.013). Salinity was the environmental variable correlated with both taxa T index (salinity T index for *Podon* (r= 0.750, *p*= 0.001) and summer salinity for appendicularians (r= 0.789, *p*< 0.001)). Only one taxon T anomaly evidenced a significant negative correlation with year, and this was *Evadne* (r= -0.543, *p*= 0.037) at SH. This cladocerans peaked during the summer and their T index was positively correlated to the timing of the seasonal peak of Chl *a* (r= 0.639, *p*= 0.01). At L4 site no taxa T index showed a significant unidirectional trend of variation during the study period.

The coherence in the interannual patterns of zooplankton taxa T index from the same site is shown in **Figure 4**. The threshold distance to select the clusters or taxa groups varied between sites and depended on the observed distance values between taxa (higher at B35 and U35 and lower at L4 and SH). The highest coherence between zooplankton taxa T index was observed at SH and the lowest one at U35.

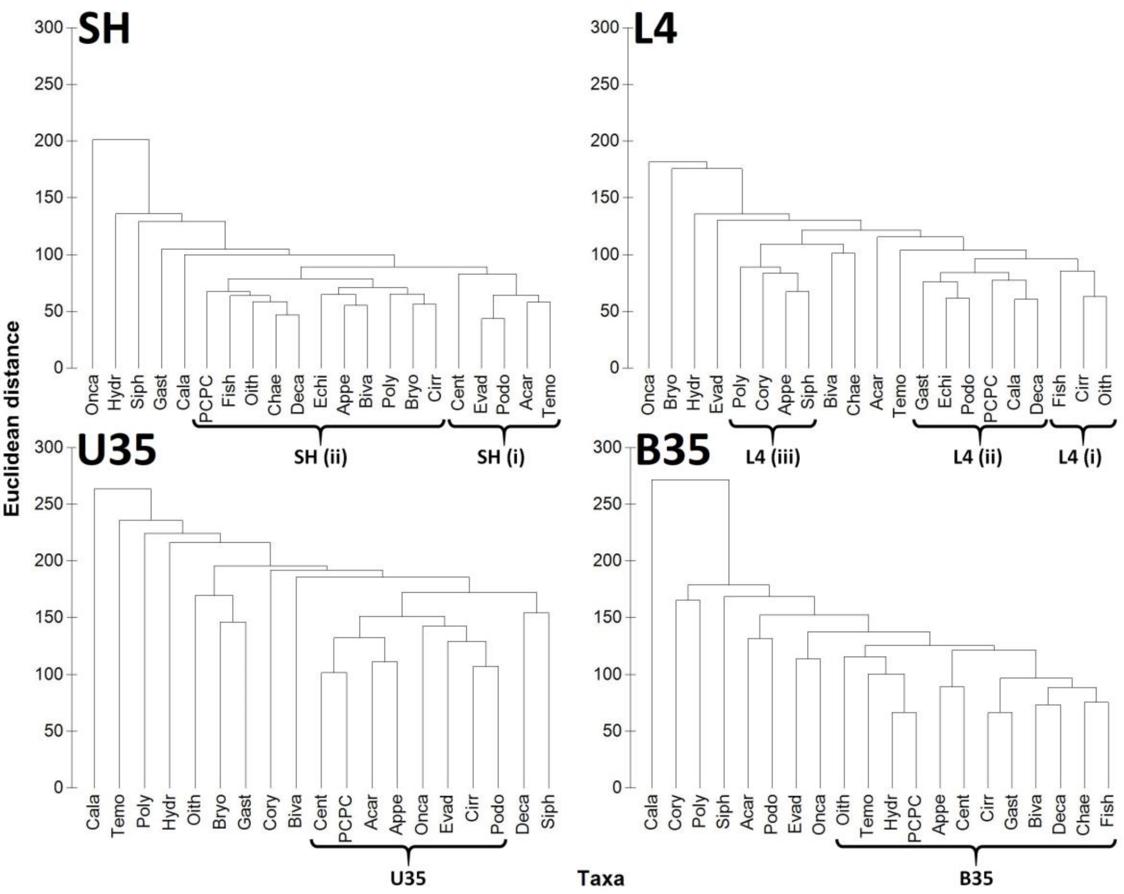

Figure 4. Dendrograms based on Euclidean distance similarities of zooplankton taxa T index anomalies at each of the sites under study (SH, L4, U35 and B35). Clusters of taxa with highest similarity are indicated (SH (i); SH (ii); L4 (i); L4 (ii); L4 (iii); U35 and B35). Chae (chaetognaths), Appe (appendicularians), Doli (doliolids), Siph (siphonophores), Acar (*Acartia* sp.), Cala (Calanidae), PCPC (PCPC-calanus), Cory (*Corycaeus* sp.), Onca (*Oncaea* sp.), Temo (*Temora* sp.), Cent (*Centropages* sp.), Evad (*Evadne* sp.), Podo (*Podon* sp.), Cirr (cirripede larvae), Echi (echinoderm larvae), Fish (fish egg and larvae), Deca (decapod larvae), Bryo (bryozoan larvae), Medu (hydromedusae), Gast (gastropod larvae), Biva (bivalve larvae).

The combinations of environmental variables with the highest correlation (Spearman’s rank) with the T index of zooplankton taxa from each of the clusters selected according to their similarity have been shown in **Table 3** and the results from the PCA showing relationships between the environmental factors can be found in **Figure 5**. The patterns of year-to-year variation in the T index of zooplankton taxa (individual taxa and group average patterns) from each cluster and the environmental factors that best correlated (see **Table 3**) have been depicted in **Figures 6**, **7**, **8** and **9**.

Table 3. Combinations of environmental variables with the highest correlation with the T index of zooplankton taxa from each of the clusters selected according to their similarity. (Wi = winter, Sp = spring, Su = summer, T = T index anomaly).

Group	Variables	Correlation factor
B35	Sp EA	0.051
U35	Wi AMO, Su Sal, T AMO	0.509
L4 (i)	Wi Chl *a*, Su WT	0.381
L4 (ii)	Wi WT, Sp WT, Su Chl *a*	0.304
L4 (iii)	Wi EA, Su Sal	0.072
SH (i)	Sp EA, Su EA, NAO	0.486
SH (ii)	Sp Chl *a*, Sp EA, Sp Sal, Su Sal	0.506

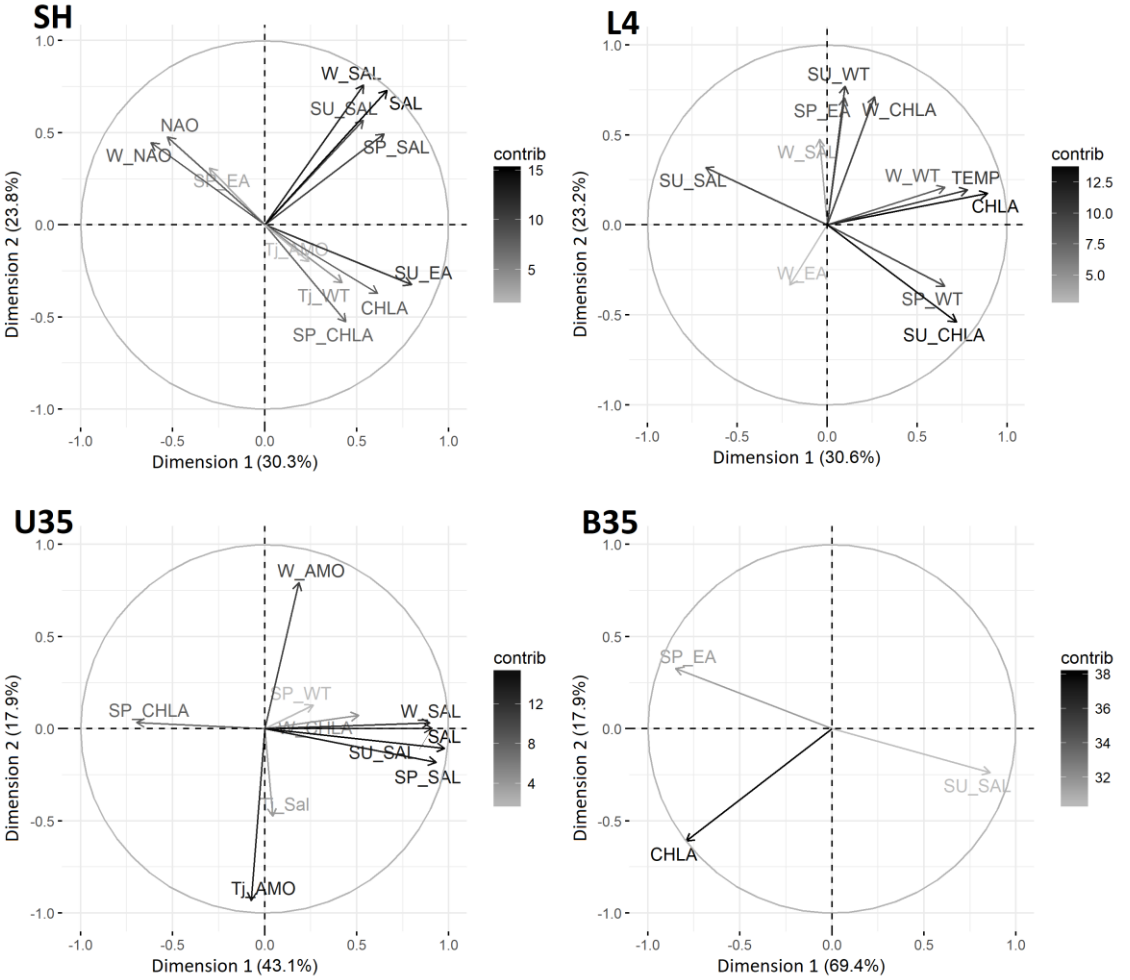

Figure 5. PCA of environmental variables at SH (A), L4 (B), U35 (C) and B35 (D). The darker the arrow and name of a variable plus the longer the vector, the higher the contribution to an axis. Also, small angles between variables denote close relationship. The variables used were the climatic indices EA, NAO and AMO, and the environmental variables measured *in situ*: salinity (SAL), water temperature (TEMP for the annual mean and WT for seasonal means) and Chlorophyll *a* (CHLA). T stands for T index anomaly, W for winter, SU for summer, SP for spring; when no season is specified the value corresponds to the annual mean.

The B35 taxa cluster was comprised of appendicularians, bivalve larvae, hydromedusae, gastropod larvae, cirripede larvae, decapod larvae, *Centropages*, *Temora*, PCPC-calanus, *Oithona*, chaetognaths and fish eggs and larvae (**Figure 4**). All these were summer taxa, except for hydromedusae, which were abundant during summer, but reached their annual maxima in spring and PCPC-calanus, *Oithona* and *Temora* that peaked in early autumn (see Chapter 1, this work). The average interannual pattern of T index did not show a significant unidirectional trend of variation, but the overall tendency for most of the period was of an increase with a decrease towards the end of the study period (**Figure 6**). The environmental factor that was selected as explanatory variable was spring EA index (**Table 3**) and spring EA index was negatively correlated with summer salinity (**Figure 5**).

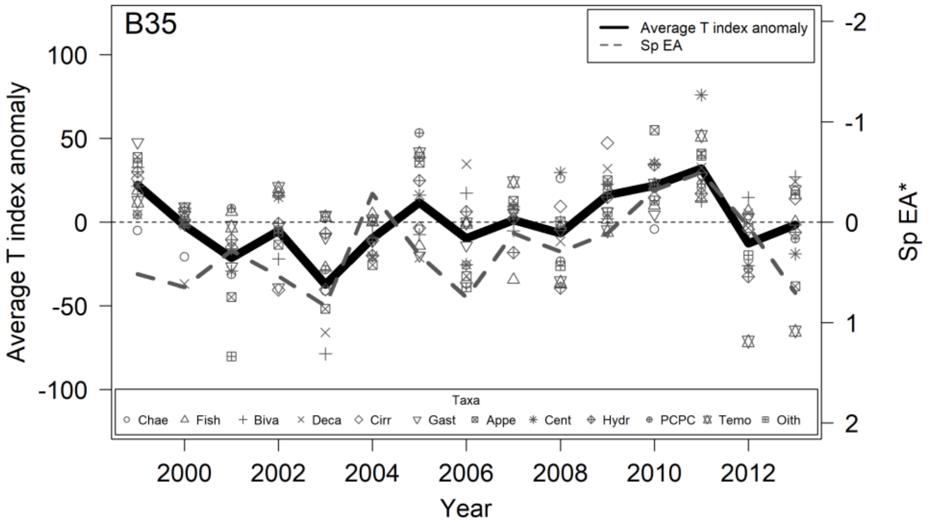

Figure 6. Interannual pattern of T index anomalies for individual taxa (symbols) and for the average pattern (continuous black line) of the zooplankton taxa cluster identified with the similarity analysis at B35 and the environmental variable that best correlated with it, i.e. spring EA (Sp EA). The asterisk (*) stands for reversed axis values, used because of the negative correlation with the group average zooplankton T index anomalies. Taxa code: Chae (chaetognaths), Fish (fish eggs and larvae), Biva (bivalve larvae), Deca (decapod larvae), Cirr (cirripede larvae), Gast (gastropod larvae), Appe (appendicularians), Cent (*Centropages*), Hydr (hydromedusae), PCPC (PCPC-calanus), Temo (*Temora*) and Oith (*Oithona*).

The U35 taxa cluster was comprised of cirripede larvae, appendicularians, *Evadne*, *Podon*, *Oncaea*, *Acartia*, *Centropages* and PCPC-calanus (**Figure 4**). *Centropages* and PCPC-calanus peaked during summer, *Oncaea* in autumn, and the rest were early spring taxa (see Chapter 1). As for the rest of clusters that were identified, the average interannual pattern of T index for this taxa cluster did not show a significant unidirectional trend of variation. As for the B35 cluster pattern, the overall tendency for most of the period was of an increase, with a decrease towards the end of the study period (**Figure 7**). The combination of environmental factors that best correlated with the T index of these taxa were winter AMO, summer salinity and T index of AMO

(**Table 3**). Salinities of different seasons were highly correlated and spring salinity was negatively correlated (p= 0.023) with spring chlorophyll *a* concentration (**Figure 5**).

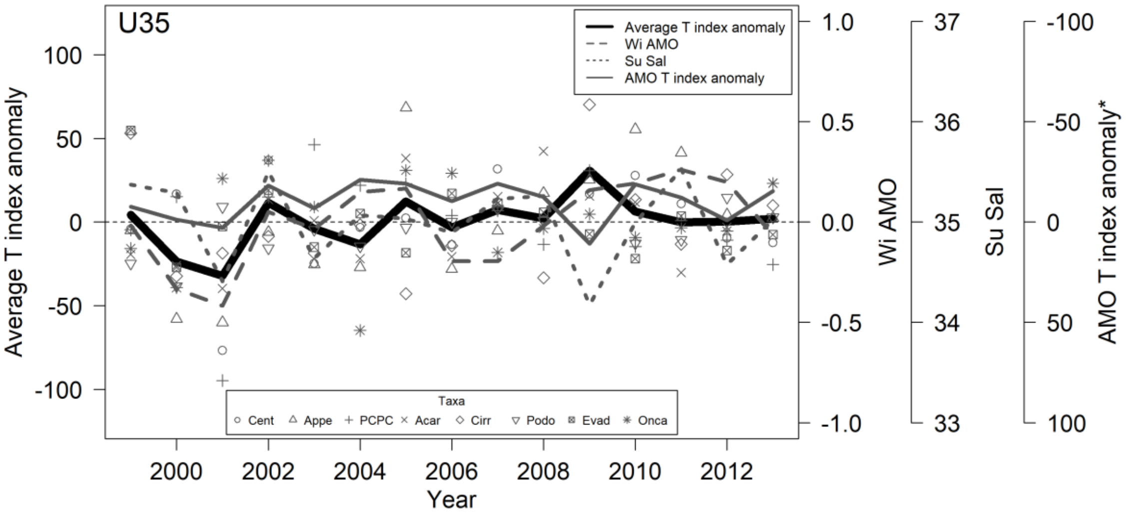

Figure 7. Interannual pattern of T index anomalies for individual taxa (symbols) and continuous for the average pattern (black line) of the zooplankton taxa cluster identified with the similarity analysis at U35 and the environmental variables that best correlated with it. Winter AMO (Wi AMO), summer salinity (Su Sal) and the AMO T index anomaly. The asterisk (*) stands for reversed axis values, used for environmental variables with negative correlation with the group average zooplankton T index anomalies. Taxa code: Cent (*Centropages*), Appe (appendicularians), PCPC (PCPC-calanus), Acar (*Acartia*), Cirr (cirripede larvae), Podo (*Podon*), Evad (*Evadne*) and Onca (*Oncaea*).

The three clusters distinguished at L4 (**Figure 8**) included the following taxa: (i) cirripede larvae, fish eggs and larvae and *Oithona*, which were all spring peaking taxa (see Chapter 1); (ii) Calanidae, *Podon*, PCPC-calanus, gastropod larvae, echinoderm larvae and decapod larvae, all of them summer peaking taxa, except for PCPC-calanus that showed two peaks, but the larger one in spring (see Chapter 1); and (iii) appendicularians, *Corycaeus*, polychaete larvae and siphonophores. These latter taxa cluster was rather heterogeneous with taxa peaking in spring (polychaete larvae and appendicularians), summer (siphonophores) and autumn (*Corycaeus*; see Chapter 1). No significant unidirectional patterns for any of the three clusters were observed and the patterns were less clear than for the taxa clusters identified at the rest of sites, smallest changes been observed for L4 (ii) cluster (**Figure 8**). Water temperature in summer or winter-spring and chlorophyll *a* in summer or winter appeared as the main environmental factors correlated with the T index of taxa that belonged to L4 (i) and L4 (ii) clusters (**Table 3**). For L4 (iii) cluster the combination of variables selected was winter EA and summer salinity (**Table 3**).

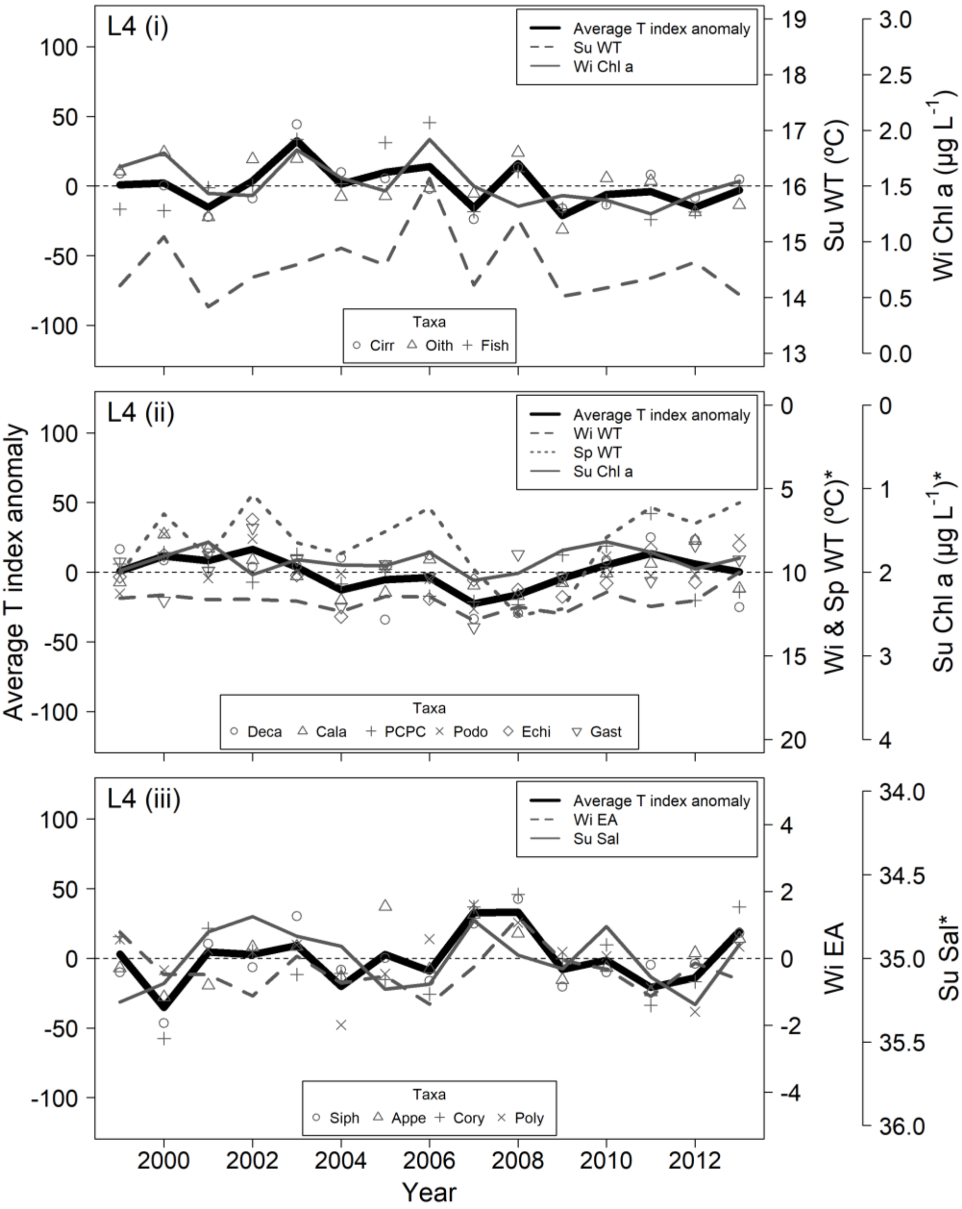

Figure 8. Interannual patterns of T index anomalies for individual taxa (symbols) and for the average pattern (continuous black line) of each of the three zooplankton taxa clusters identified with the similarity analysis at L4 (L4 (i) (top panel), L4 (ii) (middle panel) and L4 (iii) (bottom panel)) and the environmental variables that best correlated with them. Top panel: summer WT (Su WT) and winter chlorophyll *a* (Wi Chl a). Middle panel: winter water temperature (Wi WT), spring water temperature (Sp WT) and summer chlorophyll *a* (Su Chl a). Bottom panel: winter EA (Wi EA) and summer salinidad (Su Sal). The asterisk (*) stands for reversed axis values, used for environmental variables with negative correlation with the group average zooplankton T index anomalies. Taxa code: Cirr (cirripede larvae), Oith (*Oithona*), Fish (fish eggs and larvae), Deca (decapod larvae), Cala (Calanidae), PCPC (PCPC-calanus), Podo (*Podon*), Echi (echinoderm larvae), Gast (gastropod larvae), Siph (siphonophores), Appe (appendicularians), Cory (*Corycaeus*) and Poly (polychaete larvae).

At SH the two zooplankton taxa clusters with the highest similarity in their T indices (**Figure 9**) were constituted by (i) *Evadne*, *Acartia*, *Podon*, *Temora* and *Centropages*, all summer taxa; and (ii) fish eggs and larvae, bryozoan larvae, cirripede larvae, echinoderm larvae, PCPC-calanus and bivalve larvae, which peak in spring, and polychaete larvae, decapod larvae. chaetognaths, *Oithona* and appendicularians, which peak in summer (see Chapter 1). Although, as for the rest of clusters, the average patterns of the T index of each of these clusters did not show significant linear trends of variation, there was an overall tendency for a decline (advance in timing), except for the last three years in which an increase was observed in both SH cluster patterns, in contrast to the B35 and U35 cluster patterns. Year-to-year changes were more marked for the SH (i) than for the SH (ii) cluster (**Figure 9**). For the SH (i) taxa cluster spring and summer EA indices and the mean annual NAO index (which was, in turn, well correlated with winter NAO index (**Figure 5**)) was the combination of environmental factors with the highest correlation with their T index (**Table 3**). For the SH (ii) cluster spring chlorophyll *a*, spring EA index, spring salinity and summer salinity was the combination of explanatory variables selected (**Table 3**). Summer EA index, in turn was well correlated with mean annual chlorophyll *a* concentration and spring chlorophyll *a* concentration at SH (**Figure 5**).

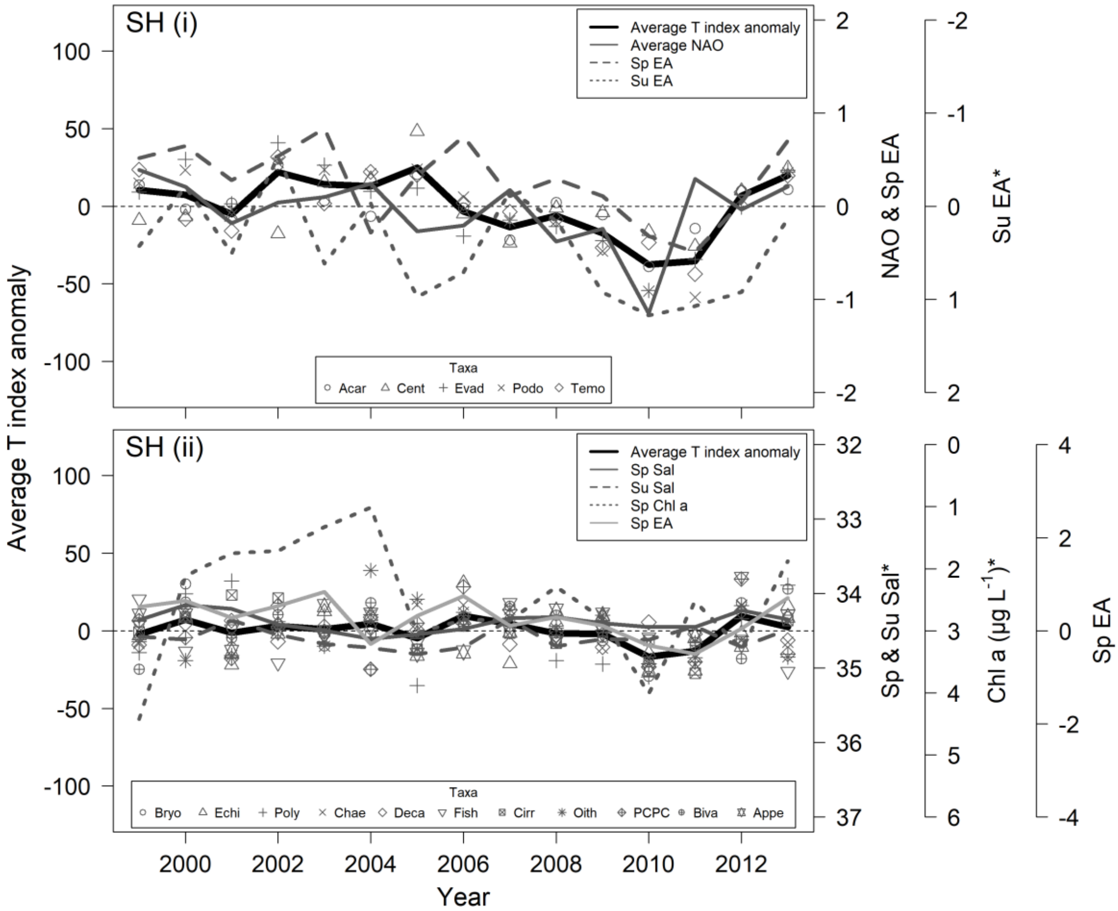

Figure 9. Interannual patterns of T index anomalies for individual taxa (symbols) and for the average pattern (continuous black line) of each of the two zooplankton taxa clusters identified with the similarity analysis at SH (SH (i) (top panel) and SH (ii) (bottom panel)) and the environmental variables that best correlated with them. Top panel: mean annual NAO (Average NAO), spring EA (Sp EA) and summer EA (Su EA). Bottom panel: spring salinity (Sp Sal), summer salinity (Su Sal), spring chlorophyll *a* (Sp Chl a) and spring EA (Sp EA). The asterisk

(*) stands for reversed axis values, used for environmental variables with negative correlation with the group average zooplankton T index. Taxa code: Acar (*Acartia*), Cent (*Centropages*), Evad (*Evadne*), Podo (*Podon*), Temo (*Temora*), Bryo (Bryozoan larvae), Echi (echinoderm larvae), Poly (polychaete larvae). Chae (chaetognaths), Deca (decapod larvae), Fish (fish eggs and larvae), Cirr (cirripede larvae), Oith (*Oithona*), PCPC (PCPC-calanus), Biva (bivalve larvae) and Appe (appendicularians).

Significant positive correlations were observed between the average T index values of the L4 (i) and SH (i) clusters, as well as between those of the B35 and U35 clusters, but the best correlation was found between the average T index values of the B35 and SH (ii) taxa clusters and it was negative (**Table 4**), further evidencing the north-south differences.

Table 4. Results of Spearman's rank correlation tests between the average patterns of T index variations of the zooplankton taxa clusters.

		SH (i)	SH (ii)	L4 (i)	L4 (ii)	L4 (iii)	U35
SH (ii)	r	0,286					
	p	0,302					
L4 (i)	r	0,546	0,139				
	p	**0,035**	0,621				
L4 (ii)	r	0,029	-0,043	-0,054			
	p	0,919	0,879	0,850			
L4 (iii)	r	0,121	-0,182	0,100	-0,407		
	p	0,666	0,516	0,723	0,132		
U35	r	-0,025	-0,421	-0,193	-0,300	0,279	
	p	0,930	0,118	0,491	0,277	0,315	
B35	r	-0,314	-0,654	-0,339	-0,018	-0,143	0,579
	p	0,254	**0,008**	0,216	0,950	0,612	**0,024**

3.3.4. Magnitude of interannual variation of zooplankton T index by sites and seasons

Although linear regression fits of T index versus year were non-significant (*p*> 0.05) for most taxa, regression equations were used to obtain rough estimates of change in T index during the study period, i.e. from 1999 to 2013. Changes in the seasonal timing ranged from -45 days (lead) to +67 days (lag). These estimates also showed that most zooplankton taxa tended to advance the timing of their seasonal peak (T index) at the northern sites (17 taxa out of 23 at SH and 14 out of 23 at L4), whereas at the southern sites, for most taxa (14 taxa out of 23 at U35 and 16 taxa out of 23 at B35), the seasonal peak tended to occur later along the study period (**Figure 10**). These differences between north and south were clearest for taxa that peaked earlier. Copepods, on average, showed a delay in their T index of 21 days at B35 and U35, and an advance of 9 days at L4 and SH, being the only group of taxa that showed statistically significant differences between sites (Kruskal Wallis test, *p*= 0.007) in the magnitude of seasonal change from 1999 to 2013. Overall, largest changes in the T index were observed for taxa from U35 (mean change of 33 and

23 days in taxa that delayed and advanced their seasonal timings, respectively) and smallest ones for taxa from SH (mean change of 9 and 17 days in taxa that delayed and advanced their seasonal timings, respectively). Within each site there were not statistically significant differences (*p*> 0.05) in T index between zooplankton groups (copepods, cladocerans, other holozooplankton and meroplankton), in the year (springtime T index value) and for copepods.

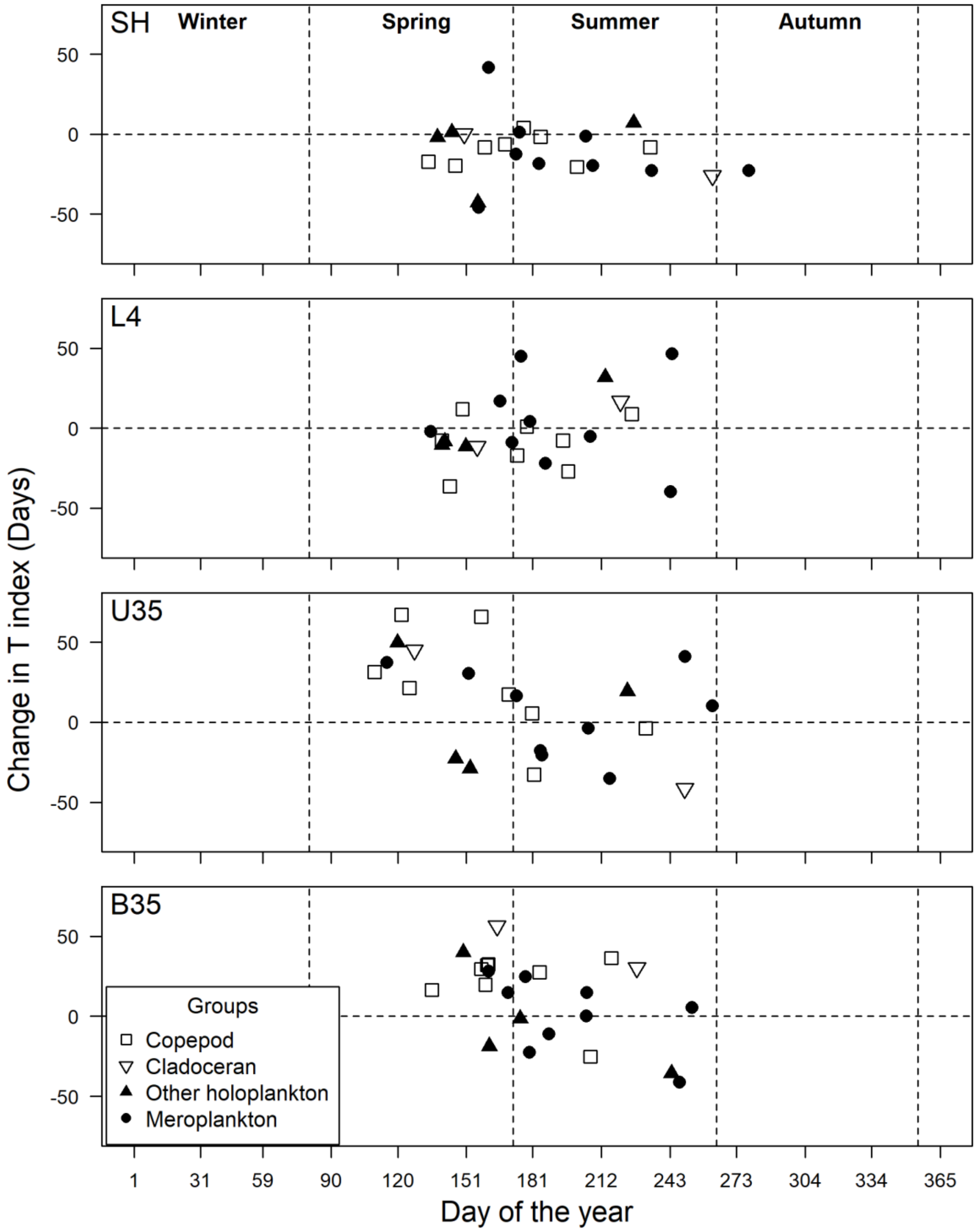

Figure 10. Change in the T index from 1999 to 2013 for copepod and cladoceran genera and other holoplankton and meroplankton groups at each of the sites under study.

At U35, and to a less extent at B35, the major T index delays were observed in taxa that peaked earliest in the year, in spring, whereas the major advances were for summer taxa. This phonological behaviour was more evident for copepod and cladoceran genera at U35 but for other holoplanktonic and meroplanktonic groups at B35. In contrast with the southern sites of U35 and B35, summer taxa showed most evident delays at L4, whereas no clear differences in the delay or advance of taxa were observed at SH between seasons.

3.4. Discussion

In The magnitude of the year-to-year variability of the phenological T index (as shown by values of standard deviation) was, on average, higher at the southeastern Bay of Biscay sites (B35 and U35, highest at the latter site) than at the English Channel (L4) and North Sea (SH) sites (lowest at the latter site). The reason behind these differences between sites is likely the increase in the year-to-year regularity of environmental factors such as water temperature and chlorophyll *a* concentration from U35 and B35 to L4 and SH (See Chapter 2). This increase in regularity may be partly a latitudinal effect, but it is likely related also to differences in depth and distance offshore. Both B35 and U35 are shallow nearshore sites and are more affected by anthropogenic perturbations and short-lived high frequency hydrological events occurring at the land-ocean interface, which promote a higher residual variability of plankton abundance (Cloern and Jassby, 2010) than at deeper and further offshore sites such as L4 and SH (Chapter 1, this work). Also, phenological variability was on average higher at U35 than at B35, at least at the coarse zooplankton taxonomic level, and this may be related to a lower year-to-year variability of the seasonal pattern of chlorophyll *a* at the mesotrophic B35 site (Chapter 2, this work) and also to the fact that, owing largely to the influence of an estuarine plume that prevents summer depletion of nutrients and phytoplankton biomass, the seasonal patterns of many zooplankton taxa tend to be unimodal at B35, in contrast to being bimodal at U35, following the bimodal pattern of chlorophyll *a* concentration (spring (primary) and autumn (secondary) peaks) at this latter site (Villate *et al*., 2017). This north *vs* south and B35 *vs* U35 differences were clearer at the broad taxonomic group level for both holoplankton and meroplankton than at the copepod and cladoceran genera level.

Advances in zooplankton seasonal timing as a response to warming during the last decades have been reported from freshwater (Adrian *et al*., 2006; Thackeray *et al*., 2012) and marine ecosystems (Edwards and Richardson, 2004; Molinero *et al*., 2005; Conversi *et al*., 2009; Atkinson *et al*., 2015; Reygondeau *et al*., 2015). But in some of these studies few taxa showed statistically significant linear trends in seasonal timing (Conversi *et al*., 2009; Atkinson *et al*., 2015), as was the case in the present study, in which almost no zooplankton taxa showed significant linear trends of phenological advance. In agreement, during the present study period (1999 – 2013) no significant trend in water temperature increase was observed at any of the sites under study. In fact, during this time period globally there has been a reduction in radiative forcing and a cooling due to natural internal climate variability, as compared to the period 1952 to 2012 (IPCC, 2015). Therefore, given the non-homogeneous pace of climate change (IPCC, 2015), the time-window of analysis appears critical for the observation of trends in phenology variation. However, even though very few zooplankton taxa showed significant unidirectional trends of phenological variation during our study period, many of them exhibited measurable variations, thus showing that most zooplankton can give a fast response in terms of seasonal adjustments to year-to-year environmental changes (Richardson 2008; Rice and Stewart, 2016).

There were no significant differences in the magnitude of phenological change between copepod genera, cladoceran genera, broad taxonomic groups of other holoplankton and meroplankton within each site. In contrast to these findings, Edwards and Richardson (2004) observed that during the 1958 – 2002 period in the North Sea meroplankton had larger advances in their seasonal timing than copepods and non-copepod holoplankton. Also, although spring taxa showed lowest within-site variability in phenological change, in the present study they were the ones that showed highest between-site differences in phenological index changes. Accordingly, Morse *et al*. (2017) found that the abundance response of spring zooplankton communities was less similar than for fall communities among ecoregions from the Northeast continental shelf Large Marine Ecosystem. However, due to the lack of significant unidirectional trends in T index for most zooplankton taxa, the values of change in the timing of the seasonal peak (from the beginning to the end of the study period) obtained in the present work have to be considered as rough estimates and, therefore, they should be taken with some caution. In spite of this, the values of changes in T index have been useful to obtain a general view of zooplankton phenology patterns of variation, showing that zooplankton phenology has had opposite overall tendencies from the southern sites to the northern ones during the 1999 – 2013 period, most taxa seasonal peaks tending to occur earlier at L4 and SH (predominantly at SH) and later at B35 and U35, this differences being most evident for zooplankton taxa with springtime mean T index values.

Analyzing the specific year-to-year pattern of T index variation of each zooplankton taxa, it was clear that at each site there were groups of taxa that had a common or very similar pattern of phenological variation and, predominantly, these groups were constituted by taxa that peaked in the same season (mostly spring or summer peaking taxa clusters), likely as a result of seasonally heterogeneous interannual variations in environmental factors (Straile *et al.*, 2015). These zooplankton taxa clusters with maximum coherence amongst them were used to analyze the main environmental drivers of the specific patterns of interannual variations in the T index, which were shown to vary between sites and between zooplankton taxa clusters at each site. Associations between environmental factors and phenological indices have been reported to differ among zooplankton taxonomic groups and across regions by other authors too (McGinty *et al*., 2011).

Spring EA index was the variable that best explained the T index variability for the B35 cluster (mostly summer or early autumn peaking taxa). It is claimed that large-scale climate indices can do better than individual local weather variables when predicting ecological processes (Hallett *et al*., 2004), because they integrate various spatial and temporal features of different weather components (Stenseth and Mysterud 2005). Our observations showed that higher positive spring EA index values were linked to earlier peaks of many summer/early autumn peaking zooplankton taxa at B35. The EA pattern is often interpreted as a southward shifted NAO pattern and positive values are associated with above-average surface temperatures in Europe in all months (http://www.cpc.ncep.noaa.gov/data/teledoc/ea.shtml). In addition, spring EA index was negatively correlated with summer salinity. This would agree with the fact that positive phases of EA pattern are linked to southwesterly winds and downwelling in the Bay of Biscay (Borja *et al*., 2008). Upwelling on the Iberian Basque coast can be considered to be weak as compared to regions located further to the west on the Iberian coast (Valencia *et al*., 2004), however, it may still cause a noticeable dispersion of plankton off the coast (Llope *et al*., 2006). Therefore, less intense upwelling or more intense downwelling could help retain zooplankton at B35 in spring, causing an advance in the T index.

Winter AMO index and salinity (the model selected summer salinity, but this was highly correlated with winter and spring salinities) were two of the variables that best correlated with the T index for the U35 taxa cluster. The AMO index is correlated to air temperature and rainfall over much of the Northern Hemisphere. U35 is located at the mouth of the estuary of Urdaibai and the relationship between AMO and salinity could be because in estuarine ecosystems the AMO index is mainly associated to changes in river flow and estuarine mixing dynamics (Nye *et al*., 2014). Higher winter, spring and summer salinities were associated with delays in the seasonal timing of the U35 cluster that grouped mainly spring peaking taxa but also some early summer and early autumn taxa. This could be related to the fact that spring salinity was negatively correlated (p = 0.023) with spring chlorophyll *a* concentration at U35 and it would agree with the paradigm that lower food availability can cause phenological shifts (Greve *et al*., 2001). The AMO index has also been shown to be associated with changes in wind and current regimes (Delworth *et al*., 2007; Häkkinen *et al*., 2011) and passive dispersal of marine plankton can be affected, resulting in changes in spatial distribution. In previous studies AMO was found to make a significant contribution to explain zooplankton variability in the estuary of Bilbao, in the Bay of Biscay (Uriarte *et al*., 2016). It was also shown to be a significant driver of zooplankton community composition in Northeast United States continental shelf waters (Morse *et al.*, 2017). In other areas in the Atlantic some zooplankton taxa abundances were found to be positively correlated to surface water temperature and the AMO index (Kane, 2011) and so were northward geographical shifts of Northeast Atlantic calanoid copepods (Beaugrand *et al.*, 2009).

At L4 a cluster of mostly summer peaking zooplankton taxa (L4 (ii)), that included meroplankton taxa (gastropod, echinoderm and decapod larva) as well as copepods such as Calanidae and PCPC-calanus and the cladoceran *Podon*, was related to the combination of winter and spring water temperatures, together with summer chlorophyll *a* concentration. This finding conforms to the general view that warming in preceding months enhances advances in the seasonal timing of zooplankton (Greve *et al*., 2001) and agrees with previous findings for L4 zooplankton (Atkinson *et al*., 2015). Many vital processes such as the development, reproduction, and onset/termination of seasonal dormancy all depend on temperature and shift earlier in the year when the environment is warmer (Greve *et al*., 2001; Mackas and Beaugrand, 2010; Beaugrand *et al*., 2014). Our findings also agree with the paradigm that enhanced primary production contributes to leads in zooplankton phenology (Greve *et al*., 2001), although other authors have suggested no effect or at least no prominent role of the timing and magnitude of the phytoplankton bloom on zooplankton phenology for a wide range of regions including L4 (Mackas *et al*., 2012; Atkinson *et al*., 2015). For the other two L4 taxa clusters (i and iii) the relationships with environmental factors (positive correlation to winter chlorophyll *a* and summer water temperature in the case of L4 (i) and winter EA index and summer salinity in the case of L4 (iii)) were not easily explainable. In a previous work with the L4 time-series, spanning a longer time period (1988 – 2012), it was concluded that there was a weak tendency for earlier timings of spring taxa and later timings of autumn taxa during warm years, but with many exceptions (Atkinson *et al*., 2015). Other factors, such as mortality through predation, which have not been addressed in the present work, have been shown to significantly affect temporal variations in zooplankton abundance at L4 (Irigoien and Harris, 2003; Hirst *et al*., 2007; Atkinson *et al*., 2015; Cornwell *et al*., 2018) and under intense predation zooplankton dynamics can be decoupled from environmental abiotic conditions (Casini *et al*., 2009).

At SH most zooplankton taxa showed small interannual variations in the timing of the seasonal maximum (SH (ii) cluster), but relevant summer copepod (*Acartia*, *Temora*, *Centropages*) and cladoceran (*Podon*, *Evadne*) genera showed larger variations with an overall (though non-significant) tendency for an advance during the study period, in contrast to the overall delay observed for B35 and U35 taxa clusters. For the SH (i) taxa cluster spring and summer EA indices together with the mean annual NAO index (which was, in turn, well correlated with winter NAO index) appeared to be the combination of environmental factors that best correlated with their T index. Thus, the NAO index had a higher influence at SH than at other sites, which agrees with the fact that the NAO index is strongly weighted in the North Sea (Harris *et al*., 2014). Molinero *et al*. (2005) also found significant correlation between the phenology of copepod species and NAO index in the Mediterranean Sea, where the NAO has a strong effect but with inverse conditions to those of the North Sea (Visbeck et al., 2001), In the south of the Bay of Biscay, where B35 and U35 are located, environmental conditions respond to the NAO in the same way that in the Mediterranean region but weakly (Aravena et al., 2009). At SH summer EA index was, in turn, positively correlated with mean annual chlorophyll *a* concentration (p= 0.038), which was mainly driven by variations in spring chlorophyll *a* concentration. Higher spring chlorophyll *a* concentration at SH could thus be associated to an advance in the timing of the annual maximum of the above-mentioned summer zooplankton taxa, in agreement with findings for other areas (see Greve *et al*., 2001), but other effects associated to changes in water circulation cannot be excluded. It is noteworthy that there was a significant trend of increase in chlorophyll *a* concentration at SH over the study period, whilst these copepods and cladocerans, in general, tended to show a phenological advance, in contrast to the overall delay observed at B35 and U35 sites. The best example was *Evadne*, which showed a statistically significant trend of phenological advance. These cladocerans are grazers and chlorophyll *a* has been suggested as one of the most important factors affecting their distribution and abundance (Xiong *et al*., 2012). Our study has therefore shown that the phenology of *Evadne* (mostly comprised of *Evadne nordmani*) at SH is particularly sensitive to environmental changes. This agrees with findings by Edwards and Richardson (2004) who also reported advances in the timing of the seasonal peak of this genus in the North Sea during the 1958 – 2002 period, although they showed a clear correlation with the increase in the sea surface temperature that occurred during that period.

Overall, our data have shown that climate teleconnection patterns significantly correlate to coastal zooplankton phenology variations in the Northeast Atlantic, suggesting the importance of large-scale atmospheric processes. However, in agreement with findings by Mackas *et al*. (2012), the between-location similarities of zooplankton phenology and climatic anomaly time series decrease with increasing site-to-site spatial separation. Thus, in the present study the resulting seasonal timings varied spatially, being overall tendencies almost opposite at the southernmost and northernmost sites, same zooplankton genera like *Acartia*, *Podon* or *Centropages*, tending (albeit in a non-statistically significantly) to advance their timing at SH and to retard at U35. However, *Podon* is mainly *Podon intermedius* at U35 and *Podon leuckartii* at SH and *Centropages* is mainly *Centropages typicus* at U35 and *Centropages hamatus* at SH, while in the case of *Acartia*, it is mainly composed of *A. clausi* at both sites (see Chapter 1). Therefore, it seems that the differential effect on zooplankton phenology could be the consequence of the different expression (in terms of temperature, precipitation and wind patterns) of basin-wide climate teleconnection indices at regional scales in the North Atlantic (Mackas *et al*., 2012). Latitudinal differences in seasonality of species with fixed thermal niche, e.g. *Acartia* is a spring taxon at the lowest latitude U35 site and a summer one at the highest latitude SH (Chapter 1, this work), could also be influential, because of the seasonally heterogeneous interannual variations in climate and related factors (Straile *et al*., 2015).

3.5. References

Adrian, R., Whilhelm, S., Gerten, D.. (2006). Life-history traits of lake plankton species may govern their phenological response to climate warming. *Global Change Biology*, *20*, 652–661. doi: 10.1111/j.1365-2486.2006.01125.x.

Aravena, G., Villate, F., Uriarte, I., Iriarte, A., Ibañez, B. (2009). Influence of the North Atlantic Oscillation (NAO) on climatic factors and estuarine water temperature on the Basque coast (Bay of Biscay): Comparative analysis of three seasonal NAO indices. *Continental Shelf Research*, *29*, 750–758. https://doi.org/10.1016/j.csr.2008.12.001.

Atkinson, A., Harmer, R. A., Widdicombe, C. E., McEvoy, A. J., Smyth, T. J., Cummings, D. G., Somerfield, P. J., Maud, J. L., McConville, K. (2015). Questioning the role of phenology shifts and trophic mismatching in a planktonic food web. *Progress in Oceanography*, *137*, 498–512. doi:10.1016/j.pocean.2015.04.023.

Beaugrand, G., Luczak, C., Edwards, M. (2009). Rapid Biogeographical Plankton Shifts in the North Atlantic Ocean. *Global Change Biology* *15*(7), 1790–1803. https://doi.org/10.1111/j.1365-2486.2009.01848.x.

Beaugrand, G., Luczak, C., Martin, E. (2009). Rapid biogeographical plankton shifts in the North Atlantic Ocean. *Global Change Biology*, *15*(7), 1790–1803. https://doi.org/10.1111/j.1365-2486.2009.01848.x.

Beaugrand, G., Martin, E., Legendre, L. (2010). Marine Biodiversity, Ecosystem Functioning, and Carbon Cycles. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(22), 10120–24. https://doi.org/10.1073/pnas.0913855107.

Beaugrand, G., Goberville, E., Luczak, E., Kirby, R. R. (2014). Marine biological shifts and climate. *Proceedings of the Royal Society B*, *281*(1783), 20133350. http://dx.doi.org/10.1098/rspb.2013.3350.

Behrenfeld, M. J. (2014). Climate-mediated dance of the plankton. *Nature Climate Change*, *4*, 880–887. https://doi.org/10.1038/nclimate2349.

Borja, A., Fontán, A., Sáenz, J., Valencia, V. (2008). Climate, oceanography, and recruitment: The case of the Bay of Biscay anchovy (*Engraulis encrasicolus*). *Fisheries Oceanography*, *17*(6), 477–493. https://doi.org/10.1111/j.1365-2419.2008.00494.x.

Bresnan, E., Cook, K. B., Hughes, S. L., Hay, S. J., Smith, K., Walsham, P., Webster, L. (2015). Seasonality of the plankton community at an east and west coast monitoring site in Scottish waters. *Journal of Sea Research*, *105*, 16–29. doi:10.1016/j.seares.2015.06.009.

Casini, M., Hjelm, J., Molinero, J.-C., Lövgren, J., Cardinale, M., Bartolino, V., Belgrano, A., Kornilovs, G. (2009). Trophic cascades promote threshold-like shifts in pelagic marine ecosystems. *PNAS*, *106*(1), 197–202. https://doi.org/10.1073/pnas.0806649105.

Chivers, W. J., Walner, A. W., Hays, C. (2017). Mismatch between Marine Plankton Range Movements and the Velocity of Climate Change. *Nature Communications*, *8*, 1–8. http://dx.doi.org/10.1038/ncomms14434.

Cloern, J. E., Jassby, A. D. (2010). Patterns and scales of phytoplankton variability in estuarine-coastal ecosystems. *Estuaries and Coasts*, *33*, 230–241. doi:10.1007/s12237-009-9195-3.

Conversi, A., Peluso, T., Fonda-Umani, S. (2009). Gulf of Trieste: A Changing Ecosystem. *Journal of Geophysical Research: Oceans* *114*(7), 1–10. https://doi.org/10.1029/2008JC004763.

Cornwell, L. E.,Findlay, H. S., Smyth, T. J., Hirst, A. G., Bruun, J. T., McEvoy, A. J., Widdicombe, C. E., Castellani, C., Lewis, C., Atkinson, A. (2018). Seasonality of *Oithona Similis* and *Calanus Helgolandicus* Reproduction and Abundance: Contrasting Responses to Environmental Variation at a Shelf Site. *Journal of Plankton Research*, *40*(3), 295–310. https://doi.org/10.1093/plankt/fby007.

Cushing, D. H. (1990). Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Advances in Marine Biology*, *26*, 249–293. doi:10.1016/S0065-2881(08)60202-3.

Delworth, T. L., Zhang, R., Mann, M. E. (2007). Decadal to Centennial Variability of the Atlantic from Observations and Models. *Geophysical Monograph Series* 173, 131–48. https://doi.org/10.1029/173GM10.

Edwards, M., Richardson, A. J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature*, *430*, 881–884. https://doi.org/10.1038/nature02808.

Ferrer, L., Fontán, A., Mader, J., Chust, G., González, M., Valencia, V., Uriarte, A., Collins, M. B. (2009). Low-salinity plumes in the oceanic region of the Basque Country. *Continental Shelf Research*, *29*(8), 970–984. https://doi.org/10.1016/j.csr.2008.12.014.

Greve, W., Prinage, S., Zidowitz, H., Nast, J., Reiners, F. (2005). On the Phenology of North Sea Ichthyoplankton. *ICES Journal of Marine Science 62*(7), 1216–23. https://doi.org/10.1016/j.icesjms.2005.03.011.

Greve, W., Lange, U., Reiners, F., Nast, J. (2001). Predicting the Seasonality of North Sea Zooplankton. *Senckenbergiana Maritima*, *31*(2), 263–68. https://doi.org/10.1007/BF03043035.

Häkkinen, S., Rhines, P. B., Worthen, D. L. (2011). Atmospheric Blocking and Atlantic Multidecadal Ocean Variability. *Science 334*(6056), 655–59. doi: 10.1126/science.1205683.

Hallet, T. B., Coulson, T., Pilkington, J. G., Clutton-Brock, T. H., Pemberton, J. M., Grenfell, B. T. (2004). Why large-scale climate indices seem to predict ecological processes better than local weather. *Nature*, *430*, 71-75. https://doi.org/10.1038/nature02708.

Harris, V., Edwards, M., Olhede, S. C. (2014). Multidecadal Atlantic climate variability and its impact on marine pelagic communities. *Journal of Marine Systems*, *133*, 55–69. https://doi.org/10.1016/j.jmarsys.2013.07.001.

Hirst, A. G., Bonnet, D., Harris, R. P. (2007), Seasonal dynamics and mortality rates of Calanus helgolandicus over two years at a station in the English Channel. *Marine Ecology Progress Series*, *340*, 189–205. https://doi.org/10.3354/meps340189.

Irigoien, X., Harris, R. P. (2003). Interannual Variability of *Calanus Helgolandicus* in the English Channel. *Fisheries Oceanography*, *12*(4–5): 317–26. https://doi.org/10.1046/j.1365–2419.2003.00247.x.

IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland. 151 pp.

Kane, J. (2011). Inter-decadal variability of zooplankton abundance in the Middle Atlantic Bight. *Journal of Northwest Atlantic Fishery Science*, *43*, 81–92. https://doi.org/10.2960/J.v43.m674.

Llope, M., Anadón, R., Viesca, L., Quevedo, M., González-Quirós, R., Stenseth, N. C. (2006). Hydrography of the southern Bay of Biscay shelf-break region: Integrating the multiscale physical variability over the period 1993-2003. *Journal of Geophysical Research: Oceans*, *111*(9), 1–14. https://doi.org/10.1029/2005JC002963.

Longhurst, A. R. (1998). Ecological geography of the sea, 1st ed. Academic Press, San Diego. 398 pp.

Mackas, D. L., Beaugrand, G. (2010). Comparisons of zooplankton time series. *Journal of Marine Systems*, *79,* 286–304. doi:10.1016/j.jmarsys.2008.11.030.

Mackas, D. L., Greve, W., Edwards, M., Chiba, S., Tadokoro, K., Eloire, D., Mazzochi, M. G., Batten, S., Richardson, A. J., Johnson, C., Head, E., Conversi, A., Peluso, T. (2012). Changing zooplankton seasonality in a changing ocean: Comparing time series of zooplankton phenology. *Progress in Oceanography, 97–100,* 31–62. https://doi.org/10.1016/j.pocean.2011.11.005.

Marqués, S. C., Primo, A. L., Martinho, F., Azeiteiro, U. M., Pardal, M. A. (2014). Shifts in Estuarine Zooplankton Variability Following Extreme Climate Events: A Comparison between Drought and Regular Years. *Marine Ecology Progress Series*, *499,* 65–76. doi: 10.3354/meps10635.

McGinty, N., Power, A.M., Johnson, M.P. (2011). Variation among Northeast Atlantic regions in the responses of zooplankton to climate change: not all areas follow the same path. J*ournal of Experimental Marine Biology and Ecology*, *400,* 120–131. doi: 10.1016/j.jembe.2011.02.013.

Molinero, J. C., Ibañez, F., Souissi, S., Chifflet, M., Nival, P. (2005). Phenological changes in the Northwestern Mediterranean copepods Centropages typicus and *Temora stylifera* linked to climate forcing. *Oceologia*, *145*(4), 640-649. doi: 10.1007/s00442-005-0130-4.

Morse, R. E., Friedland, K. D., Tommasi, D., Stock, C., Nye, J. (2017). Distinct zooplankton regime shift patterns across ecoregions of the U.S. Northeast continental shelf Large Marine Ecosystem. *Journal of Marine Systems, 165,* 77–91. https://doi.org/10.1016/j.jmarsys.2016.09.011.

Nye, J. A., Baker, M. R., Bell, R., Kenny, A., Kilbourne, K. H., Friedland, K. D., Martino, E., Stachura, M. M., Van Houtan, K. S., Wood, R. (2014). Ecosystem effects of the Atlantic Multidecadal Oscillation. *Journal of Marine Systems, 133,* 103–116. https://doi.org/10.1016/j.jmarsys.2013.02.006.

O'Brien, T. D., Wiebe, P. H., Falkenhaug, T. (2013). ICES Zooplankton Status Report 2010/2011. *ICES Cooperative Research Report*, *318*. 208 pp. ISSN 1017-6195.

Post, E. (2017). Implications of earlier sea ice melt for phenological cascades in arctic marine food webs. *Food Webs*, *13,* 60-66. https://doi.org/10.1016/j.fooweb.2016.11.002.

Reygondeau, G., Molinero, J. C., Coombs, S., MacKenzie, B. R., Bonnet, D. (2015). Progressive changes in the Western English Channel foster a reorganization in the plankton food web. *Progress in Oceanography*, *137B,* 524–532. https://doi.org/10.1016/j.pocean.2015.04.025.

Rice, E., Stewart, G. (2016). Decadal changes in zooplankton abundance and phenology of Long Island Sound reflect interacting changes in temperature and community composition. *Marine Environmental Research*, *120*, 154–165. doi: 10.1016/j.marenvres.2016.08.003.

Richardson, A. J. (2008). In hot water: zooplankton and climate change. *ICES Journal of Marine Science*, *65*, 279–295. doi: 10.1093/icesjms/fsn028.

Smith, V. H., Tilman, G. D., Nekola, J. C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution*, *100*, 179–196. doi:10.1016/S0269-7491(99)00091-3.

Stenseth, N. C., Mysterud, A. (2005). Weather packages: finding the right scale and composition of climate in ecology. *Journal of Animal Ecology*, *74,* 1195–1198. https://doi.org/10.1111/j.1365-2656.2005.01005.x.

Straile, D., Kerimoglu, O., Peeters, F. (2015). Trophic mismatch requires seasonal heterogeneity of warming. *Ecology*, 96(10), 2794–2805. https://doi.org/10.1890/14-0839.1.

Thackeray, S. J., Henrys, P. A., Jones, I. D., Feuchtmayr, H. (2012). Eight decades of phenological change for a freshwater cladoceran: what are the consequences of our definition of seasonal timing? *Freshwater Biology*, 57(2), 345–359. https://doi.org/10.1111/j.1365-2427.2011.02614.x.

Uriarte, I., Villate, F., Iriarte, A. (2016). Zooplankton recolonization of the inner estuary of Bilbao: influence of pollution abatement, climate and non-indigenous species. *Journal of Plankton Research*, *38*(3), 718–731. https://doi.org/10.1093/plankt/fbv060.

Usov, N., Kutcheva, I., Primakov, I., Martynova, D. (2013). Every Species Is Good in Its Season: Do the Shifts in the Annual Temperature Dynamics Affect the Phenology of the Zooplankton Species in the White Sea? *Hydrobiologia*, *706*(1), 11–33. https://doi.org/10.1007/s10750-012-1435-z.

Valencia, V., Franco, J., Borja, A., Fontán, A. (2004). Hydrography of the southeastern Bay of Biscay. In Borja, A., Collins, M. (eds.), *Oceanography and marine environment of the Basque Country*. Elsevier Oceanography Series, *70*, 616 pp.

Villate, F., Iriarte, A., Uriarte, I., Sanchez, I. (2017). Seasonal and interannual variability of mesozooplankton in two contrasting estuaries of the Bay of Biscay: relationship to environmental factors. *Journal of Sea Research*, *130*, 189–203. doi: 10.1016/j.seares.2017.05.002.

Visbeck, M. H., Hurrell, J. W., Polvani, L., Cullen, H. M. (2001). The North Atlantic oscillation: past, present, and future. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 12876–12877. https://doi.org/10.1073/pnas.231391598.

Xiong, L.L., Yin, J.Q., Huang, L.M., Li, K.Z., Lian, S.M. (2012). Seasonal and spatial variations of cladocerans on the northwest continental shelf of the South China Sea. *Crustaceana*, *85*, 473–496. doi:10.2307/23212676.

Chapter 4. *Modelling multi-annual zooplankton variations*

Changes in climate affect plankton, potentially altering the temporal trajectory of ocean ecosystems. Thus, identifying drivers of change and exploring their effects is of key importance. We modelled the temporal variation of zooplankton (1999 – 2013) at the coastal sites of Stonehaven (SH), Plymouth L4 (L4), Urdaibai35 (U35) and Bilbao35 (B35) in the North Atlantic to test for changes beyond the annual cycle throughout time. We summarized the state of zooplankton communities using principal component analysis and we modelled temporal variations of zooplankton using Generalized Additive Mixed Models (GAMMs), in which three environmental variables: water temperature, salinity and chlorophyll *a* were included as covariates. The first principal component reflected the dynamics of taxa with abundance maxima in the main growing period from spring to summer, while the second principal component reflected those of taxa that peak later in the year (summer – autumn) in opposition to taxa that peak earliest. Gradual changes (trends) were found for the zooplankton assemblage of the main growing period at L4 but for the later and earliest assemblages at the other three sites, being this trend restricted to the 1999 – 2005 period at SH, likely connected to a shift initiated previously. Regarding individual taxa, increasing and decreasing trends were observed in several taxa, but no individual taxon showed the same pattern of change at the four sites, Outstandingly, *Acartia* showed inverse trends at the northernmost (SH) and the southernmost (U35/B35) sites and no trend at the intermediate latitude site (L4). The selection of environmental variables by the models indicates that zooplankton dynamics were mainly shaped by temperature at all sites, although the patterns of change in zooplankton differed between sites. The interaction between temperature and month was always selected as the main driver of community trends in all cases, except at U35, and also of individual taxa variations at L4. Salinity and chlorophyll *a*, singly or in interaction, had a more variable role depending on the site, and they were mainly selected to account for zooplankton changes at B35 and SH, respectively. The almost absence of selected environmental variables in the models for U35 was attributed to the lower coherence of environmental seasonal patterns at this site.

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4.1. Introduction

Human interaction with the natural world is causing rapid changes in biodiversity (McGill *et al*., 2015), and climate change is becoming more evident than ever, with warming oceans leading to profound changes in marine communities (Beaugrand *et al*., 2002). Long-term monitoring programs are required to help to track temporal variability in natural biological assemblages and assess whether potential drivers can explain the observed patterns (Magurran *et al*., 2012), as predicting the impacts of environmental change requires that we identify the drivers of ecological variation. Regime shifts, the definition of which should include at least the following: “sudden, high-amplitude, infrequent events, which are detectable in multiple aspects of the physical and biological components and on large spatial scales” (Lees *et al*., 2006), have recently attracted a lot of interest. Such shifts have been observed for example in the 1980s in studies of long-term climate dynamics and they represent major changes in the Earth's biophysical systems all around the globe (Reid *et al*., 2016). Regarding long-term biota dynamics (Clare *et al*., 2017; Spencer *et al*., 2012), these shifts could potentially result in irreversible changes in species composition and community functioning (Clare *et al*., 2017). Another plausible response of these communities is to track underlying gradual changes in environmental factors causing gradual trends of change in the biotic component (Molinero *et al*., 2013; Reygondeau *et al*., 2015).

The abundance of zooplankton may be highly variable at different time scales (Haury *et al*., 1978) in response to environmental drivers (Villate *et al*., 2016). Several studies have tried to untangle how long-term interannual variations in the mesozooplankton community are linked to patterns of environmental descriptors in the Northeast Atlantic (Beaugrand *et al*., 2000; Beaugrand and Ibanez, 2004; Aravena *et al*., 2009; Hátún *et al*., 2009; Rombouts *et al*., 2010; Eloire *et al*., 2010). In order to understand these variations, a logical approach is to focus on the factors that are expected to exert the greatest ecological impact. Untangling the roles of extrinsic factors is one of the biggest challenges faced by those tasked with predicting the ecological impacts of environmental change (Stenseth *et al*., 2002). Water temperature has been found to be the most important factor driving both spatial and temporal changes in plankton (Beaugrand *et al*., 2002; Rombouts *et al*., 2010; Reygondeau and Beaugrand, 2011). Salinity has been related to regime shifts of marine ecosystems (Weijerman *et al*., 2005). Also, salinity-induced stratification alters the exchange of nutrients and suspended particulate matter. Changes in salinity through advective processes, in combination with changes in temperature, alter the density and the stratification of the water column, thus affecting zooplankton populations distribution (Cross *et al*., 2015). Chlorophyll *a*, as a measure of phytoplankton biomass, plays a major role for some marine species as it represents the main food source for zooplankton grazers (Kleppel and Pieper, 1984), and filter feeders, affecting their recruitment (Highfield *et al*., 2010).

The aims of the present study were 1) to assess multiannual patterns of variation in mesozooplankton series from four different coastal sites of the Northeast Atlantic during the 1999 – 2013 period and 2) to determine if these multiannual patterns were related to underlying variations of three major environmental drivers: water temperature, salinity and chlorophyll *a* concentration.

4.2. Methods

4.2.1. Data analysis

Year Principal component analyses (PCAs) were performed on the abundance series of the selected zooplankton taxa for each site: non-crustacean holoplankton (appendicularians, chaetognaths, siphonophores and doliolids), cladocerans and copepods (*Evadne*, *Podon*, *Acartia*, *Centropages*, *Temora*, *Oithona*, *Oncaea*, *Corycaeus*, the Calanidae family and the PCPC-calanus assemblage) and meroplankton (cirripede larvae, decapod larvae, gastropod larvae, bivalve larvae, polychaete larvae, fish eggs and larvae, bryozoan larvae, echinoderm larvae and hydromedusae).

Abundance data were log-transformed (log(x+1)). Data were then centered and scaled (mean 0 and standard deviation 1). Therefore, the principal components (PCs) were calculated from the correlation matrix of these transformed data. PCs are low-dimensional representations of how the zooplankton community has changed along the time series at each site. Only PCs 1 and 2 were considered for this analysis.

With the purpose of detecting trends or shifts in the main modes of zooplankton community changes extracted by the PCs (PCs 1 and 2) and in the abundance of the taxa selected by their contribution to such community changes, Generalized Additive Mixed Models (GAMMs; Wood 2006, section 6.6) were fitted. Their advantage over other methods such as Rodionov's regime shift detection algorithm (RSD; Rodionov, 2004), is that they are not limited by assumptions such as time series stationarity except at change points. Furthermore, GAMMs do not assume that trends must be linear, and allow more than one change point in contrast to Spencer *et al*. (2012). They also make it easy to include seasonality and the effects of environmental variables. Our approach is based on Orr *et al*. (2015) and Clare *et al*. (2017).

Time (year of the time series + the corresponding fraction of 1/12 for each one of the months, i.e. January 1999 = 1999 + 0, February 1999 = 1999 + 0.08333, March 1999 = 1999 + 0.16666, etc.) was used as a smoothed covariate. The initial model took the form:

$$y_{i,j} = \beta_{0,j} + f_{1,j}s(time_i) + f_{2,j}(month_i) + \varepsilon_{i,j}$$

where $\boldsymbol{y_{i,j}}$ is the value of the $\boldsymbol{j}$th principal component ($\boldsymbol{j} = 1,2$) at time $\boldsymbol{i}$, $\boldsymbol{\beta_{0,j}}$ is the intercept, $\boldsymbol{f_{1,j}s(time_i)}$ is the smoothed time function, $\boldsymbol{f_{2,j}(month_i)}$ is the month in which sampling was conducted and $\boldsymbol{\varepsilon_{i,j}}$ is a residual error. We used a first-order continuous-time autoregressive (CAR(1)) model for the error term (Pinheiro and Bates, 2000, section 5.3). GAMMs were fitted using the mgcv package (Wood, 2017) in R (version 3.2.2; R Core Team, 2017), with the default maximum likelihood (ML) estimation method. We used graphical analyses of residuals to check model assumptions. Simultaneous 95% confidence intervals on the first and second derivatives of the smoothed time effect were estimated by a Monte Carlo method, as described in Clare *et al.* (2017). Time periods where these confidence intervals do not contain zero indicate temporal trends (for the first derivative) or changes in temporal trend (for the second derivative), which may be of more interest.

The environmental variables to be included in the model for the two main modes of zooplankton community variations and for the abundance of selected taxa were water temperature (Temp), salinity (Sal) and chlorophyll *a* (Chl *a*), which were standardized (expressed in terms of standard deviations from their monthly means) to remove seasonality. In order to determine whether any of those was a driver of community or individual taxa dynamics, the initial models were modified to include the latter as (non-smoothed) covariates. The selected environmental variables were added into the initial model, along with all two-way interactions among fixed effects:

$$y_{i,j} = \beta_{0,j} + f_{1,j}s(time_i) + \left(f_{2,j}(month_i) + f_{3,j}(Sal_i) + f_{4,j}(Temp) + f_{5,j}(Chla_i)\right)^2 + \varepsilon_{i,j}$$

where $\boldsymbol{f_{3,j}(Sal_i)}$ is standardised salinity, $\boldsymbol{f_{4,j}(Temp_i)}$ is standardised water temperature and $\boldsymbol{f_{5,j}(Chla_i)}$ is standardised chlorophyll *a*. Approximate *p*-values were obtained from Wald-like tests implemented in the anova.gam() function (Wood, 2017). Non-significant effects were removed from the models. Terms which themselves were not statistically significant ($p > 0.05$) but took part in a significant interaction with another term, were retained in the final model. Confidence intervals for first and second derivatives of the time effect were obtained as above. The presence of significant second derivatives in the initial model that were absent in the final model may indicate that environmental variables could account for changes in temporal trend. GAMMs were fitted for the two principal components of the zooplankton community abundance and some selected and relevant zooplankton taxa.

In order to visualize the change over time of the environmental variables and the log-transformed (log (x+1)) abundance of taxa, locally weighted polynomial regression (LOESS) fits were applied. The non-parametric Spearman's rank correlation coefficient method (rho) was used to test for trends. Plots with LOESS fit and Spearman's rho tests were both coded using R (version 3.2.2; R Core Team, 2017).

4.3. Results

4.3.1. Environmental variables

Significant trends in the variation of the environmental variables (**Figure 1**) during the study period were only found in three cases: the decreasing trend for chlorophyll *a* at B35 (p= 0.0185), and the increasing trends for salinity at U35 (p<0.001) and at B35 (p<0.001).

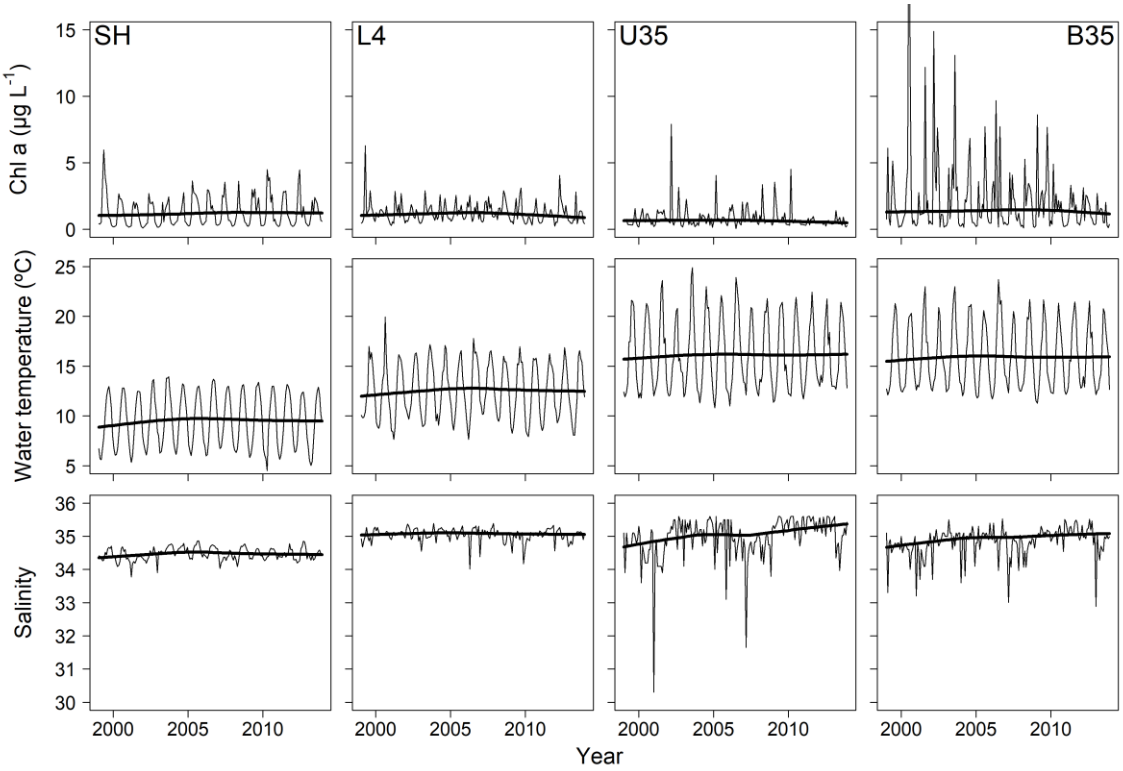

Figure 1. Temporal variation in water temperature (ºC), chlorophyll *a* (µg L^{-1}) and salinity at SH, L4, U35 and B35 sites. Bold line is the LOESS smoother (bold line).

However, the linear regressions performed between environmental variables and year (independently for each season; **Figure 2**) revealed decreasing trends, although non-significant ones, for temperature in the warmest summer months at all sites. This decrease was most evident, and almost reached statistical significance (p= 0.07), at L4 and least evident at SH. Non-significant temperature increases were mainly observed in autumn at L4, in winter at U35 and in spring at B35. The decrease of chlorophyll *a* throughout the study period at B35 was very strong in summer for the annual maxima (p< 0.001). There is a chlorophyll *a* decrease, although non-significant, in winter and spring at B35, spring and autumn at U35 and spring at L4. In contrast, chlorophyll *a* increased significantly in winter (*p*= 0.015) and autumn (*p*= 0.028), and non-significantly in spring, at SH. Salinity values increased throughout time at B35 and U35 in most months, with significant trends for winter (*p*= 0.003) at U35 and for winter (*p*= 0.047) and summer (*p*= 0.013) at B35.

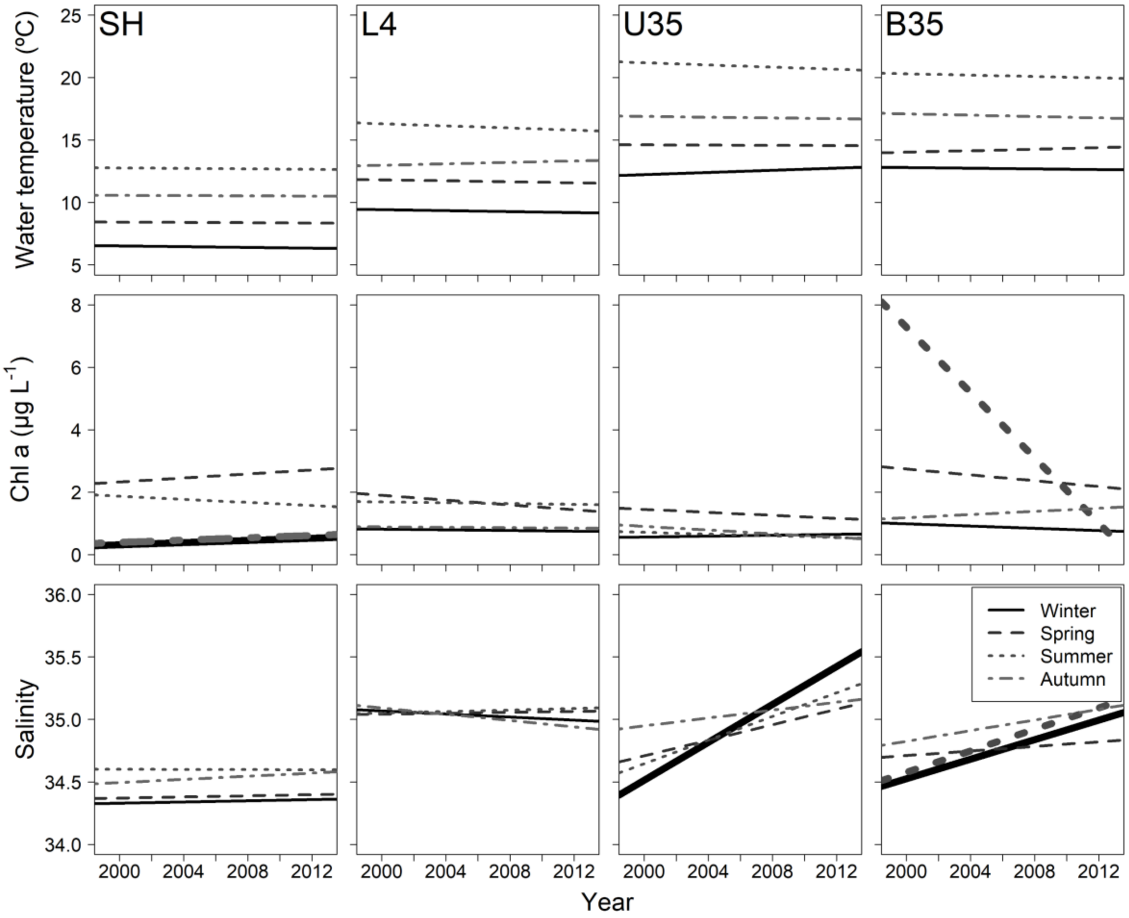

Figure 2. Linear regression models of water temperature, chlorophyll *a* and salinity vs. year for each season at the SH, L4, U35 and B35 sites. Thick lines stand for significant increasing/decreasing trends along the study period.

4.3.2. Zooplankton community

The percentage of variation captured by the two first components of the zooplankton taxa abundances PCA was: 42.93% for PC1 and 13.25% for PC2 at SH, 35.63% for PC1 and 15.05% for PC2 at L4, 23.54% for PC1 and 14.35% for PC2 at U35 and, 33.3% for PC1 and 12.8% for PC2 at B35. Taxa loaded on PC1 (**Table 1A**) mostly showed positive loading values, with only three exceptions with negative but very close to zero loadings (*Oncaea* at SH and L4 and *Corycaeus* at SH). Taxa with highest positive loadings were *Temora*, decapod larvae, *Acartia*, *Centropages* and appendicularians at SH, echinoderm larvae, *Podon*, *Temora*, Calanidae, *Acartia*, appendicularians and *Evadne* at L4, appendicularians, gastropod larvae, *Acartia* and cirripede larvae at U35 and PCPC-calanus, bivalve larvae, siphonophores, cirripede larvae, *Centropages*, appendicularians, *Podon* and decapod larvae at B35. The annual maxima of these taxa occurred in summer (July – August) at SH, from spring to summer (May – August) at L4, mainly in early spring (March) at U35 and mainly in early summer (June) at B35.

Table 1. Taxa loadings (positive and negative values separated by the dashed line) on the PC1 and PC2 of the zooplankton abundance at SH, L4, U35 and B35 sites from 1999 to 2013, with indication of the month of the annual maximum abundance (Month max.). Taxa: ACAR (*Acartia*), APPE (Appendicularians), BIVA (Bivalve larvae), BRYO (Bryozoan larvae), CALA (*Calanus*), CENT (*Centropages*), CHAE (Chaetognaths), CIRRI (Cirripede larvae), CORY (*Corycaeus*), DECA (Decapod larvae), DOLI (Doliolids), ECHI (Echinoderm larvae), EVAD (*Evadne*), GAST (Gastropod larvae), MEDU (hydromedusae), OITH (*Oithona*), ONCA (*Oncaea*), PCPC-calanus (*Pseudo–Cteno–Para–Clausocalanus*), FISH (Fish eggs and larvae), PODO (*Podon*), POLY (Polychaete larvae), SIPH (Siphonophores), TEMO (*Temora*).

A) PC1

SH			L4			U35			B35		
Taxon	PC loading	Month Max	Taxon	PC loading	Month Max	Taxon	PC loading	Month Max	Taxon	PC loading	Month Max
TEMO	0,28395	AUG	ECHI	0,29065	JUL	APPE	0,31676	MAR	PCPC	0,26937	SEP
DECA	0,28158	AUG	PODO	0,29009	JUL	GAST	0,30402	JUN	BIVA	0,26702	JUL
ACAR	0,27675	JUL	TEMO	0,28764	AUG	ACAR	0,29242	MAR	SIPH	0,26397	MAY
CENT	0,26743	JUL	CALA	0,27780	AUG	CIRR	0,29182	MAR	CIRR	0,25951	JUN
APPE	0,26577	JUL	ACAR	0,27487	MAY	EVAD	0,26841	MAR	CENT	0,25781	JUN
POLY	0,25869	JUL	APPE	0,26834	JUN	PCPC	0,25776	JUL	APPE	0,25749	JUN
BIVA	0,24774	JUN	EVAD	0,26801	MAY	PODO	0,25317	MAR	PODO	0,25680	JUN
ECHI	0,24448	JUN	DECA	0,25186	JUL	OITH	0,24550	MAR	DECA	0,25208	JUN
EVAD	0,23374	AUG	POLY	0,24237	JUN	CENT	0,23902	JUN	GAST	0,24544	JUN
CALA	0,23183	SEP	GAST	0,24031	AUG	BIVA	0,23553	MAR	FISH	0,22680	JUN
OITH	0,22240	JUL	CENT	0,21245	AUG	SIPH	0,22913	MAY	EVAD	0,22660	MAY
PODO	0,22136	AUG	OITH	0,20657	APR	MEDU	0,22656	APR	MEDU	0,21413	APR
CIRR	0,20837	APR	MEDU	0,20501	JUL	ECHI	0,16108	MAY	ACAR	0,18962	MAR
CHAE	0,20527	SEP	CIRR	0,19476	APR	BRYO	0,15712	JUN	CHAE	0,18150	AUG
GAST	0,19945	SEP	SIPH	0,15165	SEP	TEMO	0,14624	SEP	TEMO	0,17302	SEP
BRYO	0,19070	APR	CHAE	0,14076	SEP	CALA	0,13743	APR	DOLI	0,17128	SEP
PCPC	0,16707	MAY	PCPC	0,13713	APR	FISH	0,13573	MAY	ONCA	0,16855	SEP
MEDU	0,14213	APR	FISH	0,13186	JUN	DOLI	0,12955	AUG	OITH	0,16761	SEP
FISH	0,11674	APR	BIVA	0,07734	SEP	CHAE	0,12063	AUG	ECHI	0,14740	MAY
SIPH	0,03959	SEP	CORY	0,06145	OCT	DECA	0,11058	FEB	CALA	0,13656	JUN
DOLI	0,03565	SEP	DOLI	0,05716	SEP	CORY	0,05707	AUG	BRYO	0,10644	MAY
ONCA	-0,00409	MAR	BRYO	0,05008	MAR	ONCA	0,03030	OCT	CORY	0,09735	AUG
CORY	-0,01193	OCT	ONCA	-0,09916	NOV	POLY	0,01441	MAR	POLY	0,04618	FEB

B) PC2

SH			L4			U35			B35		
Taxon	PC loading	Month Max	Taxon	PC loading	Month Max	Taxon	PC loading	Month Max	Taxon	PC loading	Month Max
CIRR	0,29641	APR	FISH	0,24113	JUN	ACAR	0,24380	MAR	ACAR	0,33264	MAR
FISH	0,29415	APR	CIRR	0,23110	APR	BRYO	0,18544	JUN	EVAD	0,20470	MAY
APPE	0,16426	JUL	OITH	0,20039	APR	PODO	0,18385	MAR	BRYO	0,18812	MAY
EVAD	0,14031	AUG	DECA	0,19635	JUL	EVAD	0,17816	MAR	APPE	0,17044	JUN
PODO	0,13317	AUG	BRYO	0,15554	MAR	CIRR	0,15294	MAR	FISH	0,16376	JUN
BRYO	0,12877	APR	APPE	0,12898	JUN	POLY	0,13842	MAR	CIRR	0,14951	JUN
POLY	0,08220	JUL	CALA	0,09676	AUG	APPE	0,12576	MAR	CALA	0,12953	JUN
ACAR	0,02920	JUL	POLY	0,08445	JUN	FISH	0,10902	MAY	GAST	0,11891	JUN
DECA	0,01237	AUG	EVAD	0,06968	MAY	ECHI	0,07802	MAY	ECHI	0,10903	MAY
CENT	0,01128	JUL	PODO	0,03652	JUL	DECA	0,03955	FEB	PODO	0,10890	JUN
OITH	-0,02860	JUL	PCPC	0,03475	APR	CENT	0,00490	JUN	CENT	0,08390	JUN
ECHI	-0,04560	JUN	TEMO	-0,00113	AUG	GAST	0,00449	JUN	POLY	0,05933	FEB
TEMO	-0,04808	AUG	ACAR	-0,01088	MAY	CALA	-0,00389	APR	BIVA	-0,00466	JUL
BIVA	-0,08116	JUN	ECHI	-0,07969	JUL	OITH	-0,06418	MAR	MEDU	-0,03234	APR
PCPC	-0,12069	MAY	DOLI	-0,16171	SEP	BIVA	-0,07081	MAR	SIPH	-0,04260	MAY
MEDU	-0,13037	APR	CENT	-0,16833	AUG	SIPH	-0,10846	MAY	DECA	-0,05729	JUN
CALA	-0,14384	SEP	GAST	-0,18810	AUG	MEDU	-0,11695	APR	OITH	-0,09457	SEP
DOLI	-0,19703	SEP	MEDU	-0,19372	JUL	PCPC	-0,23910	JUL	PCPC	-0,12411	SEP
ONCA	-0,25221	MAR	ONCA	-0,25665	NOV	CHAE	-0,33256	AUG	TEMO	-0,33007	SEP
GAST	-0,28204	SEP	BIVA	-0,31986	SEP	TEMO	-0,33963	SEP	DOLI	-0,33011	SEP
CHAE	-0,31001	SEP	CHAE	-0,37457	SEP	DOLI	-0,35255	AUG	CHAE	-0,35683	AUG
CORY	-0,42498	OCT	CORY	-0,38767	OCT	CORY	-0,37063	AUG	CORY	-0,36197	AUG
SIPH	-0,46261	SEP	SIPH	-0,39037	SEP	ONCA	-0,42816	OCT	ONCA	-0,40228	SEP

At all sites and for PC1, both the initial model (i.e. smoothed time and month) and the final model (including the selected environmental variables as covariates) fitted linear time effects. The significant interactions with environmental variables (**Table 2A**) were with chlorophyll *a* at SH, with water temperature in interaction with month at L4 and with salinity at B35, while no covariate was retained at U35. Only at L4 and for the entire study period, the first derivative of the smoothed time effect was significantly different from zero (**Figure 3C**, bold line). Among the top taxa with highest positive loadings, echinoderm larvae and appendicularians increased in abundance during the study period and *Podon*, *Temora* and *Acartia* decreased (**Figure 4**).

Table 2. Generalized additive mixed models (GAMMs) output showing variation in the principal components 1 (A) and 2 (B) of zooplankton taxa abundance at SH, L4, U35 and B35 sites, from 1999 to 2013. Relationships between the PCs and standardized (standard deviations from the monthly mean) chlorophyll *a* (Chl *a*), water temperature (WT) and salinity (S), and also month, are shown. All possible two-way interactions were tested. Non-significant interactions and non-significant main effects, when no interactions were involved, were removed. Significant *p*-values (< 0.05) are in bold. For smoothed time, d.f. stands for the effective degrees of freedom. Dashes indicate terms removed from the final model.

A

	SH			L4			U35			B35		
Source	d.f.	F	*p*	d.f.	F	*p*	d.f.	F	*p*	d.f.	F	*p*
(Smoothed) Time	1	0.39	0.533	1	5.815	**0.017**	1	2.76	0.098	1	0.626	0.43
WT	-	-	-	1	0.757	0.386	-	-	-	-	-	-
S	-	-	-	-	-	-	-	-	-	1	4.948	**0.027**
Chl *a*	1	4.836	**0.029**	-	-	-	-	-	-	-	-	-
Month	11	47.73	< **0.001**	11	46.08	< **0.001**	11	13.54	< **0.001**	11	25.85	< **0.001**
WT*S	-	-	-	-	-	-	-	-	-	-	-	-
WT*Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
WT*Month	-	-	-	11	1.901	**0.043**	-	-	-	-	-	-
S*Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
S*Month	-	-	-	-	-	-	-	-	-	-	-	-
Chl *a* *Month	-	-	-	-	-	-	-	-	-	-	-	-

B

	SH			L4			U35			B35		
Source	d.f.	F	*p*	d.f.	F	*p*	d.f.	F	*p*	d.f.	F	*p*
(Smoothed) Time	1.901	3.805	0.06	1	0.474	0.492	1	9.186	**0.003**	1	13.64	< **0.001**
WT	1	0.004	0.947	1	0.694	0.406	-	-	-	-	-	-
S	-	-	-	1	0.305	0.581	-	-	-	1	3.908	**0.0498**
Chl *a*	-	-	-	1	0.132	0.716	-	-	-	-	-	-
Month	11	36.96	< **0.001**	11	25.88	< **0.001**	11	25.87	< **0.001**	11	30.69	< **0.001**
WT*S	-	-	-	-	-	-	-	-	-	-	-	-
WT*Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
WT*Month	11	2.418	**0.008**	11	2.233	**0.015**	-	-	-	11	3.173	< **0.001**
S*Chl *a*	-	-	-	1	5.078	**0.026**	-	-	-	-	-	-
S*Month	-	-	-	-	-	-	-	-	-	-	-	-
Chl *a**Month	-	-	-	-	-	-	-	-	-	-	-	-

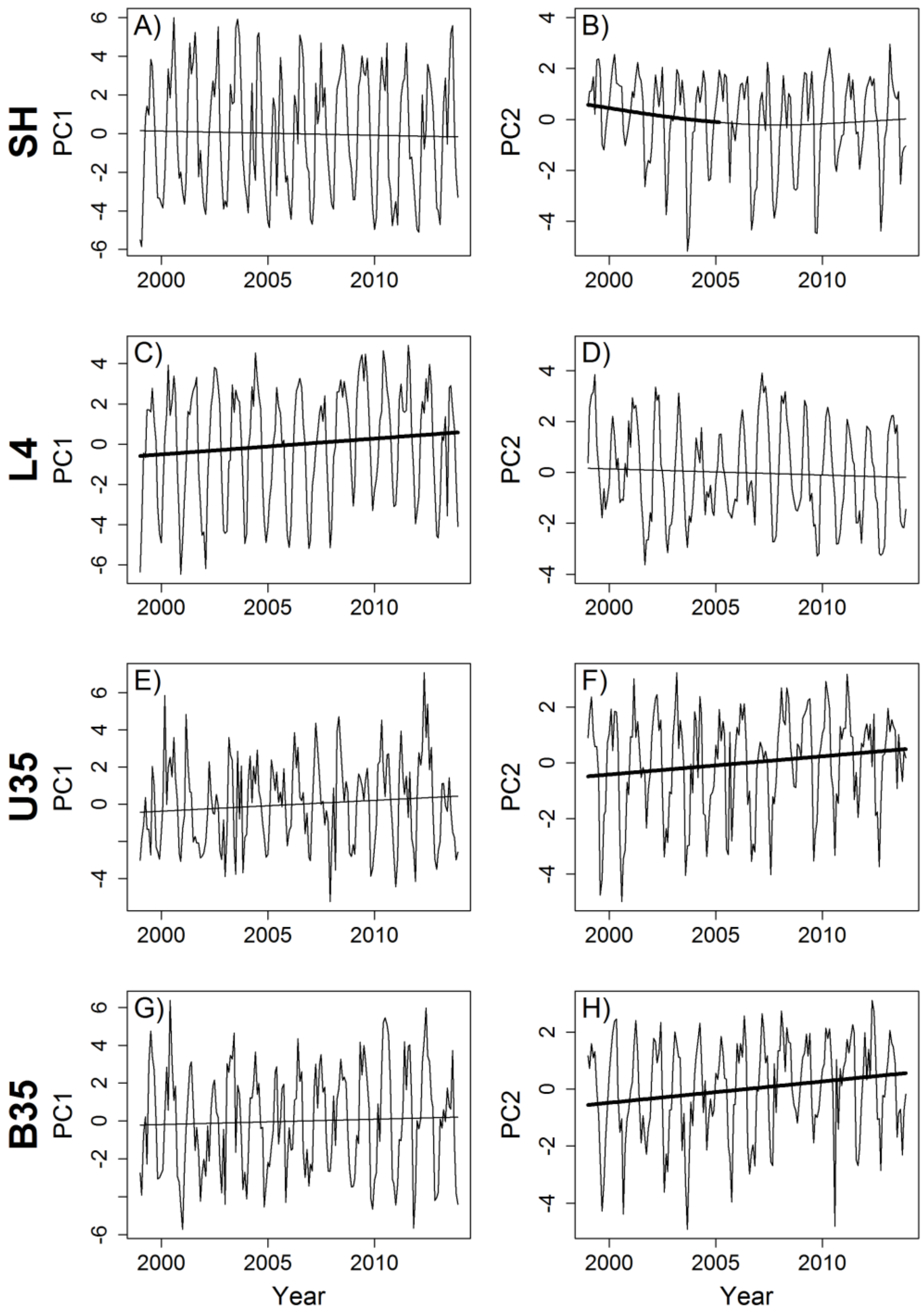

Figure 3. Variation in the first principal component and the second principal component of taxa abundances over (smoothed) time for SH site (A, B, respectively), L4 site (C, D, respectively), U35 site (E, F, respectively) and B35 site (G, H, respectively). Lines with seasonal fluctuations are the observed values of the principal components, and lines without seasonal fluctuations are the smoothed time effects, which represent the relationships in the final models (including (smoothed) time, month and extrinsic drivers (water temperature, Chl *a* and salinity) that were significantly related ($p < 0.05$) to the principal components (including any significant interactions), as terms in the linear predictor matrix). Periods where the first derivative of the smoothed time effect was significantly different from zero are represented with a thick bold line.

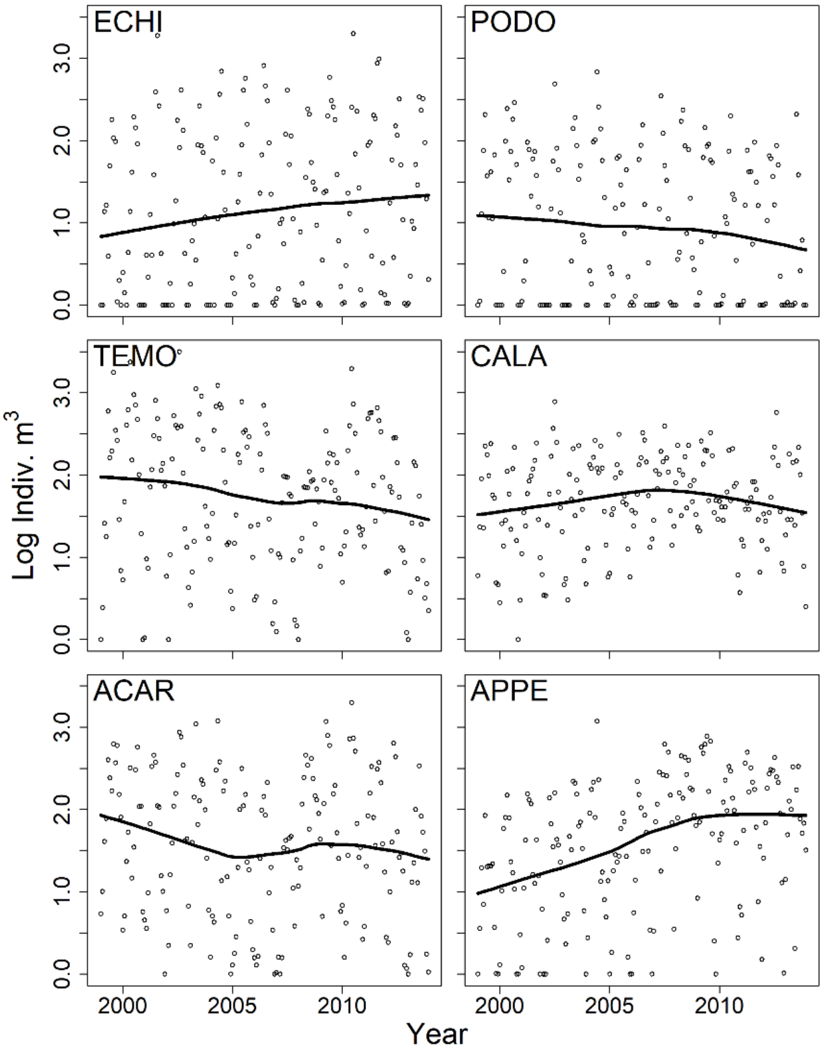

Figure 4. Monthly taxa abundance (as Log of individuals/m^3; empty circles) with a LOESS smoother (bold line) to visualize the variations along the study period at the L4 site. Selected taxa were those with the most positive loadings for the first principal component: ECHI (0.29065), PODO (0.29009), TEMO (0.28764), CALA (0.27780), ACAR (0.27487) and APPE (0. 26834). Taxa abbreviations as in **Table 1**.

On the PC2 (**Table 1B**), the taxa with largest positive loadings had an early annual maximum during early spring at all the sites (April at SH and L4, March at U35 and B35), while the taxa with the most negative loadings, showed the latest annual maxima (September-October at SH and L4, and August – October at B35 and U35). The taxa with highest positive loading were cirripede larvae and fish eggs and larvae at SH and L4, and *Acartia* at U35 and B35. The taxa with highest negative loading included siphonophores, *Corycaeus*, chaetognaths and *Oncaea* at SH and L4, and *Oncaea*, *Corycaeus*, chaetognaths, doliolids and *Temora* at U35 and B35.

At SH, the final model included the interaction between water temperature and month (**Table 1B**) and showed a decreasing trend (significantly negative first derivative) during the 1999 – 2005 period (**Figure 3B**, bold line), which became non-significant since 2005 (non-significant second derivative, i.e. no changes to the temporal trend). From 1999 to 2005, there was a clear increase in abundance for *Corycaeus* and chaetognaths, and a decrease for cirripede larvae (**Figure 5**).

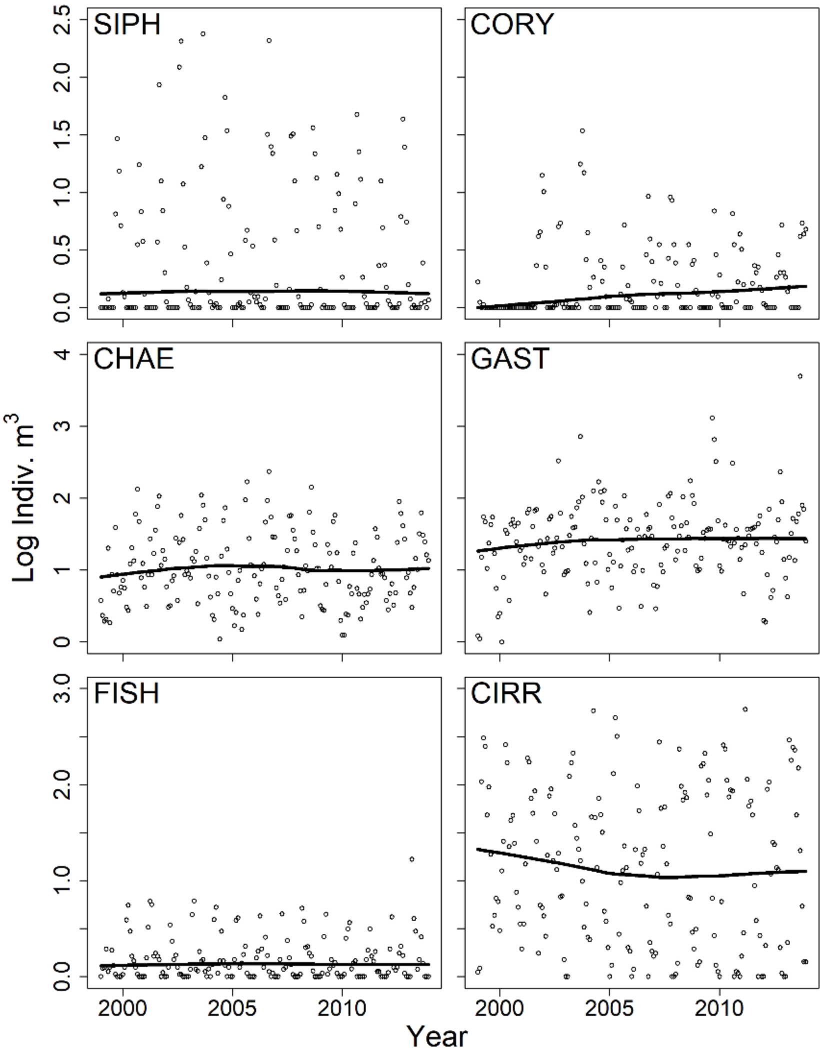

Figure 5. Monthly taxa abundance (as Log of individuals/m^3; empty circles) with a LOESS smoother (bold line) to better visualize the variations along the study period at the SH site. Selected taxa were those with the most positive and most negative loadings for the second principal component: SIPH (-0.46261), CORY (-0.42498), CHAE (-0.310005), GAST (-0.28204), FISH (0.29415) and CIRR (0.29641). Taxa abbreviations as in **Table 1**.

At L4, the smoothed time effect was not significant for PC2, with the final model including a significant association with the interaction between water temperature and month and to a lesser extent also with the interaction between salinity and chlorophyll *a*. At U35 and B35, there was a significant trend for the entire study period (**Figure 3F** and **3H**, bold line). The final model did not retain any environmental covariable at U35 but included a major effect of the interaction between water temperature and month and the weaker effect of salinity at B35 (**Table 2B**). At both sites the abundance of *Oncaea*, *Corycaeus*, *Temora* and chaetognaths decreased in the last years of the series, while *Acartia* increased (**Figures 6** and **7**).

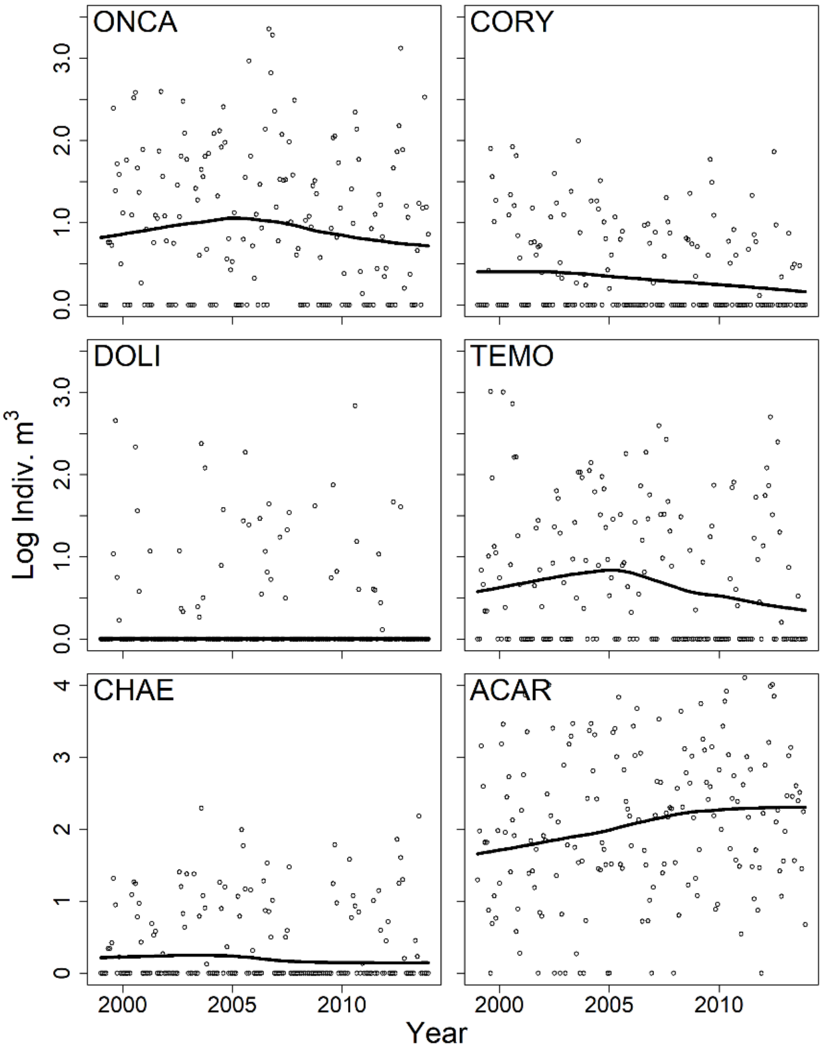

Figure 6. Monthly taxa abundance (as Log of individuals/m^3; empty circles) with a LOESS smoother (bold line) to visualize the variations along the study period at the U35 site. The taxa selected were those with the most positive and most negative loadings for the second principal component: ONCA (-0.42816), CORY (-0.37063), DOLI (-0.35255), TEMO (-0.33963), CHAE (-0.33256) and ACAR (0.24380). Taxa abbreviations as in **Table 1**.

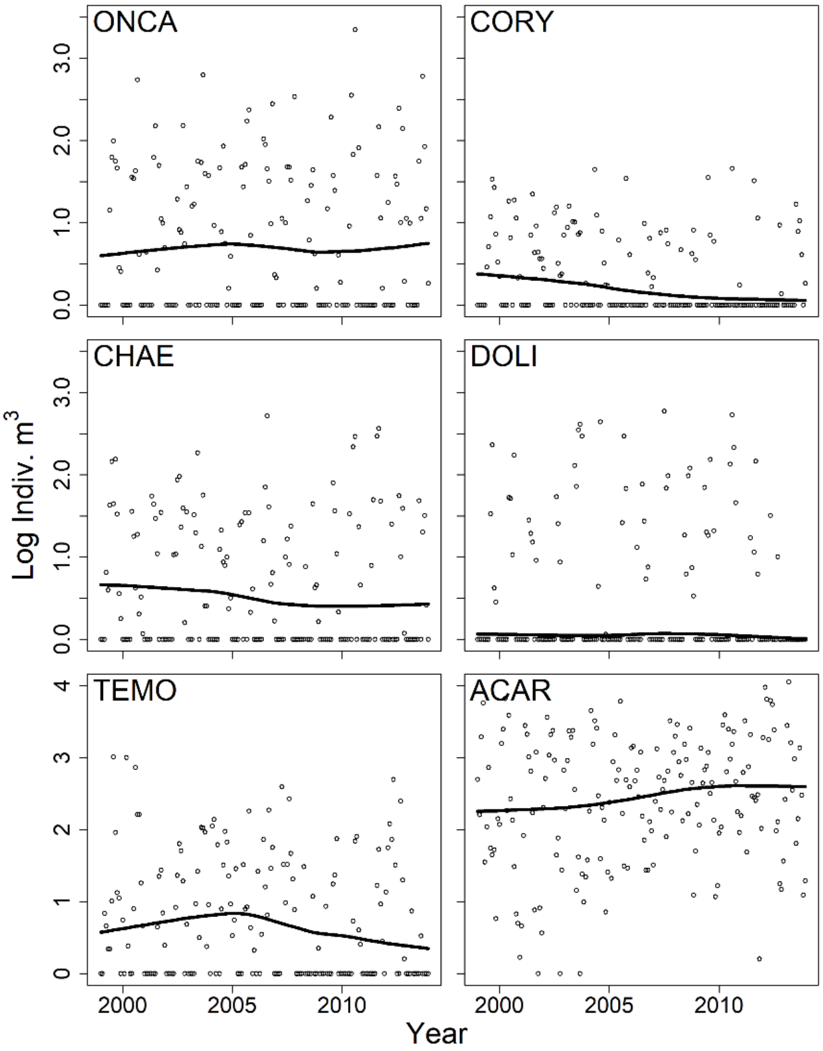

Figure 7. Monthly taxa abundance (as Log of individuals/m^3; empty circles) with a LOESS smoother (bold line) to visualize the variations along the study period at the B35 site. Selected taxa were those with the most positive and most negative loadings for the second principal component: ONCA (-0.40228), CORY (-0.36197), CHAE (-0.35683), DOLI (-0.33011), TEMO (-0.33007) and ACAR (0.33264). Taxa abbreviations as in **Table 1**.

4.3.3. Zooplankton taxa

None of the taxa to test the pattern of change in abundance in relation to environmental factors (i.e. *Acartia*, appendicularians, *Podon*, chaetognaths and *Centropages*) showed the same trend at all sites during the study period (**Figure 8**). Different taxa showed different significant trends: *Acartia* increased at B35 and U35 and decreased at SH. Chaetognaths decreased at B35 and increased at L4. Appendicularians also increased at L4, while *Podon* decreased and *Centropages* increased at B35. The relationships of environmental variables or between-variable interactions with taxa, abundance (**Table 3**) showed that, at SH, the decrease of *Acartia* could not be associated to any of the selected environmental variables. At L4 the increase of appendicularians and chaetognaths, and the decrease of *Podon* were all associated to the interaction between temperature and month. At U35, as for SH, the increase of *Acartia* could not be related to any variable. At B35, *Acartia* and *Centropages* increase were associated to water temperature and the interaction between water temperature and salinity, respectively, and chaetognaths decrease to the interaction between salinity and month.

Furthermore, significant relationships with environmental factors were also found for taxa that did not show significant trends of abundance variation during the study period. At SH, the abundance of appendicularians was mainly explained by the interaction between temperature and month and also chlorophyll *a*, the abundance of *Podon* by the interaction between chlorophyll *a* and month, and water temperature. At L4, the interaction between temperature and month explained the abundance changes of *Acartia*, as for the other taxa at this site. At U35, the abundance changes of *Centropages* and chaetognaths were associated to the interaction between temperature and month and to temperature, respectively. At B35, changes in the abundance of appendicularians were associated to chlorophyll *a* and those of *Podon* to water temperature.

Table 3. Generalized additive mixed models (GAMMs) output showing variation in the abundance of selected zooplankton taxa at SH, L4, U35 and B35 sites, from 1999 to 2013. Relationships between the abundance and standardized (standard deviations from the monthly mean) chlorophyll *a* (Chl *a*), water temperature (WT) and salinity (S), and also month, are shown. All possible two-way interactions were tested. Non-significant interactions and non-significant main effects, when no interaction involved, were removed. Significant *p*-values (< 0.05) are in bold. For smoothed time, d.f. stands for the effective degrees of freedom. Dashes indicate terms removed from the final model.

		SH			L4			U35			B35		
		d.f.	F	*p*	d.f.	F	*p*	d.f.	F	*p*	d.f.	F	*p*
Acartia	(Smoothed) Time	1	4.270	**0.040**	1	1.128	0.290	1	14.76	< **0.001**	1	6.812	**0.009**
	WT	-	-	-	1	0.882	0.349	-	-	-	1	4.984	**0.027**
	S	-	-	-	-	-	-	-	-	-	-	-	-
	Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
	Month	11	33.99	< **0.001**	11	15.54	< **0.001**	11	13.46	< **0.001**	11	11.06	< **0.001**
	WT*S	-	-	-	-	-	-	-	-	-	-	-	-
	WT*Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
	WT*Month	-	-	-	11	1.888	**0.045**	-	-	-	-	-	-
	S*Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
	S*Month	-	-	-	-	-	-	-	-	-	-	-	-
	Chl *a**Month	-	-	-	-	-	-	-	-	-	-	-	-
Appendicularians	(Smoothed) Time	1	0.645	0.423	1	30.56	< **0.001**	1	0.703	0.403	1	2.680	0.103
	WT	1	0.475	0.492	1	1.327	0.251	-	-	-	-	-	-
	S	-	-	-	-	-	-	-	-	-	-	-	-
	Chl *a*	1	10.87	**0.001**	-	-	-	-	-	-	1	5.210	**0.024**
	Month	11	24.04	< **0.001**	11	14.20	< **0.001**	11	8.298	< **0.001**	11	5.881	< **0.001**
	WT*S	-	-	-	-	-	-	-	-	-	-	-	-
	WT*Chl *a*	1	5.065	**0.026**	-	-	-	-	-	-	-	-	-
	WT*Month	11	3.642	< **0.001**	11	3.437	< **0.001**	-	-	-	-	-	-
	S*Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
	S*Month	-	-	-	-	-	-	-	-	-	-	-	-
	Chl *a**Month	-	-	-	-	-	-	-	-	-	-	-	-
Podon	(Smoothed) Time	1.000	0.848	0.359	1	5.322	**0.022**	1	0.028	0.868	1	2.993	0.086
	WT	1	2.134	0.146	1	3.275	0.072	-	-	-	1	5.555	**0.020**
	S	-	-	-	-	-	-	-	-	-	-	-	-
	Chl *a*	1	1.207	0.274	-	-	-	-	-	-	-	-	-
	Month	11	13.84	< **0.001**	11	36.08	< **0.001**	11	10.81	< **0.001**	11	14.42	< **0.001**
	WT*S	-	-	-	-	-	-	-	-	-			
	WT*Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
	WT*Month	11	2.239	**0.015**	11	2.411	**0.009**	-	-	-	-	-	-
	S*Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
	S*Month	-	-	-	-	-	-	-	-	-	-	-	-
	Chl *a**Month	11	3.116	< **0.001**	-	-	-	-	-	-	-	-	-
Chaetognaths	(Smoothed) Time	1	0.049	0.826	1	4.690	**0.032**	1	0.173	0.678	1	7.542	**0.006**
	WT	-	-	-	1	0.871	0.352	1	4.081	**0.045**	-	-	-
	S	-	-	-	-	-	-	-	-	-	1	2.370	0.126
	Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
	Month	11	15.68	< **0.001**	11	8.425	< **0.001**	11	5.110	< **0.001**	11	**13.75**	< **0.001**
	WT*S	-	-	-	-	-	-	-	-	-	-	-	-
	WT*Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
	WT*Month	-	-	-	11	1.865	**0.048**	-	-	-	-	-	-
	S*Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
	S*Month	-	-	-	-	-	-	-	-	-	11	2.066	**0.026**
	Chl *a**Month	-	-	-	-	-	-	-	-	-	-	-	-
Centropages	(Smoothed) Time	1	0.101	0.751	1	2.586	0.110	1	1.090	0.298	1	3.658	**0.057**
	WT	-	-	-	1	0.858	0.355	1	2.390	0.124	1	0.100	0.753
	S	-	-	-	-	-	-	-	-	-	1	0.291	0.592
	Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
	Month	11	20.22	< **0.001**	11	13.63	< **0.001**	11	6.490	< **0.001**	11	**5.436**	< **0.001**
	WT*S	-	-	-	-	-	-	-	-	-	1	4.845	**0.029**
	WT*Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
	WT*Month	-	-	-	11	1.889	**0.045**	11	2.387	**0.009**	-	-	-
	S*Chl *a*	-	-	-	-	-	-	-	-	-	-	-	-
	S*Month	-	-	-	-	-	-	-	-	-	-	-	-
	Chl *a**Month	-	-	-	-	-	-	-	-	-	-	-	-

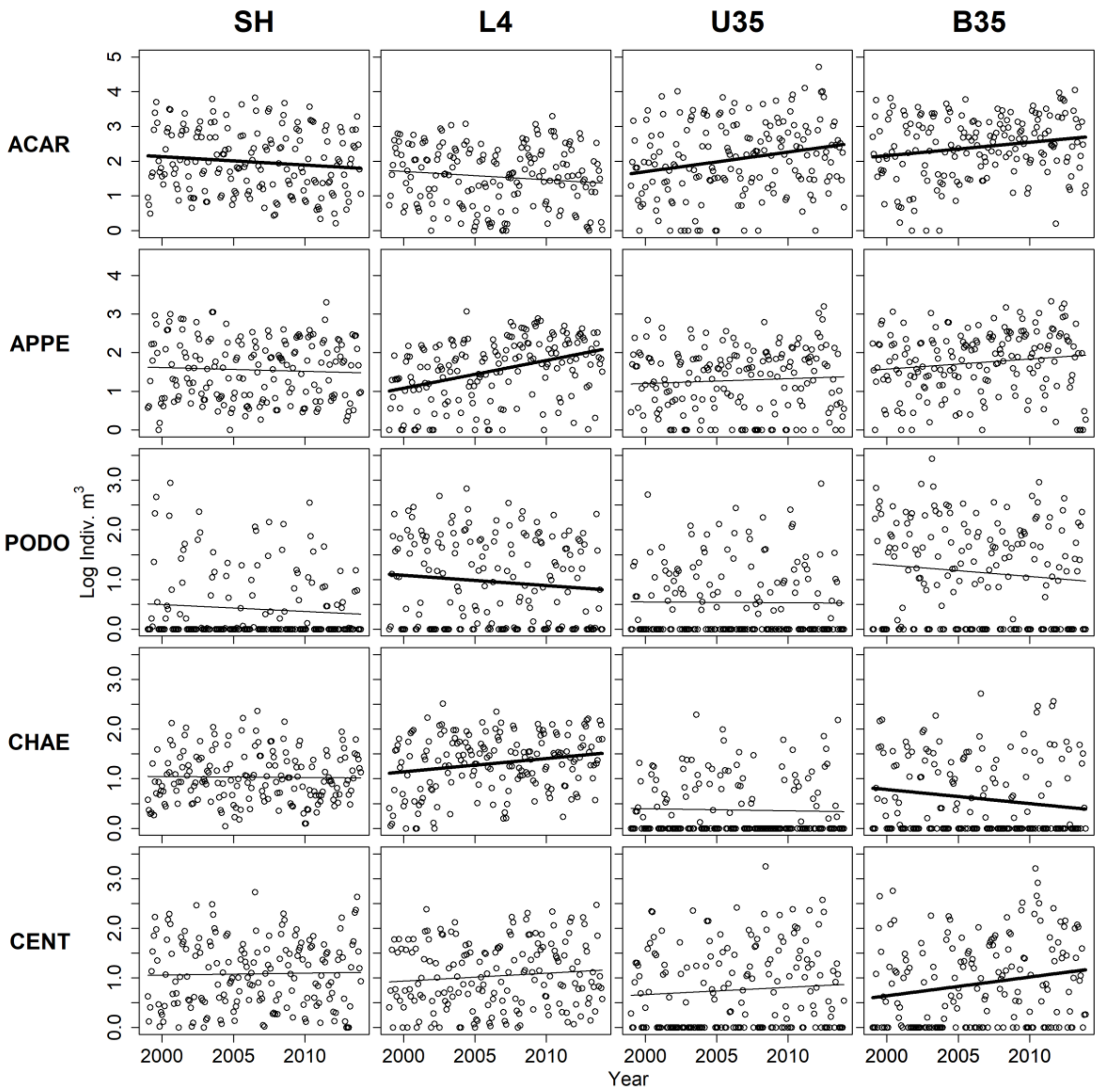

Figure 8. Variation in the abundance of selected taxa over (smoothed) time at the SH, L4, U35 and B35 sites: ACAR (*Acartia*), APPE (appendicularians), PODO (*Podon*), CHAE (chaetognaths) and CENT (*Centropages*). Empty circles represent the abundance values obtained during the study period and the lines are the smoothed time effects. Periods where the first derivative of the smoothed time effect was significantly different from zero are represented with a thick bold line.

4.4. Discussion

4.4.1. Zooplankton community

Zooplankton community patterns of multiannual change identified by GAMMs were either random or gradual (trend) changes at the studied sites. Thus, no abrupt deviations from temporal trends, or shifts, in species composition and abundance were detected, this denoting no noticeable regime changes affecting the zooplankton of the European shelf area covered by our four monitoring sites for the period 1999 – 2013. Rapid shifts have been reported in synchrony during the late 1980s and early 1990s at different marine regions of the Northern hemisphere, and attributed mainly to common large-scale climate drivers which modify sea temperature regimes (Möllmann and Diekmann, 2012), but also to a major event of global scope and scale which involved natural and anthropogenic multiplier effects (Reid *et al*., 2016). In the North Sea, where the SH site is located, these substantial regime shifts affected all trophic levels of the pelagic system (Alheit *et al*., 2005; Weijerman *et al*., 2005), and a cold-biological regime until 1982 and a warm-biological dynamic regime since 1984 were distinguished in time-series until 1999 (Beaugrand and Ibañez, 2004). The extension of the analysis of the North Sea plankton community to the 2000s, however, has allowed the detection of a later regime change around 1998 that was evidenced by a shift with variable timing within the 1996 – 2003 period depending on the planktonic group and also on species within a taxonomic group (Alvarez-Fernandez *et al*., 2012; Beaugrand *et al*., 2014). But this shift also preceded time-series analyzed in our study, which start in 1999 and cover the 2000s and early 2010s, and our results revealed trends in some components of the zooplankton community but not shifts since 2000.

The trends observed in the present work differed between the sites under study in the zooplankton components responsible for the gradual change or in the duration of the trend. The zooplankton assemblages responsible for the trends differed mainly from L4 to the other three sites. At L4 the trend was detected for the zooplankton assemblage responsible for the first principal component that included taxa with annual maxima in spring (*Podon*, *Acartia*, appendicularians and *Evadne*) and summer (echinoderm larvae, *Temora* and *Calanus*), while at SH, U35 and B35 the trend was detected for zooplankton assemblages responsible for the second principal component, and included primarily common late summer – autumn taxa (*Corycaeus*, *Oncaea*, and chaetognaths) in opposition to earliest peaking ones (cirripede and fish larvae and eggs at SH and *Acartia* at U35 and B35). Morse *et al*, (2017), comparing long-term patterns of zooplankton variations among adjacent ecoregions of the western Atlantic, also found that the composition and response of spring zooplankton was more variable across ecoregions in contrast to an autumn community characterized by a more concise grouping of taxa with similar patterns of change across all ecoregions.

Final models for both the trend of the spring – summer assemblage at the L4 and the trends of late-summer – autumn assemblages at the other sites included the interaction between water temperature and month as the main explanatory variable, except at U35 where no variables were selected by the model. Previous studies at the L4 site have found an overall gradual warming of 0.68 ºC per decade (Highfield *et al*., 2010; Smyth *et al*., 2010), but for the period analyzed (1999 – 2013) water temperature did not show an increasing trend at any site, and the model actually stated that it was not temperature but the interaction between temperature and month the driving factor of gradual changes in zooplankton communities. This means that only the changes of temperature at given months of a specific season drove the observed zooplankton trends. The comparison of water temperature by seasons at each site reveals that temperature trends differ between seasons at all sites. The trends of the spring – summer assemblage at L4 and the summer – autumn assemblage at the other sites agreed with the decrease of temperature in spring – summer and the increase in autumn at L4, and with the predominant decrease of temperature in summer – autumn and the increase or lack of trend in spring at the other sites. Similar season-related relationships between zooplankton and temperature have been reported by Dippner *et al.* (2000) in the Central Baltic Sea, where the interannual variability of zooplankton species in spring was found to be controlled by the spring sea surface temperature.

The gradual change in the spring – summer zooplankton community at L4 was mainly related to the increase in echinoderm larvae and appendicularians, and the decrease in three abundant crustacean zooplankters i.e. *Podon*, *Temora* and *Acartia*, which occurred at the same time water temperature decreased (although the trend for the latter was non-significant). Some of these results, however, are in conflict with the expected response to the decrease of temperature in spring – summer. For echinoderm larvae a positive effect of temperature on their abundance peak and development rates has been previously described (Edwards and Richardson, 2004; Kirby *et al*., 2007), but our results do not support a positive relation of echinoderm larvae with temperature over the period analyzed at this site, this suggesting that other factors not taken into account in the present study, like wind (Eloire *et al*., 2010), might be affecting their dynamics. The decreasing abundance of *Temora* and *Acartia* can be consistent with a temperature effect if the response of these taxa to temperature at L4 operates as in the Baltic Sea, where the reproduction of *Temora* and *Acartia* in spring is favored by higher water temperature (Dippner *et al.*, 2000). This season-linked effect of temperature should be differentiated from that of the general warming, that is claimed to affect negatively *Temora longicornis* (99.88% of the total *Temora* at L4) in this area (Kane and Prezioso, 2008; Eloire *et al*., 2010), since our study is restricted to a set of years where a general warming was not detected. Relationships of zooplankton with environmental conditions have been found to be more evident when predation pressure decreases

(Dippner *et al*., 2000). In this context, an unexpected relationship with temperature could be plausible if a top-down control is occurring. The significant increase of chaetognaths at L4 during the study period might support the hypothesis of a predation driven decrease of *Temora* and *Acartia*, because the enhancement of predators could explain the decrease in the abundance of co-occurring prey taxa (Nicholas and Frid, 1999, Bonnet *et al*., 2010). Chaetognath species found at L4 prey selectively and primarily, with higher predation impact, on copepods (Oresland, 1987; Tönnesson and Tiselius, 2005), and show higher densities from June to October with a temporal coherence with the higher densities of Temora and Acartia from May to September (Chapter 1, this work).

At SH there was a significant trend only for the 1999 – 2005 period that was mainly accounted for by the increase of late summer – early autumn taxa like chaetognaths, gastropod larvae and *Corycaeus* and the decrease of early spring taxa like cirripede larvae. As for L4, the final model selected temperature in interaction with month as the main driver of zooplankton changes. However, temperature did not increase nor decrease significantly at any season during the entire study period, but for the 1999 – 2005 period there was an overall significant increase (p= 0.05) that could presumably favor warm water taxa but could have a negative effect on cirripede larvae, whose larval size and survival decrease with increasing temperature (Anil and Kurian, 1996; Harms, 1986). The fact that this zooplankton changing trend stopped in 2005 suggests a relationship with the shifts in plankton ecosystems of the North Sea detected between 1996 and 2003 by Beaugrand *et al.* (2014), and previously suggested by Weijerman *et al.* (2005). The former authors attribute the shifts of several planktonic groups in the North Sea between 1996 and 2003 to a major discontinuity in the intensity of warming (Levitus *et al*., 2009; Raitsos *et al*., 2010; Raid and Beaugrand, 2012) which agrees with the increasing trend of temperature observed at SH from 1999 to 2004 in our study.

The trends obtained at both U35 and B35 showed great similarity and the main taxa responsible for the change were those peaking later in the year in opposition to those peaking earliest, as at SH. However, in contrast to findings for SH, at U35 and B35 the most representative taxon of the early spring assemblage was *Acartia*, which showed an increasing trend over the entire study period, and among the most influential taxa of the late assemblage were doliolids and *Temora*, in addition to chaetognaths, *Oncaea* and *Corycaeus*. which showed mostly decreasing trends. Although there were no variables selected by the model at U35, the temperature in interaction with month was included in the final model at B35, and the season-related behavior of temperature was similar in both sites. Although not significant, the common increase of spring temperature might be the cause of the increase of *Acartia* (mostly *Acartia clausi*), as was also observed at L4, and the common decrease of summer temperature the cause of the decrease of *Temora* (dominated by *Temora stylifera*), *Oncaea* and *Corycaeus* (*Ditrichocorycaeus anglicus*). Temperature has a positive effect on *A. clausi* species growth rates (Leandro *et al*., 2006), while thermophilic opportunistic species such as *Temora stylifera* and *Ditrichocorycaeus anglicus* (Di Capua and Mazzocchi, 2004; Valdés *et al*., 2007) might be negatively affected by the decrease of temperature in summer. It is important to note also, that *T. stylifera* is an omnivore copepod, but mainly opportunistic herbivore (Turner, 1984), that might have suffered the decrease of Chl *a* during summer, especially at B35 but to a lower extent also at U35.

4.4.2. Zooplankton taxa

None of the individual taxa showed the same pattern of change at the four sites under study, but most of them had a same trend at the two Bay of Biscay sites as stated above for *Acartia*, *Temora* and *Corycaeus*. This denotes, that such trends were driven to a larger extent by hydroclimatic forces acting at regional scale that affect similarly U35 and B35 sites, than by local anthropogenic changes which differed between sites during the study period. Also, in the case of *Acartia*, findings can be related to the dynamics of a single species i.e. *A. clausi* which was dominant in the genus *Acartia* at all sites. For *A. Clausi* opposite trends of change were observed, with significant increasing trends at both U35 and B35 and a significant decreasing trend at SH and a non-significant trend of decrease at L4. Although no environmental variables were selected by models at SH and U35, the findings that relate *A. clausi* dynamics to temperature at L4 and B35, in spite of the higher variations showed by salinity and chlorophyll *a*, suggest an overall major control of this species by temperature in relation to the other two environmental factors analyzed in this study. The relationship between *Acartia* and temperature derived from this study is in accordance with previous findings at L4, where its phenology was found to be particularly temperature-sensitive (Atkinson *et al*. 2015). In addition, *A. clausi* showed a common pattern of variation in abundance with temperature for the four sites (Chapter 5, this work) that determines an annual optimum for this species in early spring at the southernmost sites (U35 and B35) but in summer at the northernmost site (SH; Chapter 1, this work). This delay with latitude implies that *A. clausi* supra-annual variations may be primarily governed by temperature changes differing between seasons. This may lead to opposite trends at the same time at different regions of the North Atlantic.

Appendicularians and *Podon* showed increasing and decreasing trends, respectively, directly related to the interaction between temperature and month at L4. At this site both taxa show the standard annual peak in late spring – early summer when temperature decreased, this suggesting an opposite response to temperature. Non-significant increases of appendicularians and decreases of *Podon* were also observed at B35, where both taxa have the standard annual peak at the same time in June, but in this case the increase of appendicularians was related to chlorophyll *a* and the decrease of *Podon* to temperature. In addition, both temperature and chlorophyll *a* (alone, in interaction between them or in interaction with month) were selected by the models to explain the variations of appendicularians and *Podon* at SH. Similarly, the increasing trend of chaetognaths and the non-significant increase of *Centropages* at L4 were related to temperature in interaction with month, while the decreasing trend of chaetognaths and the increasing trend of *Centropages* at B35 were related to salinity in interaction with month and to the interaction between temperature and salinity, respectively. All this draws a complex picture of the relationships between these taxa and environmental factors, and prevents the selection of a single variable as major driver of a given taxa at all sites under study.

At the L4 site, and for a longer period that overlaps the time-series we used until 2007, Eloire *et al*. (2010) already found a decreasing trend for *Podon*, which could not be related to any environmental parameter but was negatively correlated to the winter NAO index, and an increase of chaetognaths that could not be related to any environmental parameter either. Our results did not show a relationship between these taxa abundance trends and overall temperature, but they showed an association with the interaction of temperature with month, that is to say, they showed a season-dependent effect of temperature. Season-dependent effects of temperature on plankton series from the North Atlantic have been reported by other workers too in recent years (Feng *et al.*, 2014).

4.4.3. Environmental variables

The interaction between temperature and month was selected as the only or main driver of zooplankton trends at community level in all cases, except at U35, and also of individual taxa trends at L4. Temperature, without interaction or in interaction with another variable, was also selected by the models in the case of some taxa trends at SH, B35 and U35. These results indicate that temperature was the major driver of zooplankton changes at all sites, although no clear trends of temperature were obtained at any of the studied sites. This corroborates the predominant role of temperature as driver of the most relevant multiannual changes in the zooplankton of different regions of the Northeastern Atlantic (e.g. Alheit *et al.*, 2005; Weijerman *et al.*, 2005; Beaugrand *et al.*, 2014). In contrast, chlorophyll *a*, alone or in interaction with month or another variable, was only selected by the models at SH and B35, the two sites where this variable showed clear trends in some seasons. Similarly, salinity, alone or in interaction with month or another variable, was only selected by the models occasionally at L4, and in several cases at B35, where a signicant increasing trend of of salinity was found. This suggests that both chlorophyll *a* and salinity play secondary roles , and are more important at more local scales.

A remarkable result is the lack of retained environmental variables in the models that revealed zooplankton changes at community level and in most of the models for individual taxa at U35. This could be due to the higher year-to-year variability of the seasonal patterns of environmental variables, and the lower synchrony of the annual cycles of temperature and chlorophyll *a* at this site, in which the contribution of environmental variables to explain the observed zooplankton variability has been found to be the lowest of the four sites under study (Chapter 1, this work), However, the high coincidence in the trends at community level and individual taxa between the sites of U35 and B35 suggests that zooplankton changes mostly respond to the same environmental drivers at both sites.

4.5. References

Anil, A. C., Kurian, J. (1996). Influence of food concentration, temperature and salinity on the larval development of *Balanus amphitrite*. *Marine Biology, 127*(1), 115–124. https://doi.org/10.1007/BF00993651.

Aravena, G., Villate, F., Uriarte, I., Iriarte, A., Ibáñez, B. (2009). Response of *Acartia* populations to environmental variability and effects of invasive congenerics in the estuary of Bilbao, Bay of Biscay. *Estuarine, Coastal and Shelf Science, 83*(4), 621–628. https://doi.org/10.1016/j.ecss.2009.05.013.

Atkinson, A., Harmer, R. A., Widdicombe, C. E., McEvoy, A. J., Smyth, T. J., Cummings, D. G., Somerfield, P. J., Maud, J. L., McConville, K. (2015). Questioning the role of phenology shifts and trophic mismatching in a planktonic food web. *Progress in Oceanography, 137,* 498–512. doi:10.1016/j.pocean.2015.04.023.

Beaugrand, G., Ibañez, F., Reid, P.C. (2000). Spatial, seasonal and long-term fluctuations of plankton in relation to hydroclimatic features in the English Channel, Celtic Sea and Bay of Biscay. *Marine Ecology Progress Series, 200,* 93–102. doi: 10.3354/meps200093.

Beaugrand, G., Ibanez, F. (2004). Monitoring marine plankton ecosystems. II: Long-term changes in North Sea calanoid copepods in relation to hydro-climatic variability. *Marine Ecology Progress Series, 284,* 35–47. https://doi.org/10.3354/meps284035.

Beaugrand, G., Reid, P. C., Ibañez, F., Lindley, J. A., Edwards, M. (2002). Reorganization of North Atlantic marine copepod biodiversity and climate. *Science, 296*(5573), 1692–1694. https://doi.org/10.1126/science.1071329.

Bonnet, D., Lindeque, P. K., Harris, R. P. (2010). *Sagitta setosa* predation on *Calanus helgolandicus* in the English Channel. *Journal of Plankton Research, 32*(5), 725–737. https://doi.org/10.1093/plankt/fbq008.

Clare, D. S., Spencer, M., Robinson, L. A., Frid, C. L. J. (2017). Explaining ecological shifts: the roles of temperature and primary production in the long-term dynamics of benthic faunal composition. *Oikos, 1*, 1–11. https://doi.org/10.1111/oik.03661.

Cross, J., Nimmo-Smith, W. A. M., Hosegood, P. J., Torres, R. (2015). The role of advection in the distribution of plankton populations at a moored 1-D coastal observatory. *Progress in Oceanography, 137,* 342–359. https://doi.org/10.1016/j.pocean.2015.04.016.

Di Capua, I., and Mazzocchi, M. G. (2004). Population structure of the copepods Centropages typicus and Temora stylifera in different environmental conditions. *ICES Journal of Marine Science, 61*(4), 632–644. https://doi.org/10.1016/j.icesjms.2004.03.007.

Eloire, D., Somerfield, P. J., Conway, D. V. P., Halsband-Lenk, C., Harris, R., Bonnet, D. (2010). Temporal variability and community composition of zooplankton at station L4 in the Western Channel: 20 years of sampling. *Journal of Plankton Research, 32*(5), 657–679. https://doi.org/10.1093/plankt/fbq009.

Feng, J., Stige, L. C., Durant, J. M., Hessen, D. O., Zhun L., Hjermann, D. Ø, Llope, M., Stenseth, N. C. (2014). Large-scale season-dependent effects of temperature and zooplankton on phytoplankton in the North Atlantic. *Marine Ecology Pregress Series, 502,* 25–37. https://doi.org/10.3354/meps10724.

Harms, J. (1986). Effects of temperature and salinity on larval development of Elminius modestus (Crustacea, Cirripedia) from Helgoland (North Sea) and New Zealand. *Helgoländer Meeresuntersuchungen, 40*(4), 355–376. https://doi.org/10.1007/BF01983818.

Hátún, H., Payne, M. R., Beaugrand, G., Reid, P. C., Sandø, A. B., Drange, H., Hansen, B., Jacobsen, J. A., Bloch, D. (2009). Large bio-geographical shifts in the north-eastern Atlantic

Ocean: From the subpolar gyre, via plankton, to blue whiting and pilot whales. *Progress in Oceanography*, *80*(3–4), 149–162. https://doi.org/10.1016/j.pocean.2009.03.001.

Haury, L., McGowan, J., Wiebe, P. (1978). Patterns and process in the time-space scales of plankton distributions. *Spatial Pattern in Plankton Communities*, *3*, 277–327. https://doi.org/10.1017/CBO9781107415324.004.

Highfield, J. M., Eloire, D., Conway, D. V. P., Lindeque, P. K., Attrill, M. J., Somerfield, P. J. (2010). Seasonal dynamics of meroplankton assemblages at station L4. *Journal of Plankton Research*, *32*(5), 681–691. https://doi.org/10.1093/plankt/fbp139.

Kirby, R. R., Beaugrand, G., Lindley, J. a., Richardson, A. J., Edwards, M., Reid, P. C. (2007). Climate effects and benthic-pelagic coupling in the North Sea. *Marine Ecology Progress Series, 330*, 31–38. https://doi.org/10.3354/meps330031.

Kleppel, G. S., Pieper, R. E. (1984). Phytoplankton pigments in the gut contents of planktonic copepods from coastal waters off southern California. *Marine Biology*, *78*(2), 193–198. https://doi.org/10.1007/BF00394700.

Leandro, S. M., Queiroga, H., Rodríguez-Graña, L., Tiselius, P. (2006). Temperature-dependent development and somatic growth in two allopatric populations of *Acartia clausi* (Copepoda: Calanoida). *Marine Ecology Progress Series, 322*, 189–197. https://doi.org/10.3354/meps322189.

Levitus, S., Antonov, J. I., Boyer, T. P., Locarnini, R. A., García, H. E., Mishonov, A. V. (2009). Global ocean heat content 1955 – 2008 in light of recently revealed instrumentation problems. *Geophysical Research Letters*, *36*(7), 1–5. https://doi.org/10.1029/2008GL037155.

Lees, K., Pitois, S., Scott, C., Frid, C., MacKinson, S. (2006). Characterizing regime shifts in the marine environment. *Fish and Fisheries*, *7*(2), 104–127. https://doi.org/10.1111/j.1467-2979.2006.00215.x.

Litt, E. J., Hardman-Mountford, N. J., Blackford, J. C., Mitchelson-Jacob, G., Goodman, A., Moore, G. F., Cummings, D. G., Butenschön, M. (2010). Biological control of pCO2 at station L4 in the Western English Channel over 3 years. *Journal of Plankton Research*, *32*(5), 621–629. https://doi.org/10.1093/plankt/fbp133.

Magurran, A. E., Baillie, S. R., Buckland, S. T., Dick, J. McP., Elston, D. A., Scott, E. M., Smith, R. I., Sommerfield, P. J., Watt, A. D. (2012). Long-term datasets in biodiversity research and monitoring: assessing change in ecological communities through time. *Trends in Ecology and Evolution*, *25*(10), 574–582. https://doi.org/10.1016/j.tree.2010.06.016.

McGill, B. J., Dornelas, M., Gotelli, N. J., Magurran, A. E. (2015). Fifteen forms of biodiversity trend in the anthropocene. *Trends in Ecology and Evolution*, *30*(2), 104–113. https://doi.org/10.1016/j.tree.2014.11.006.

Molinero, J. C., Reygondeau, G., Bonnet, D. (2013). Climate variance influence on the non-stationary plankton dynamics. *Marine Environmental Research*, *89*, 91–96. https://doi.org/10.1016/j.marenvres.2013.04.006.

Morse, R. E., Friedland, K. D., Tommasi, D., Nye, J. (2017). Distinct zooplankton regime shift patterns across ecoregions of the U.S. Northeast continental shelf Large Marine Ecosystem. *Journal of Marine Systems*, *165*, 77–91. https://doi.org/10.1016/j.jmarsys.2016.09.011.

Nicholas, K. R., Frid, C. L. J. (1999). Occurrence of hydromedusae in the plankton off Northumberland (western central North Sea) and the role of planktonic predators. *Journal of the Marine Biological Association of the United Kingdom*, *79*(6), 979–992. https://doi.org/10.1017/S0025315499001216.

Raitsos, D. E., Beaugrand, G., Georgopoulos, D., Zenetos, A., Pancucci-Papadopoulou, M., Theocharis, A., Papathanassiou, E. (2010). Global climate change amplifies the entry of tropical species into the Eastern Mediterranean Sea. *Limnology and Oceanography*, *55*(4), 1478–1484. doi:10.4319/lo.2010.55.4.1478.

Reid, P. C., Beaugrand, G. (2012). Global synchrony of an accelerating rise in sea surface temperature. *Journal of the Marine Biological Association of the United Kingdom*, *92*(7), 1435–1450. https://doi.org/10.1017/S0025315412000549.

Reid, P. C., Hari, R. E., Beaugrand, G., Livingstone, D. M., Marty, C., Straile, D., Bariwich, J., Goberville, E., Adrian, R., Aono, Y., Brown, R., Foster, J., Groisman, P., Héalouet, P., Hsu, H.-H., Kirby, R., Knight, J., Kraberg, A., Li, J., Lo, T.-T., Myneni, R. B., North, R. P., Pounds, J. A., Sparks, T., Stübi, R., Tian, Y., Wiltshire, K. H., Xiao, D., Zhu, Z. (2016). Global impacts of the 1980s regime shift. *Global Change Biology*, *22*(2), 682–703. https://doi.org/10.1111/gcb.13106.

Reygondeau, G., Beaugrand, G. (2011). Future climate-driven shifts in distribution of *Calanus finmarchicus*. *Global Change Biology*, *17*(2), 756–766. https://doi.org/10.1111/j.1365-2486.2010.02310.x.

Reygondeau, G., Molinero, J. C., Coombs, S., MacKenzie, B. R., Bonnet, D. (2015). Progressive changes in the Western English Channel foster a reorganization in the plankton food web. *Progress in Oceanography*, *137B*, 524–532. https://doi.org/10.1016/j.pocean.2015.04.025.

Rombouts, I., Beaugrand, G., Ibañez, F., Gasparini, S., Chiba, S., Legendre, L. (2010). A multivariate approach to large-scale variation in marine planktonic copepod diversity and its environmental correlates. *Limnology and Oceanography*, *55*(5), 2219–2229. https://doi.org/10.4319/lo.2010.55.5.2219.

Smyth, T. J., Fishwick, J. R., Al-Moosawi, L., Cummings, D. G., Harris, C., Kitidis, V., Rees. A., Martinez-Vicente, V., Woodward, E. M. S. (2010). A broad spatio-temporal view of the Western English Channel observatory. *Journal of Plankton Research*, *32*(5), 585–601. https://doi.org/10.1093/plankt/fbp128.

Spencer, M., Mieszkowska, N., Robinson, L. A., Simpson, S. D., Burrows, M. T., Birchenough, S. N. R., Capasso, E., Cleall-Harding, P., Crummy, J., Duck, C., Eloire, D., Frost, M., Hall, A. J., Hawkins, S. J., Johns, D. G., Sims, D. W., Smyth, T. J., Frid, C. L. J. (2012). Region-wide changes in marine ecosystem dynamics: State-space models to distinguish trends from step changes. *Global Change Biology*, *18*(4), 1270–1281. https://doi.org/10.1111/j.1365-2486.2011.02620.x.

Stenseth, N. C., Mysterud, A., Ottersen, G., Hurrell, J. W., Chan, K. S., Lima, M. (2002). Ecological effects of climate fluctuations. *Science*, *297*(5585), 1292–1296. Retrieved from /home/siccha/Science/Library/Stenseth%5Cn2002.pdf.

Turner, J. T. (1984). Zooplankton feeding ecology: contents of fecal pellets of the copepods Temora turbinata and T. stylifera from continental shelf and slope waters near the mouth of the Mississippi River. *Marine Biology*, *82*(1), 73–83. https://doi.org/10.1007/BF00392765.

Valdés, L., López-Urrutia, A., Cabal, J., Alvarez-Ossorio, M., Bode, A., Miranda, A., Cabanas, M., Huskin, I., Anadón, R., Alvarez-Marqués, F., Llope, M., Rodríguez, N. (2007). A decade of sampling in the Bay of Biscay: What are the zooplankton time series telling us? *Progress in Oceanography*, *74*, 98–114. doi:10.1016/j.pocean.2007.04.016.

Villate, F., Iriarte, A., Uriarte, I., Sanchez, I. (2017). Seasonal and interannual variability of mesozooplankton in two contrasting estuaries of the Bay of Biscay: relationship to environmental factors. *Journal of Sea Research*, *130,* 189–203. doi: 10.1016/j.seares.2017.05.002.

Weijerman, M., Lindeboom, H., Zuur, A. F. (2005). Regime shifts in marine ecosystems of the

North Sea and Wadden Sea. *Marine Ecology Progress Series*, *298*, 21–39. https://doi.org/Doi 10.3354/Meps298021.

Wood, S.N. (2004). Stable and efficient multiple smoothing parameter estimation for generalized additive models. *Journal of the American Statistical Association*. 99, 673–686.

Wood, S.N. (2017) Generalized Additive Models: An Introduction with R. *Chapman and Hall/CRC*. 476 pp.

Chapter 5. *Interacting effects of latitude, temperature, salinity, depth and distance offshore in modulating zooplankton assemblages*

Interacting effects of environmental factors in modulating zooplankton assemblages across the NE Atlantic Shelves Province, from the Bay of Biscay (B35 and U35) to the English Channel (L4) and the North Sea (SH), were assessed mainly by redundancy analysis. For coarse zooplankton groups latitude explained the main between-site differences, and meroplankton contributed more than holoplankton. Latitudinal differences were best indicated by contrasting abundances of doliolids (most abundant at the lowest latitude sites) and bryozoan and polychaete larvae (most abundant at the highest latitude site). Doliolids were best indicators of temperature-mediated latitudinal differences. The interaction between latitude and distance offshore or salinity and phytoplankton biomass explained a smaller percentage of the variability. The main differences in copepod and cladoceran genera reflected the oceanic influence, with *Corycaeus* and *Oncaea* more associated to L4, likely reflecting the higher influence of off-shelf water intrusions, and the neritic *Acartia* dominating at SH, U35 and B35. *Podon* and *Evadne,* which decreased from south to north, reflected latitude-related differences driven more by salinity than by temperature. While the consistency of identification limited our taxonomic resolution, instances where a single species (e.g. *Acartia clausi*) dominated showed common relationships with temperature, consistent with a common thermal niche.

***Fanjul*, A.**, *Iriarte, A., Villate, F., Uriarte, I., Artiach, M., Atkinson, A., & Cook, K. (2018). Interacting effects of latitude, temperature, salinity, depth and distance offshore in modulating zooplankton assemblages.*

5.1. Introduction

Knowledge of the effect of environmental drivers on marine plankton is essential to be able to predict the response of pelagic ecosystems to environmental change (Pepin *et al*., 2015). Coastal plankton communities exhibit higher variability (Ribera d'Alcalà *et al*., 2004) than oceanic ones because, in addition to larger-scale oceanographic and atmospheric forcing effects, they are also subject to smaller-scale processes affected mainly by interactions between water circulation and bathymetry, benthic-pelagic interactions and terrestrial inputs of freshwater, nutrients and pollutants through rivers and estuarine plumes (Pepin *et al*., 2015).

Zooplankton play key roles in food webs and biogeochemical cycles (Mackas and Beaugrand, 2010) and the study of zooplankton communities of shelf ecosystems is crucial, because these are high productivity areas, supporting over half of the world's marine fisheries (Caddy *et al*., 1998; Mossop, 2007). The composition of zooplankton communities varies with latitude, when spanning different climatic zones (Xu *et al*., 2016). Inshore-offshore gradients along shelf waters also show corresponding zooplankton community gradients, with water depth and intrusions of oceanic water onto the shelf being very influential (Tremblay and Roff, 1983; Blachowiak-Samolyk *et al*., 2008; Pepin *et al*., 2015; Dvoretsky and Dvoretsky, 2015). Furthermore, there are also zooplankton community differences related to the level of nutrient enrichment and pollution of nearshore coastal waters (Uriarte and Villate, 2004).

For the study of the effect of environmental factors on shelf zooplankton communities it is, therefore, desirable that comparisons between sites of different characteristics are made both within and across regions. Zooplankton are being regularly monitored at multiple fixed sites in shelf waters around the world (Mackas and Beaugrand, 2010; O'Brien *et al*., 2013). However, up to the present, these time-series from fixed sites have been greatly underutilized for comparative purposes (e.g. Bonnet *et al*., 2007; Castellani *et al*., 2016). This may be due to difficulties in accessing archived zooplankton data, to differences in sampling methodologies and taxonomic discrimination between time-series, and to difficulties in getting so many different institutions and researchers involved in collaborative work with common objectives and data analysis methodologies.

Several of these monitoring sites are located in the eco-geographical unit of the Northeast Atlantic Shelves Province (NECS; Longhurst, 1998), where zooplankton show a lack of coherence between sites in their interannual variations and seasonal cycles, suggesting a higher influence of local factors over large-scale environmental drivers (Chapters 1 and 2, this work). The aim of the present study was to assess which are the main differences in the zooplankton community structure and the environmental drivers that can account for these differences at four selected coastal sites in the NECS province. These sites are located in the Bay of Biscay (Bilbao 35 and Urdaibai 35), the English Channel (L4) and the North Sea (Stonehaven), and differ in latitude, water depth, distance offshore and phytoplankton biomass. Emphasis was placed in establishing the relative importance of large-scale (climate) *versus* smaller scale (local) environmental drivers. For this purpose, we used a multivariate ordination approach, which helps to summarize the variance of a wide range of zooplankton and environmental data. In order to better understand the effect of latitude, we also compared the relationships between the variations of zooplankton abundance and temperature at the different sites.

5.2. Methods

5.2.1. Data preparation

Zooplankton data were grouped, as for **chapter 2,** in two categories: (i) the *Zooplankton Group* level (ZG), including six holoplankton (copepods, cladocerans, appendicularians, chaetognaths, siphonophores and doliolids) and nine meroplankton categories (cirripede larvae, decapod larvae, gastropod larvae, bivalve larvae, polychaete larvae, fish eggs and larvae, bryozoan larvae, echinoderm larvae and hydromedusae) and (ii) the *Copepod and Cladoceran Genera* level (CCGen), consisting of cladocerans (*Evadne* and *Podon*) and copepods (*Acartia*, *Centropages*, *Temora*, *Oithona*, *Oncaea*, *Corycaeus*, the PCPC-calanus assemblage and the Calanidae family). Zooplankton was expressed in units of density (individuals m^{-3}) and prior to Redundancy Analyses (RDA), the zooplankton density data were transformed using log (x + 1) (ter Braak and Šmilauer, 2002).

5.2.2. Statistical analyses

In order to assess the taxa that contributed most to between-site differences in mesozooplankton community and the environmental variables that best explained these taxa variations, multivariate ordination analyses were performed using Canoco v. 4.55 (ter Braak and Šmilauer, 2002). First, Detrended Correspondence Analyses were performed to assess whether Canonical Correspondence Analysis (CCA) or Redundancy Analysis (RDA), recommended for unimodal and linear relationships between taxa and environmental variables, respectively (ter Braak and Šmilauer, 2002), should be used. Since the length of the longest gradient was in all cases < 2, RDAs were selected. To perform the RDAs relevant water environment variables routinely monitored at all sites, i.e. water temperature (WT), chlorophyll *a* (Chl *a*) and salinity (S), together with the NAO, EA and AMO climate indices, the GSNW index, and site-specific features such as latitude, water depth and distance offshore were used as explanatory variables. Separate redundancy analyses were conducted for the two taxonomic levels tested (ZG and CCGen), but in both cases data pooled for the four sites were run. Since ordination along Axis 1 reflected mainly seasonal variations in mesozooplankton taxa for CCGen (spring – summer as opposed to winter), in order to better assess differences attributable to site-specific characteristics, partial RDAs were performed with month as covariable (thereby removing the effect of months) and sites as supplementary variables for both taxonomic levels tested. Monte Carlo tests were performed (499 permutations) under reduced model, with unrestricted permutations and blocks defined by the covariables (ter Braak and Šmilauer, 2002).

Latitude may be considered itself a surrogate for some other underlying mechanisms that are typically not well understood (Iken *et al*., 2010). Although temperature (both annual mean and range of variation) is linked to latitude, the combined effect of latitude and temperature on species distribution and community changes is still unclear. For a more accurate analysis of the quantitative response of taxa to temperature in the latitudinal context models of the relationship between temperature and taxon abundance (log abundance +1) were obtained. For this purpose, polynomial orthogonal regression analyses (to control for multicollinearity) were performed on all taxa taking temperature up to grade 3 as the independent variable. Subsequent tests on significance and constraints of equality of effects among different sites were performed in order to get the most representative and parsimonious model. Models for each site, as well as common models for the four sites were tested for each taxon.

5.3. Results

5.3.1. Zooplankton community composition

Figure 1 shows the percentage contribution of different taxa to total ZG and CCGen abundances. At every site copepods made up more than half of the total mesozooplankton abundance (52.3 – 70.4%) and cirripede larvae where the second most abundant ZG (10 – 27.5%) at B35, U35 and L4, although at B35 copepods and cirripede larvae accounted for a markedly lower and higher percentage, respectively, than at U35 and L4. It is noteworthy that SH was characterized by a substantially lower contribution of cirripede larvae, and a higher contribution of polychaete and bryozoan larvae than the other sites. Within CCGen, *Acartia* contributed most to total abundance (26.9 – 38.8%), followed by PCPC-calanus (13.3 – 16.2%) at B35, U35 and SH. At L4, however, the contribution of *Acartia* was much lower (4.4%) and PCPC-calanus was the most abundant (22.3%) CCGen, followed by *Oithona* (11.4%) and *Oncaea* (10.4%) genera. SH showed the lowest cladoceran contribution and was the only site in which *Podon* was more abundant than *Evadne*.

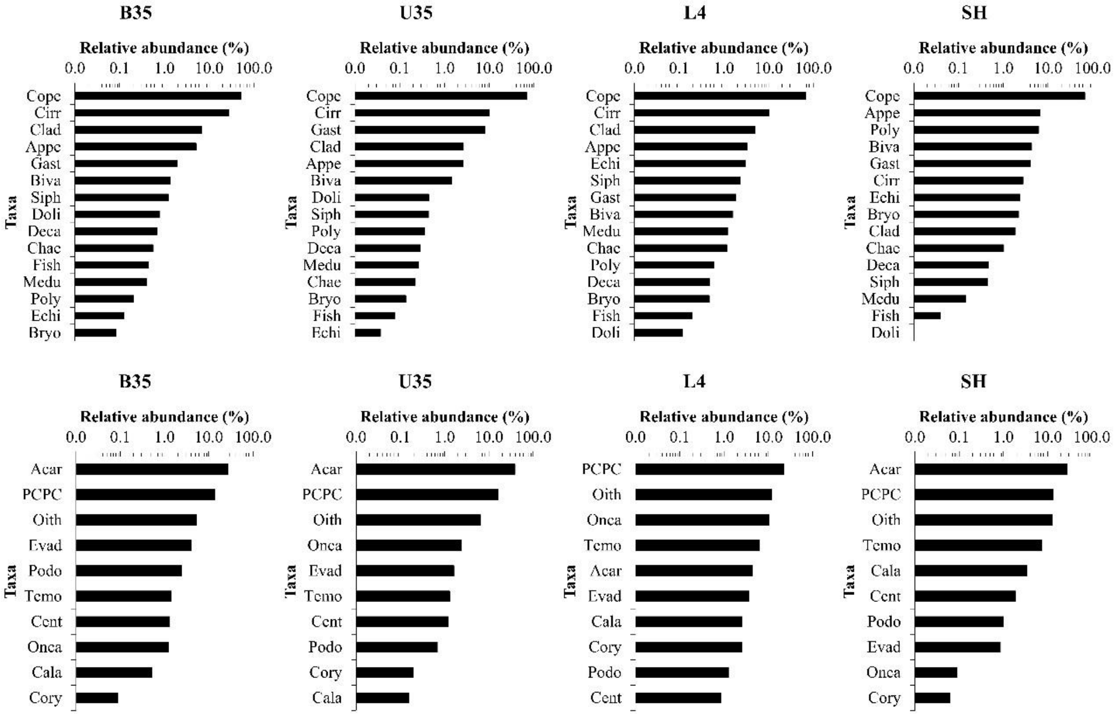

Figure 1. Relative abundance (% of total zooplankton) of ZG (upper panel) and CCGen (lower panel) taxa at the four sampling sites under study.

Information on the species composition and the relative contribution to the total abundance of each CCGen at each of the sites under study is available as supplementary material in **tables 1S**, **2S** and **3S**.

5.3.2. Differences in zooplankton community and environmental drivers

Results of the RDA for ZG revealed that the environmental variables tested explained 18% of taxa variations, and the main mode of variation (axis 1) explained 57.1% of this taxa-environment relationship. Overall, latitude, depth, distance offshore and water temperature were the factors that best explained ZG variability (**Table 1**). Ordination along axis 1 (**Figure 2A**) evidenced differences between sites, where doliolids and cirripede larvae were the taxa with the highest relationship with B35 and U35, and bryozoans, polychaete and echinoderm larvae were more related to SH and L4. Variations of ZG taxa scores on axis 1 appeared to be related primarily to latitude, and to a lesser extent to distance offshore, bottom depth and water temperature (**Table 2**). The second main mode of variation (axis 2) accounted for 31.5% of the taxa-environment relationship that could be explained by the environmental variables tested. The taxa that contributed most to this second mode of variation were fish eggs and larvae, siphonophores and hydromedusae, that showed highest abundances at L4 and lowest ones at U35 and SH, in contrast to gastropod larvae, that appeared to be more linked to SH and U35 (**Figure 2A**). Depth was the factor that best correlated with taxa scores on axis 2, followed by distance offshore, salinity and chlorophyll *a* (**Table 2**).

Table 1. Marginal effects of environmental variables for Zooplankton groups (ZG) and Copepod and Cladoceran genera (CCGen). Variables with significant effects in bold.

ZG				*CCGen*			
Variable	Lambda1	F	*p*	Variable	Lambda1	F	*p*
Latitude	**0.10**	**111.69**	**0.002**	**Distance**	**0.13**	**138.40**	**0.002**
Depth	**0.10**	**66.68**	**0.002**	**Latitude**	**0.11**	**124.85**	**0.002**
Distance	**0.09**	**16.90**	**0.002**	**Depth**	**0.07**	**15.02**	**0.002**
WT	**0.09**	**13.32**	**0.002**	**WT**	**0.07**	**13.35**	**0.002**
Sal	**0.01**	**3.09**	**0.004**	**Sal**	**0.02**	**2.44**	**0.038**
Chl *a*	**0.01**	**2.94**	**0.004**	EA	0.00	1.39	0.220
AMO	0.00	1.74	0.062	Chl *a*	0.00	1.34	0.266
EA	0.00	1.39	0.166	AMO	0.00	1.19	0.308
GSNW	0.00	1.12	0.350	GSNW	0.00	0.99	0.406
NAO	0.00	0.45	0.932	NAO	0.00	0.71	0.656

Distance: distance offshore, **WT**: water temperature, **Sal**: salinity, **Chl *a***: concentration of chlorophyll *a*, **AMO**: Atlantic Multidecadal Oscillation index, **EA**: East Atlantic index, **GSNW**: Gulf Stream North Wall index, **NAO**: North Atlantic Oscillation index.

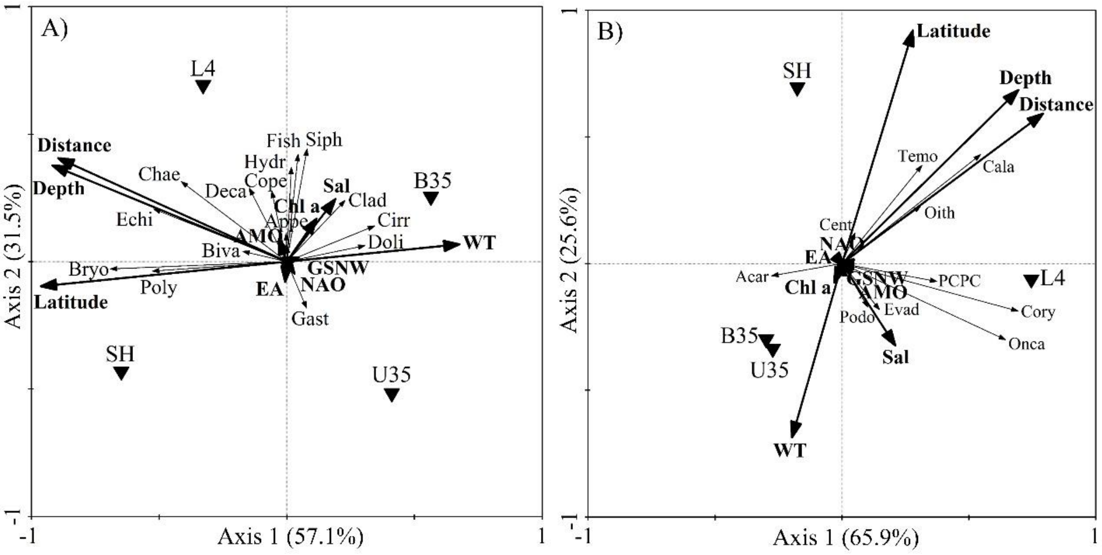

Figure 2. RDA triplot for ZG (A) and CCGen (B). Taxa are shown by thin arrows, explanatory variables by thick arrows and sites by triangles. Acar: *Acartia*, Appe: appendicularians, Biva: bivalve larvae, Bryo: bryozoans, Cala: Calanidae, Cent: *Centropages*, Chae: chaetognaths, Cirr: cirripede larvae, Clad: cladocerans, Cope: copepods, Cory: *Corycaeus*, Deca: decapod larvae, Doli: doliolids, Echi: echinoderm larvae, Evad: *Evadne*, Fish: fish eggs and larvae, Gast: gastropod larvae, Hydr: hydromedusae, Oith: *Oithona*, Onca: *Oncaea*, PCPC: PCPC-calanus, Podo: *Podon*, Poly: polychaete larvae, Siph: siphonophores, Temo: *Temora*.

Table 2. Correlations between environmental factors and scores on axis 1 and axis 2 for Zooplankton groups (ZG) and Copepod and Cladoceran genera (CCGen). Significant correlation coefficients in bold. Abbreviations as in **Table 1**.

	ZG		*CCGen*	
	Axis 1	Axis 2	Axis 1	Axis 2
Latitude	**-0.840 (<0.001)**	**-0.142 (<0.001)**	**0.332 (<0.001)**	**0.636 (<0.001)**
Distance	**-0.736 (<0.001)**	**0.329 (<0.001)**	**0.678 (<0.001)**	**0.382 (<0.001)**
Depth	**-0.669 (<0.001)**	**0.422 (<0.001)**	**0.633 (<0.001)**	**0.373 (<0.001)**
NAO	0.029 (0.436)	-0.037 (0.328)	-0.014 (0.709)	0.026 (0.490)
EA	-0.007 (0.852)	-0.072 (0.054)	-0.003 (0.941)	-0.026 (0.484)
AMO	-0.028 (0.448)	0.052 (0.164)	0.048 (0.194)	-0.045 (0.231)
GSNW	0.028 (0.446)	0.009 (0.800)	0.025 (0.508)	0.000 (0.997)
Salinity	**0.231 (<0.001)**	**0.241 (<0.001)**	**0.134 (<0.001)**	**-0.320 (<0.001)**
WT	**0.581 (<0.001)**	**0.084 (0.024)**	**-0.175 (<0.001)**	**-0.551 (<0.001)**
Chl *a*	-0.048 (0.196)	**0.226 (<0.001)**	**0.136 (<0.001)**	-0.013 (0.732)

For CCGen the environmental variables tested explained 25% of taxa variations, and the main mode of variation (axis 1) explained 65.9% of this taxa-environment relationship (**Figure 3B**). Distance offshore and latitude, followed by depth and water temperature were the environmental factors that explained a largest proportion of CCGen variability (**Table 1**). Site scores on axis 1 showed mainly differences between the CCGen at L4 and the rest of sites tested. The taxa that contributed most to these differences were *Corycaeus* and *Oncaea*, which were most related to L4, in contrast to *Acartia*, most abundant at the rest of sites. Distance offshore, together with bottom depth appeared as the main environmental factors related to these differences in CCGen between sites (**Figure 3B**; **Table 2**).

Axis 2 accounted for 25.6% of the taxa-environment relationship tested for CCGen. According to correlation analyses, latitude and temperature were the factors that best correlated with this second axis, although distance offshore, bottom depth and salinity were also significant (**Table 2**). Taxa with the highest contributions to axis 2 were Calanidae, *Temora* and *Oithona*, which were those that best correlated to distance offshore and depth, in opposition to *Oncaea*, *Corycaeus*, *Podon* and *Evadne*, which were the taxa that best correlated to salinity (**Figure 3B**).

5.3.3. Relationships between taxa abundance and water temperature

The models fitted for the relationship between the abundance of ZG and water temperature are shown in **Figure 3**. Among the taxa that showed a relationship with latitude in the RDA, doliolids evidenced a pattern of linear increase in abundance with increasing water temperature at the four sites under study and they showed the same or very similar quantitative (abundance) response to temperature at all sites. Thus, a global model could be fitted to log (abundance +1) data pooled for all sites. Cirripede larvae did not show the same relationship with water temperature along the entire range of temperatures, instead, a second-degree polynomial curve could be fitted for all sites, which showed minima at intermediate temperatures around 15 °C. Bryozoan larvae showed no significant differences in abundance over a large range of water temperatures, but they also showed increases in abundance at temperatures below 10 ºC at SH and L4. Although global models for cirripede and bryozoan larvae abundances could be fitted for data pooled for the four sites, such models could not account for the between-site differences in abundance for a given water temperature. In the case of polychaete larvae, no significant response to temperature at any of the sites under study was found, and in the case of echinoderm larvae, no common model for the four sites under study was obtained. Second and third-degree polynomials could be fitted for the abundance of echinoderm larvae at SH and L4 respectively, but no significant responses to water temperature could be observed at B35 and U35.

At the coinciding range of water temperatures, a larger number of ZG were more abundant at SH (bryozoan, polychaete, echinoderm, gastropod and bivalve larvae) and L4 (siphonophores, hydromedusae, copepods and fish eggs and larvae) than at the rest of sites. Only cirripede larvae showed a pattern of decreasing abundance with increasing latitude (most abundant at B35 or U35 than at L4 and SH) at the range of water temperatures at which they were present at all sites, but their abundance was much higher at B35 than at U35. Appendicularians and cladocerans did not show differences in abundance between sites within the same range of temperatures, but appendicularian abundance was found to be independent of water temperature at all sites, while cladocerans showed a common pattern of variation with water temperature at all sites, with the optimum temperature lying between 17 and 20 °C.

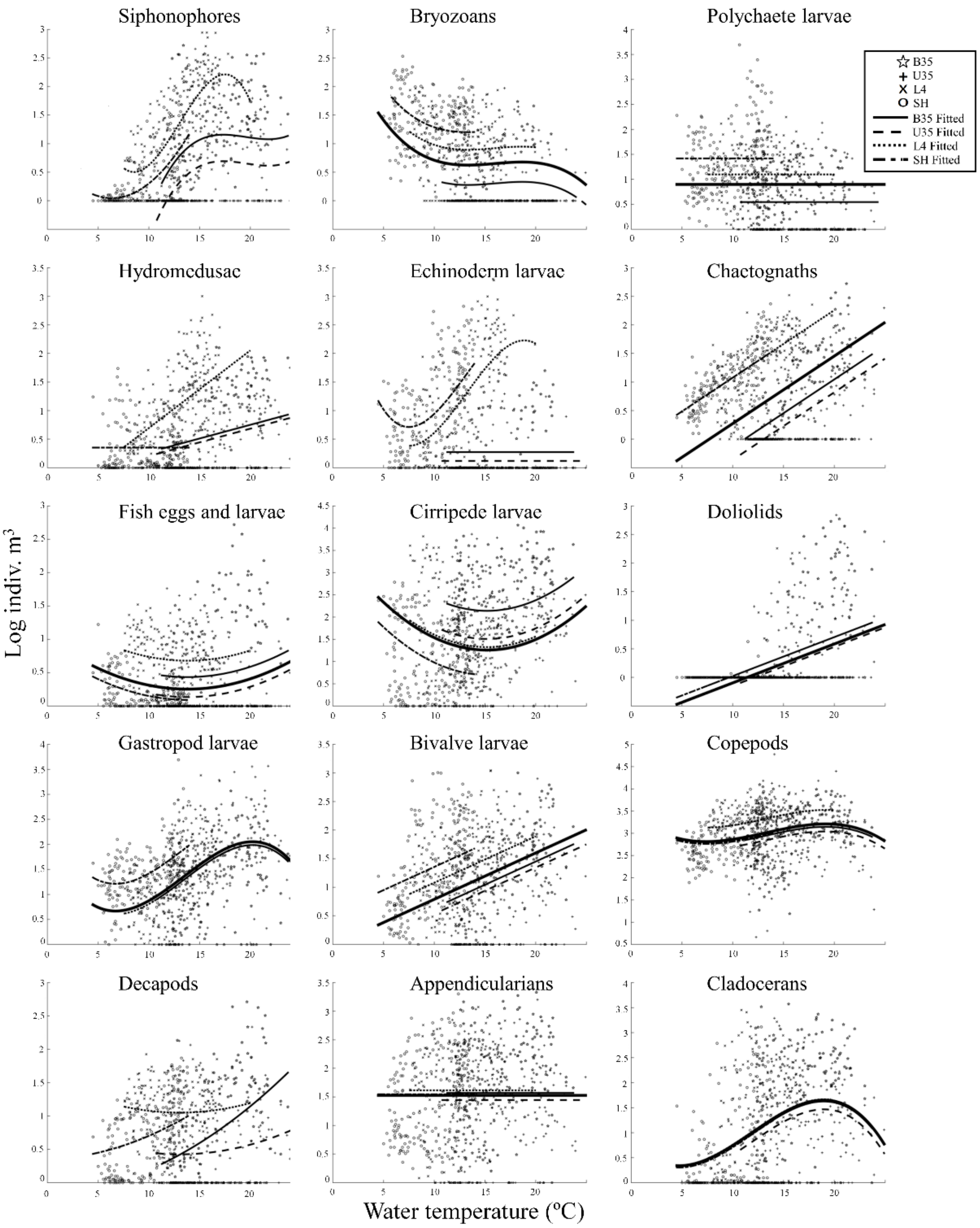

Figure 3. Models of water temperature *vs*. log (taxon abundance + 1) for ZG. Water temperature (Temp) in °C and abundance in individuals m^{-3}.

The models of the relationship between CCGen abundance and water temperature (**Figure 4**) showed that *Corycaeus* had the clearest warm water affinity and the log (abundance + 1) *versus* water temperature relationship could be fitted to linear models of common slope for the four sites under study. *Oncaea* also showed a clear increase of abundance with increasing temperature at the southernmost sites but it showed no relation to temperature at L4 and SH. However, both genera were much more abundant at L4 than at the other sites at any temperature. In fact, L4 was the site where the largest number of CCGen were most abundant at a given temperature, i.e. PCPC-calanus and *Oithona*. The abundance of *Oithona* was not related to water temperature at any site and for PCPC-calanus different non-linear models were fitted at different latitudinal locations, showing two optima, one at about 7 °C and another one at >20 °C.

For the rest of studied CCGen taxa, the abundance *versus* water temperature relationships were similar at the four sites under study. Among them, *Temora* showed the most thermophilic behaviour, followed by *Centropages*, *Podon and Evadne*. Calanidae and *Acartia* had the lowest temperature optimum. For common ranges of temperature, *Centropages* was the only CCGen that showed a pattern of increase in abundance with latitude. *Temora* and Calanidae were much more abundant at SH and L4 than at B35 and U35. *Podon* was less abundant at U35 than at the other sites and *Acartia* was the only CCGen that showed lowest abundances at L4 as compared to the rest of sites for any given temperature.

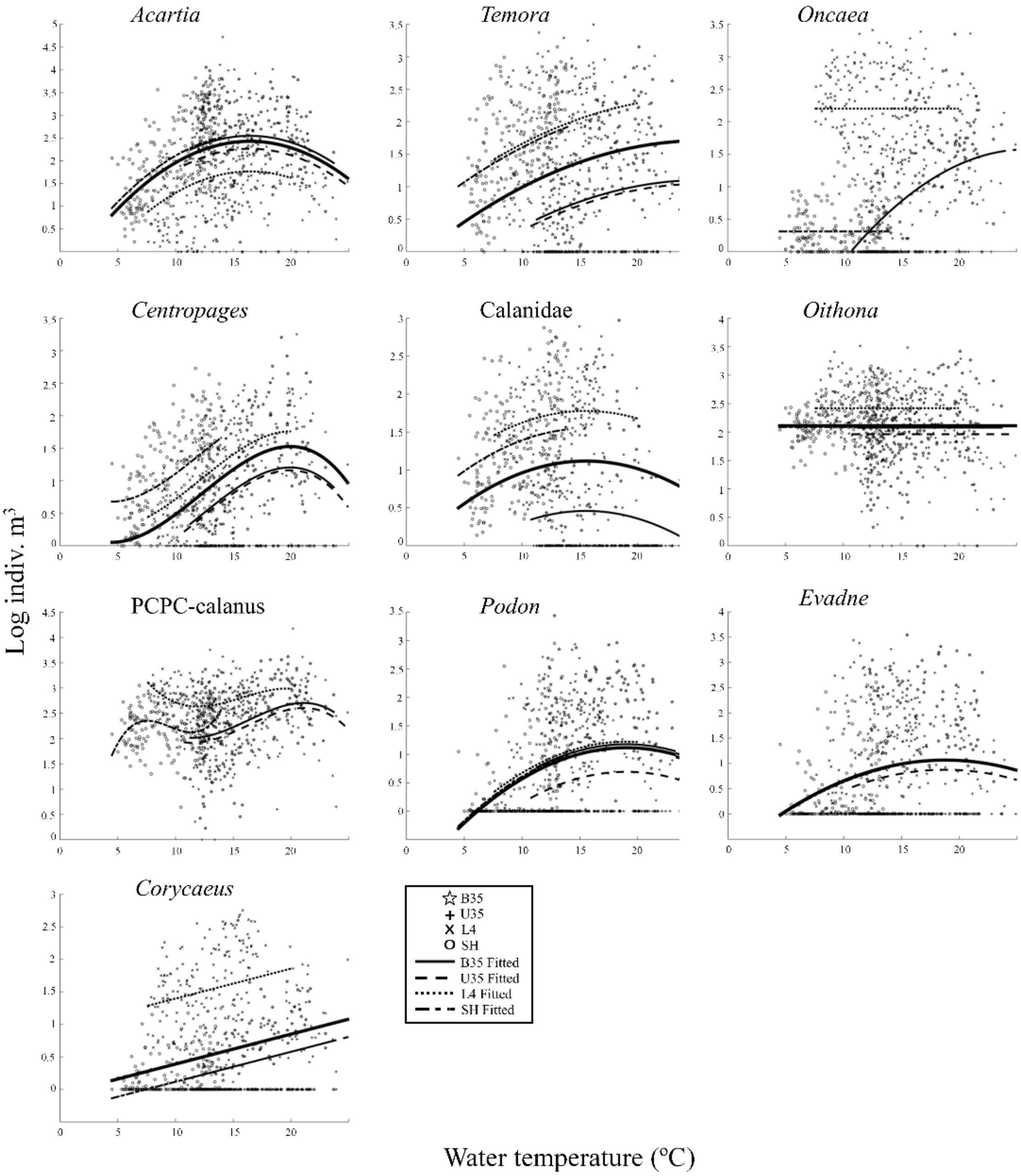

Figure 4. Models of water temperature *vs.* log (taxon abundance + 1) for CCGen. Water temperature (Temp) in ºC and abundance in individuals m^{-3}.

5.4. Discussion

5.4.1. Zooplankton differences at coarse group level (ZG)

The main between-site differences in ZG assemblages showed a gradient from the southernmost stations to the northernmost one, represented by a closer association of groups such as doliolids and cirripede larvae to B35 and U35 and bryozoan and polychaete larvae to SH. Mesoscale patchy spatial variations in doliolid abundance in shelf waters are often related to intrusions of nutrient-rich water and associated phytoplankton biomass increases (Deibel and Paffenhoffer, 2009; Liao *et al*., 2013; Villate *et al*., 2014). However, in our study area, spanning a significant latitudinal and temperature range, between-site differences in the abundance of coastal doliolids appeared to be more related to temperature than to chlorophyll *a* concentration, since at the same temperature similarly high abundances were observed at both the mesotrophic and oligotrophic sites of the southern Bay of Biscay, and minimum values in the northern North Sea site. This agrees well with the finding by Deibel and Lowen (2012) that doliolids operate at a generation time fixed primarily by temperature and secondarily by food concentration. It is interesting to note, also, that the relationship between doliolid abundance and temperature is very similar at all sites, reinforcing the view that latitudinal differences in density are linked primarily to differences in temperature.

The relationship with latitude did not seem to be temperature-mediated in the same way for the meroplankton taxa that contributed most to the main mode (axis 1) of zooplankton variability, because the water temperature *versus* abundance models for cirripede, bryozoan and polychaete larvae showed large between-site differences at a given temperature. The higher abundance of cirripede larvae at B35 and U35 may be related primarily to the fact that these sites are shallower and closer to shore than L4 and SH. Continental shelf benthos generally decreases in abundance with increasing depth (Rex *et al*., 2006; Nephin *et al*., 2014). In the case of barnacles, many of them form thick belts in intertidal rocky shores. Intertidal barnacle larvae tend to be more abundant in nearshore waters and they can become very rare or absent in areas >5 km offshore (Shanks and Shearman, 2009). However, our data showed that the highest differences in cirripede larvae abundance were between B35 and U35, this suggesting the influence of a factor linked to trophic condition, since chlorophyll *a* concentration was much lower at U35 than at B35.

Bryozoan and polychaete larvae were the ZG that showed the highest association with high latitude/low temperature sites, the densities of both taxa being highest at SH and lowest at U35 and B35 for most of the year. Furthermore, bryozoan larvae were most abundant in winter – early spring at L4 and SH and later in spring at B35 and U35 (Chapter 2, this work). Accordingly, bryozoan larvae were also found to peak in early spring in Galway Bay (Irish coast; Byrne, 1995) and in winter in a fjord in high-Arctic Svalbard (Stübner *et al*., 2016). As for cirripedes, the large between-site differences in bryozoan and polychaete larvae densities at a given temperature suggest an additional site effect unrelated to temperature. Regarding bryozoans, low temperature seems to negatively affect the growth rate of bryozoans, with higher latitude bryozoans tending to grow relatively more slowly (Smith and Lawton, 2010). Trophic condition does not seem to be relevant to account for between site differences in bryozoan larvae abundance either. Most bryozoan larvae are suggested to have little or no dependence on phytoplankton as food (Stübner *et al*., 2016), and this would agree with bryozoan larvae maxima not coinciding with the main phytoplankton spring or summer maxima at any of the sites under study. In any case, the latitude-related differences in bryozoan larvae observed in this study seem to be supported by the increasing contribution of bryozoan to benthic communities towards the Arctic region, where they are often the dominant component in hard substrate and phytal habitats (Bader and Schäfer, 2005) As for bryozoan larvae, polychaete larvae also showed decreasing densities from north to south. Accordingly, an increased abundance of benthic polychaetes from the southern to the northern North Sea was also reported by Quiroz-Martinez *et al*., (2011), although regional patterns of benthic polychaete distribution in continental shelves seem to be mainly related to bottom water stability, local distribution of sediment types and depth (Flint and Rabalais, 1980; Quiroz-Martinez *et al*., 2011). The lack of a significant relationship between polychaete larvae abundance and temperature at any site means that no local thermal optima could be determined for them. However, highest levels of polychaete larvae occur at L4 once temperature reaches 13 – 14 °C (Highfield *et al*., 2010) and their annual maxima varies from winter at B35 and U35 to summer at SH (Chapter 2, this work). Taking data pooled for the four sites, the highest abundances were found at an intermediate range of temperatures (11 – 13 °C).

Cladocerans were one of the taxa for which a common model of abundance with temperature for the four sites under study could be fitted. This model explained the between-site differences in abundance quite well. Cladocerans were also the ZG with the highest correlation with Chl *a* and salinity. They are filter-feeders feeding mainly on phytoplankton (Brown *et al*., 1997) and chlorophyll *a* concentration was suggested to be the most important factor determining the spatial distribution of cladocerans in shelf waters of the South China Sea (Xiong *et al*., 2012). Chlorophyll *a*- driven differences in cladoceran abundance can help to explain the lower density of cladocerans at the oligotrophic U35 site in relation to the mesotrophic B35. The positive relationship with salinity in the present study was due to SH showing somewhat lower salinity values and lowest cladoceran abundances. This relation has also been observed at local scales, since in estuaries of the Basque coast penetration of cladocerans in low salinity waters is also limited (Villate *et al*., 2017).

In addition to latitudinal differences, echinoderm larvae showed high correlation with depth and distance offshore, and this is because they were much more abundant at the deeper/more offshore sites (L4 and SH) than at the shallower sites close to the coast (U35 and B35). Lebour (1947) found that ophiopluteii of *Ophiothrix fragilis* were the commonest echinoderm larvae in the inshore waters of Plymouth in the 1940s. In agreement with this, in the L4 time series analysed in the present work, within the echinoderm larvae that have been identified to a coarse taxonomic level, ophiopluteii are the most abundant ones (Chapter 1, this work). These high echinoderm larval abundances are, in turn, in accordance with the occurrence of high-density aggregations in the seabed around the British Isles (particularly on the western side) of the ophiuroid species *Ophiothrix fragilis* and *Ophiocomina nigra* in areas of moderate to strong current (Aronson, 1989). No information on the type of echinoderm larvae is available for SH and the benthic community at Stonehaven has been little studied. Although ophiopluteii were more abundant than echinopluteii at B35 and U35, in the Abra bay where B35 is located, the sea urchin *Paracentrotus lividus* was suggested to be the most abundant echinoderm in the 1980s, with maximum densities up to 20 individuals m^{-2} (Arteche-Irueta, 1987). Regarding ophiuroids, on the Basque coast *Ophiotrix fragilis* and *Amphipholis squamata have been observed* in intertidal areas and *Ophioderma longicauda* and *Ophiocomina nigra* in deeper areas (Ibañez Artica, 2018), but no such dense aggregates as in waters around the British Isles have been reported. The comparison of studies carried out at different sites of the Basque coast also corroborates the offshore-inshore decrease in the contribution of echinoderm larvae to the total zooplankton (Villate *et al*., 2004).

Overall, despite their lower contribution to total zooplankton abundance, meroplankton contributed more than holoplankton to between-site differences in zooplankton structure. This seems to be due to the widespread expatriation of planktonic species, in contrast to the smaller spatial scale resolution of benthic habitat/communities, which are spatially constrained not only by water column features, but also by seafloor features, thus showing higher spatial heterogeneity than planktonic ones (Costello, 2009; Guarinello *et al*., 2010).

5.4.2. Zooplankton differences at copepod and cladoceran genera level (CCGen)

The main between-site differences in the CCGen assemblage explainable by the environmental factors under study were those between L4 and the rest of sites, and were mainly related to differences in water depth, distance offshore and salinity. The higher abundance of *Corycaeus* and *Oncaea*, and to a lesser extent also of Calanidae at L4, and the dominance of *Acartia* at B35, U35 and SH were the main features responsible for such differences. A likely explanation for the substantially higher abundance of *Corycaeus* and *Oncaea* at L4, even when compared to SH which is also a deeper and more offshore site than B35 and U35, is that L4 is affected by intrusions of saltier off-shelf water from the Atlantic that bring those taxa. In fact, *Corycaeus anglicus*

(*Ditrichiocorycaeus anglicus)* which is the dominant *Corycaeus* species at L4, is found to be a good indicator of Atlantic oceanic water inflow to the North Sea through the English Channel (Bonnet and Frid, 2004), and *Oncaea* has also been found to be representative of cross-shelf intrusions on the continental shelf of Northeastern Florida (Paffenhoffer *et al*., 1984). In contrast, *Acartia clausi* (the dominant *Acartia* at the four sites under study) is a neritic species (Wootton and Castellani, 2017). Therefore, CCGen ordination along axis 1 seemed to represent differences between sites associated to the neritic/oceanic nature of zooplankton taxa. Other studies have also highlighted the influence of inputs of oceanic water on the zooplankton species composition of shelf waters (Pedersen *et al*., 2000; Beare *et al*., 2002).

Differences explained by the higher contribution of Calanidae, *Oithona* and *Temora* to the zooplankton at SH and L4, as opposed to the higher contribution of *Podon* and *Evadne* at L4, U35 and B35 were related to water depth and salinity, respectively. *Calanus helgolandicus*, *Oithona similis* and *Temora longicornis,* the main Calanidae, *Oithona* and *Temora* species, respectively, at L4 and SH, are from intermediate or deep layers over the shelf (Vives, 1980; Villate, 1994) (although Halvorsen *et al*. (1999) reported *T. longicornis* as a surface species in shelf waters in northern Norway). *Evadne nordmanii* and *Podon intermedius*, the main *Evadne* and *Podon* species, respectively, at L4, B35 and U35 are mostly found at shallower depths than the former copepod species (Vives, 1980; Villate, 1994), but in this case, the between-site differences were mostly associated with salinity, as was the case for total cladocerans in the analysis of the ZG. Lowest *Podon* and *Evadne* abundances at SH were mainly due to the lower abundance of the species *Podon intermedius* and *Evadne nordmanni*, which was not balanced by the abundance of *Podon leuckartii*, the dominant *Podon* species at the highest latitude SH site. The distribution of this latter species, however, seems to be determined also by temperature, since *P. leuckartii* has a higher affinity for cold waters than *P. intermedius* (Onbé, 1999; Viñas *et al*. 2007) and has not been identified in the zooplankton of the western English Channel or Bay of Biscay sites under study. Furthermore, at SH, unlike at the rest of the sites in the present work, the cladoceran species *Pleopis polyphemoides* is present, which is a more euryhaline species than *P. intermedius* (Viñas *et al*. 2007).

For some CCGen, the models of the relationship between taxon abundance and temperature showed a common pattern for the four sites under study, which accounted for the between-site differences in the seasonal pattern of those taxa. This was the case for *Acartia* (almost exclusively *A. clausi* at all four sites), which showed highest abundances at intermediate temperatures within the whole range of temperatures registered at the four sites, but whose maxima coincided with the highest summer temperatures at the northernmost SH site, and were closer to the lowest temperatures in early spring at the southernmost B35 and U35 site. This common temperature optimum across a large latitudinal gradient is important, because it suggests the lack of temperature adjustment. Constancy of thermal niche is an important, but rarely-tested assumption in many species distribution models that are predicated on a fixed thermal niche (Beaugrand *et al*., 2014). Instead, the phenology of *Acartia clausi* was found to be particularly temperature-sensitive at L4 (Atkinson *et al*., 2015). Therefore, adjustments in seasonal timing, to occur at more suitable seasonal temperatures, may be a mechanism by which this particular species maintains a fixed thermal niche.

For some CCGen the models also showed a very similar shape of the temperature *versus* abundance curve at all four sites, but between-site differences in abundance for a given temperature. This may be attributed to between-site compositional differences at the species level. For example, the abundance of *Temora* was much higher at L4 and SH than at U35 and B35, and the abundance of *Centropages* was highest in the North Sea site, intermediate at the English Channel site and lowest at the Bay of Biscay sites. The dominant *Temora* at L4 and SH is *Temora longicornis* and the dominant *Centropages* at SH is *Centropages hamatus*, which are neritic boreal, cold-temperate species (Colebrook, 1964; Halsband-Lenk *et al*., 2002), whereas at U35 and B35 the dominant species is *T. stylifera* and at L4, U35 and B35 it is *C. typicus*, which are southern, warm-temperate or intermediate latitude species, respectively (Colebrook, 1964; Halsband-Lenk *et al*., 2002). The higher abundance for a given temperature of *T. longicornis* and *C. hamatus* in boreal regions than of *T. stylifera* and *C. typicus*, respectively, in warm temperate regions, that has been observed in the present work, has also been reported elsewhere (Halsband-Lenk *et al*., 2004). In the case of PCPC-calanus, very similar temperature versus abundance models could be fitted to data from U35 and B35 but no common model could be fitted for the four sites. PCPC-calanus abundance peaked both at low (i.e. around 7 °C at L4 and SH) and high temperatures (i.e. around 19 – 21 °C at L4, B35 and U35), and decreased at intermediate temperatures (i.e. 10 – 15 °C). However, peaks at low temperature correspond mainly to the cold-water species *Pseudocalanus elongatus* that is the most abundant species within this group of genera at the highest latitude SH site, and peaks at high temperature corresponded to *Paracalanus parvus*, a neritic warm-water species that was the most abundant species at the lowest latitude B35 and U35 sites. The model at L4 reflected the similar relative abundance of *P. parvus* and *P. elongatus* at this intermediate latitude site.

All this suggests that a predominant latitudinal mode of zooplankton variation, more in agreement with that observed for zooplankton groups (ZG), might have been obtained from the comparison of copepod and cladoceran assemblages, if species instead of genera could have been used. However, this was not possible in the present study because individuals of some genera were not distinguished to species level at all the four sites and/or throughout the entire time-series.

In addition, it has to be born in mind that temporal variations in zooplankton abundance can also be influenced by other factors, such as mortality through predation (Irigoien and Harris, 2003; Hirst *et al*., 2007; Cornwell, 2018), but unfortunately this is a variable that it is not routinely measured in zooplankton monitoring programmes.

5.5. Conclusions

At the level of coarse zooplankton groups (ZG), meroplankton contributed more than holoplankton to the between-site differences in zooplankton structure, which may be related to the higher degree of expatriation of plankton. At this taxonomic level, the main differences showed an association with the latitudinal gradient, but only some holoplanktonic ZG abundances evidenced temperature-mediated latitudinal differences, the warm water affinity doliolids being the best indicators of latitudinal differences based on temperature. Cladocerans were also a group for which the abundance *vs.* temperature model explained the between-site differences relatively well, although these were also affected by trophic condition, and their abundances did not show a clear-cut latitudinal gradient throughout the year. Bryozoan, polychaete, cirripede and echinoderm larvae abundances showed marked latitudinal differences, but these differences did not seem to be primarily linked to differences in temperature with latitude. Cirripede and echinoderm larvae, for example, were more affected by local features such as water depth, distance offshore and, in the case of cirripedes, also of phytoplankton availability.

At the level of copepod and cladoceran genera (CCGen), local factors appeared to have a greater influence on between-site differences, where *Corycaeus* and *Oncaea* seemed to be the best indicators of off-shelf water intrusions from the Atlantic at L4. The family Calanidae and the genera *Temora* and *Oithona* were useful indicators of bathymetry differences and the cladoceran genera *Podon* and *Evadne* of salinity differences. When the genera were dominated by a single species (e.g. *Acartia clausi*), a fixed thermal niche was found, the species adjusting its seasonal timing at different latitudes, so as to occur at more suitable temperatures.

5.6. References

Arteche-Irueta, I. (1987). (In Spanish). Resultados Programa Abra: Equinodermos. Kobie XVI: 307–314.

Aravena, G., Villate, F., Uriarte, I., Iriarte, A., Ibañez, B. (2009). Response of *Acartia* populations to environmental variability and effects of invasive congenerics in the estuary of Bilbao, Bay of Biscay. *Estuarine and Coastal Shelf Science*, *83*, 621–628. doi:10.1016/j.ecss.2009.05.013.

Aronson, R.B. (1989). Brittlestar beds: low-predation anachronisms in the British Isles. *Ecology*, *70*, 856–865. doi:10.2307/1941354.

Atkinson, A., Harmer, R. A., Widdicombe, C. E., McEvoy, A. J., Smyth, T. J., Cummings, D. G., Somerfield, P. J., Maud, J. L., McConville, K. (2015). Questioning the role of phenology shifts and trophic mismatching in a planktonic food web. *Progress in Oceanography*, *137*, 498–512. doi:10.1016/j.pocean.2015.04.023.

Bader, B., Schäfer, P. (2005). Bryozoans in polar latitudes: Arctic and Antarctic bryozoan communities and facies. *Landesmuseen Neue Serie*, *28*, 263–282.

Beare, D.J., Batten, S., Edwards, M., Reid, D.G. (2002). Prevalence of boreal Atlantic, temperate Atlantic and neritic zooplankton in the North Sea between 1958 and 1998 in relation to temperature, salinity, stratification intensity and Atlantic inflow. *Journal of Sea Research*, *48*, 29–49. doi:10.1016/S1385-1101(02)00131-4.

Beaugrand, G., Goberville, E., Luczak, C., Kirby, R.R. (2014). Marine biological shifts and climate. *Proceedings of the Royal Society B*, *281*, 20133350. doi: 10.1098/rspb.2013.3350.

Blachowiak-Samolyk, K., Søreide, J.E., Kwasniewski, S., Sundfjord, A., Hop, H., Falk-Petersen, S., Hegseth, E.N. (2008). Hydrodynamic control of mesozooplankton abundance and biomass in northern Svalbard waters (79–81°N). *Deep Sea Research II*, *55*, 2210–2224. doi:10.1016/j.dsr2.2008.05.018.

Bonnet, D., Frid, C. (2004). Seven copepod species considered as indicators of water-mass influence and changes: Results from a Northumberland coastal station. *ICES Journal of Marine Science*, *61*, 485–491. doi: 10.1016/j.icesjms.2004.03.005.

Bonnet, D., Harris, R., Lopez-Urrutia, A., Halsband-Lenk, C., Greve, W., Valdes, L., Hirche, H.J., Engel, M., Alvarez-Ossorio, M.T., Wiltshire, K.H. (2007). Comparative seasonal dynamics of *Centropages typicus* at seven coastal monitoring stations in the North Sea, English Channel and Bay of Biscay. *Progress in Oceanography*, *72*, 233–248. doi:10.1016/j.pocean.2007.01.007.

Bresnan, E., Cook, K. B., Hughes, S. L., Hay, S. J., Smith, K., Walsham, P., Webster, L. (2015). Seasonality of the plankton community at an east and west coast monitoring site in Scottish waters. *Journal of Sea Research*, *105*, 16–29. doi:10.1016/j.seares.2015.06.009.

Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A. (1997). Nutritional properties of microalgae for mariculture. *Aquaculture*, *151*, 315–331. doi: 10.1016/S0044-8486(96)01501-3.

Byrne, P. (1995). Seasonal composition of meroplankton in the Dunkellin Estuary, Galway Bay. *Proceedings of the Royal Irish Academy. Section B*, *95B*, 35–48.

Caddy, J.F., Carocci, F., Coppola, F. (1998). Have peak fishery production levels been passed in continental shelf areas? Some perspectives arising from historical trends in production per shelf area. *Journal of Northwest Atlantic Fishery Science*, *23*, 191–219.

Castellani, C., Licandro, P., Fileman, E., di Capua, I., Mazzocchi, M. G. (2016). *Oithona similis* likes it cool: evidence from two long-term time series. *Journal of Plankton Research*, *38*, 703–717. doi:10.1093/plankt/fbv104.

Colebrook, J.M., (1964). Continuous Plankton Records: a principal component analysis of the geographical distribution of zooplankton. *Bulletin of Marine Ecology*, *6*, 78–100.

Cornwell, L.E., Findlay, H.S., Fileman, E.S., Smyth, T.J., Hirst, A.G., Bruuns, J.T., McEvoy, A.J. Widdicombe, C.E., Castellani, C., Lewis, C., Atkinson, A. (2018). Seasonality of *Oithona similis* and *Calanus helgolandicus* reproduction and abundance: contrasting responses to environmental variation at a shelf site. *Journal of Plankton Research*, *3*, 295–310. doi:10.1093/plankt/fby007.

Deibel, D., Paffenhoffer, G.A. (2009). Predictability of patches of neritic salps and doliolids (Tunicata, Thaliacea). *Journal of Plankton Research*, *31*, 1571–1579. doi:10.1093/plankt/fbp091.

Deibel, D., Lowen, B. (2012). A review of the life cycles and life-history adaptations of pelagic tunicates to environmental conditions. *ICES Journal of Marine Science*, *69*, 358–369. doi:10.1093/icesjms/fsr159.

Dvoretsky, V.G., Dvoretsky, A.G. (2015). Regional differences of mesozooplankton communities in the Kara Sea. *Continental Shelf Research*, *105*, 26–41. doi:10.1016/j.csr.2015.06.004.

Ferrer, L., Fontán, A., Mader, J., Chust, G., González, M., Valencia, V., Uriarte, A., Collins, M.B. (2009). Low-salinity plumes in the oceanic region of the Basque Country. *Continental Shelf Research*, *29*, 970–984. doi: 10.1016/j.csr.2008.12.014.

Flint, R.W., Rabalais, N. N.(1980). Polychaete Ecology and Niche Patterns: Texas Continental Shelf. *Marine Ecology Progress Series*, *3*, 193–202.

Guarinello, M. L., Shumchenia, E. J., King, J. W. (2010). Marine Habitat Classification for Ecosystem-Based Management: A Proposed Hierarchical Framework. *Environmental Management*, *45*, 793–806. doi: 10.1007/s00267-010-9430-5.

Halsband-Lenk, C., Hirche, H.J., Carlotti, F. (2002). Temperature impact on reproduction and development of congener copepod populations. *Journal of Experimental Marine Biology and Ecology*, *271*, 121–153. doi:10.1016/S0022-0981(02)00025-4.

Halsband-Lenk, C., Carlotti, F., Greve, W. (2004). Life-history strategies of calanoid congeners under two different climate regimes: a comparison. *ICES Journal of Marine Science*, *6*, 709–720. doi:10.1016/j.icesjms.2004.03.020.

Halvorsen, E., Tande, K.S., Høisæter, T. (1999). Physical and biological factors influencing the seasonal variation in distributrion of zooplanton across the shelf at Nordvestbaskem, northern Norway, 1994. *Sarsia*, *84*, 279–292. doi:10.1080/00364827.1999.10420432.

Highfield, J. M., Eloire, D., Conway, D. V. P., Lindeque, P. K., Attrill, M. J. and Somerfield, P. J. (2010). Seasonal dynamics of meroplankton assemblages at station L4. *Journal of Plankton Research*, *32*, 681–691. doi:10.1093/plankt/fbp139.

Hirst, A. G., Bonnet, D. and Harris, R. P. (2007). Seasonal dynamics and mortality rates of *Calanus helgolandicus* over two years at a station in the English Channel. *Marine Ecology: Progress Series*, *340*, 189–205.

Ibañez Artica, Miguel. Zoología. Enciclopedia Auñamendi [on line], (2018). [retrieved on: 03 May 2018]. Available at: http://aunamendi.eusko-ikaskuntza.eus/es/zoologia/ar-147554/.

Iken, K., Konar, B., Benedetti-Cecchi, L., Cruz-Motta, J. J., Knowlton, A., Pohle, G., Mead, A., Miloslavich, P., Wong, M., Trott, T., Mieszkowska, N., Riosmena-Rodriguez, R., Airoldi, L., Kimani, E., Shirayama, Y., Fraschetti, S., Ortiz-Touzet, M., Silva, A. (2010). Large-Scale Spatial Distribution Patterns of Echinoderms in Nearshore Rocky Habitats. *PLoS ONE*, *5*, e13845. doi:10.1371/journal.pone.0013845.

Irigoien, X., Harris, R. P. (2003). Interannual variability of *Calanus helgolandicus* in the English Channel. *Fisheries Oceanography*, *12*, 317–326.

Lebour, M.V. (1947). Notes on the inshore plankton of Plymouth. *Journal of the Marine Biological Association of the United Kingdom*, *26,* 527–547. doi:10.1017/S0025315400013734.

Liao, Z-H., Hsieh, H-Y., Lo, W-T. (2013). Influence of monsoon-driven hydrographic features on thaliacean distribution in waters around Taiwan, western North Pacific Ocean. *Zoological Studies*, *52*, 49. doi:10.1186/1810-522X-52-49.

Longhurst, A. R. (1998). Ecological geography of the sea, 1st ed. Academic Press, San Diego. 398 pp.

Mackas, D. L. and Beaugrand, G. (2010). Comparisons of zooplankton time series. *Journal of Marine Systems*, *79*, 286–304. doi:10.1016/j.jmarsys.2008.11.030.

Mossop, J. (2007). Protecting marine biodiversity on the continental shelf beyond 200 nautical miles. *Ocean Development & International Law, 38,* 283–304. doi: 10.1080/00908320701530474.

Nephin, J., Juniper, S.K., Archambault, P. (2014). Diversity, abundance and community structure of benthic macro- and megafauna on the Beaufort Shelf and Slope. *PLoS One, 9,* e101556. doi:10.1371/journal.pone.0101556.

O'Brien, T.D., Wiebe, P.H., Falkenhaug, T. (2013). ICES Zooplankton Status Report 2010/2011. *ICES Cooperative Research Report*, *318*. 208 pp. ISSN 1017-6195.

Onbé, T., (1999). Ctenopoda and Onychopoda (=Cladocera). In: Boltovskoy D. (ed.), South Atlantic Zooplankton. Backhuys Publishers, Leiden, The Netherlands, 797–813.

Paffenhofer, G-A., Wester, B.T., Nicholas, W.D. (1984). Zooplankton abundance in relation to state and type of intrusions onto the southeastern United States shelf during summer. *Journal of Marine Research*, *42*, 995–1017. doi: 10.1357/002224084788520819.

Pedersen, O.-P., Tande, K.S., Timonin, A., Semenova, T. (2000). A possible connection between hydrography and the distribution of *Calanus finmarchicus* on the Norwegian midshelf in 1997. *ICES Journal of Marine Science*, *57*, 1645–1655. doi:10.1006/jmsc.2000.0980.

Pepin P., Johnson C.L., Harvey M., Casault B., Chassé J., Colbourne E.B., Galbraith P.S., Hebert D., Lazin G., Maillet G., Plourde S., Starr, M. (2015). A multivariate evaluation of environmental effects on zooplankton community structure in the western North Atlantic. *Progress in Oceanography, 134*, 197–220. doi: 10.1016/j.pocean.2015.01.017.

Quiroz-Martinez, B., Schmitt, F. G., Dauvin, J.-C., Dewarumez, J.-M., Foveau, A., Garcia, C. (2011). Regional patterns of continental shelf polychaete diversity: examples for the North Sea, English Channel, Irish Sea and Outer Bristol Channel areas. *Italian Journal of Zoology, 78,* sup1, 324–332. doi: 10.1080/11250003.2011.591183.

Rex, M.A., Etter, R.J., Morris, J.S., Crouse, J., McClain, C.R., Johnson, N.A., Stuart, C.T., Deming, J.W., Thies, R., Avery, R. (2006). Global bathymetric patterns of standing stock and body size in the deep-sea benthos. *Marine Ecology Progress Series*, *317,* 1–8. doi:10.3354/meps317001.

Ribera d´Alcalà, M., Conversano, F., Corato, F., Licandro, P., Mangoni, O. (2004). Seasonal patterns in plankton communities in a pluriannual time series at a coastal Mediterranean site (Gulf of Naples): an attempt to discern recurrences and trends. *Scientia Marina, 68,* 65–83. doi:10.3989/scimar.2004.68s165.

Shanks, A.L., Shearman, R.K. (2009). Paradigm lost? Cross-shelf distributions of intertidal invertebrate larvae are unaffected by upwelling or downwelling. *Marine Ecology Progress Series, 385*, 189–204. doi:10.3354/meps08043.

Smith, A.M., Lawton, E.I. (2010). Growing up in the temperate zone: age, growth, calcification and carbonate mineralogy of *Melicerita chathamensis* (Bryozoa) in southern New Zealand. *Palaeogeography, Palaeoclimatology, Palaeoecology, 298,* 271–277. doi:10.1016/j.palaeo.2010.09.033.

Smith, V. H., Tilman, G. D. and Nekola, J. C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution, 100,* 179–196. doi:10.1016/S0269-7491(99)00091-3.

Stübner, E.I., Søreide, J.E., Reigstad, M., Marquardt, M. Blachowiak-Samolyk, M. (2016). Year-round meroplankton dynamics in high-Arctic Svalbard. *Journal of Plankton Research, 38,* 522–536. doi:10.1093/plankt/fbv124.

ter Braak, C.J.F., Šmilauer, P. (2002). CANOCO Reference manual and CanoDraw for Windows User's guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power. 500pp. Ithaca, NY, USA.

Tremblay, M.J., Roff, J.C. (1983). Community gradients in the Scotian Shelf zooplankton. *Canadian Journal of Fisheries and Aquatic Sciences, 40,* 598–611. doi:10.1139/f83-079.

Uriarte, I., Villate, F. (2004). Effects of pollution on zooplankton abundance and distribution in two estuaries of the Basque coast (Bay of Biscay). *Marine Pollution Bulletin, 49,* 220–228. doi: 10.1016/j.marpolbul.2004.02.010.

Villate, F. (1994). Temporal variability of the spatial distribution of the zooplankton community in a coastal embayment of the Basque Country in relation to physical phenomena. *Hydrobiologia, 288,* 79–95. doi:10.1007/BF00007128.

Villate, F., Uriarte, I., Irigoien, X., Beaugrand, G., Cotano, U. (2004). Chapter 16: Zooplankton communities. In : Borja A, Collins M (Eds). Oceanography and marine environment of the Basque country. *Elsevier Oceanography Series, 70*, 395–424. ISBN: 9780444515810.

Villate, F., Uriarte, I., Olivar, M. P., Maynou, F., Emelianov, M., Ameztoy, I. (2014). Mesoscale structure of microplankton and mesoplankton assemblages under contrasting oceanographic conditions in the Catalan Sea (NW Mediterranean). *Journal of Marine Systems, 139,* 9–26. doi:10.1016/j.jmarsys.2014.05.004.

Villate, F., Iriarte, A., Uriarte, I., Sanchez, I. (2017). Seasonal and interannual variability of mesozooplankton in two contrasting estuaries of the Bay of Biscay: relationship to environmental factors. *Journal of Sea Research, 130,* 189–203. doi: 10.1016/j.seares.2017.05.002.

Viñas, M.D., Ramirez, F.C., Santos, B.A., Marrari, M. (2007). Spatial and temporal distribution patterns of Cladocera in the Argentine Sea. *Hydrobiologia*, 594, 59–68. doi:10.1007/s10750-007-9077-2. https://doi.org/10.1007/s10750-007-9077-2.

Vives, F. (1980). (In Spanish) Los copépodos de las aguas neríticas de las costas de Vizcaya, durante 1976. *Investigaciones Pesqueras, 44*, 313–330.

Wootton, M., Castellani, C. (2017). Crustacea: Copepoda. In Marine Plankton. A practical guide to ecology, methodology, and taxonomy, C. Castellani, C., Edwards, M. (eds). *Oxford University Press*. 267–380 pp. ISBN: 9780199233267.

Xiong, L.L., Yin, J.Q., Huang, L.M., Li, K.Z., Lian, S.M. (2012). Seasonal and spatial variations of cladocerans on the northwest continental shelf of the South China Sea. *Crustaceana, 85,* 473–496. doi:10.2307/23212676.

Xu, J.Y., Xu, Z.L., Gao, Q. (2016). Distribution of dominant zooplankton species along a latitudinal gradient in China Sea during spring. *Journal of Ocean University of China, 15,* 502–508. doi:10.1007/s11802-016-2871-6.

General conclusions

- The magnitude of zooplankton interannual, seasonal and residual components of variability did not show clear patterns of variation in relation to latitude.
- Interannual patterns of individual zooplankton taxa variations were not coherent across sites, suggesting the dominance of local forces over larger scale climatic drivers.
- Seasonal patterns of individual zooplankton taxa showed north-south trends, being the most recurrent the northward delay of early occurring taxa (spring – summer), together with the earlier occurrence of the late peak in taxa showing bimodal cycles. Taxa with coincident seasonal patterns at all sites and taxa peaking earlier with increasing latitude over the first half of the year or taxa peaking later with increasing latitude over the second half were other observed types of trends.
- The main mode of seasonal zooplankton community variability was due mainly to zooplankton taxa that peaked at the timing of the annual maximum of zooplankton at each site, and it was the mode that showed the largest between-site differences, that consisted in seasonal delays both with latitude and trophic status. These between-site differences were related primarily to the seasonal pattern of phytoplankton biomass.
- Meroplankton made a large contribution to shape the main seasonal mode of variability, cirripede larvae at B35 and U35, echinoderm larvae at L4 and decapod larvae at SH.
- Regularity of the seasonal cycle of zooplankton and the percentage of variance explained by chlorophyll *a*, temperature and salinity increased with latitude and trophic status. This may be related to the increased regularity and higher covariation of water temperature and chlorophyll a with the increase in latitude and trophic status.
- Very few zooplankton taxa showed significant linear trends of interannual variation, but, in general, opposite tendencies in phenology changes were detected between the southernmost sites (later occurrences with time) and the northernmost site (earlier occurrences with time).
- Clusters of taxa with similar interannual phenology patterns were found at every site, and they were generally composed of taxa that peaked in the same season. The phenological variations of these clusters showed a significant correlation with climatic indices (mainly EA and AMO at B35 and U35; EA and NAO at SH) or water temperature (L4). In many cases phenological variations appeared to be correlated also with phytoplankton biomass.

- Gradual changes of zooplankton communities throughout the study period were found for the assemblage occurring during the main growing period at the L4 site and for the assemblage occurring late in the year at the other three sites, restricted to the 1999 – 2005 period at SH and probably related to a shift initiated previously. The interaction between temperature and month was always selected as the main driver of those changes, with the U35 exception, likely related to a lower coherence of environmental seasonal patterns.
- None individual taxa showed the same pattern of change at the four sites, and the increasing or decreasing trends presented by some selected taxa were mainly related to the interaction of water temperature and month and Chl *a* at SH, the interaction of water temperature and month at L4. Salinity and Chl *a*, alone or in interaction, were mainly selected as explicative variables at U35 and B35.
- Meroplankton contributed more than holoplankton to the between-site differences in zooplankton community structure, which may be related to the higher degree of expatriation of holoplankton.
- At the coarse zooplankton level, between-site differences were mainly explained by latitude, but only some holoplanktonic group abundances evidenced temperature mediated latitudinal differences, doliolids being the best related to temperature. Meroplanktonic larvae, mainly those from cirripedes and echinoderms, were more affected by local features such as water depth, distance offshore and, in the case of cirripedes, also of phytoplankton availability.
- For copepod and cladoceran genera, local factors appeared to have a greater influence on between-site differences than large scale factors such as latitude. *Corycaeus* and *Oncaea* seemed to be the best indicators of off-shelf water intrusions from the Atlantic at L4. The family Calanidae and the genera *Temora* and *Oithona* were useful indicators of bathymetry differences, and the cladoceran genera *Podon* and *Evadne* of salinity differences.
- *Acartia clausi*, which was the species that dominated its genus at all four sites, showed opposite phenology tendencies at the southernmost and northernmost sites. Moreover, the relationship between the abundance of this species and temperature was consistent with a fixed thermal niche (adjustment of its seasonal timing at different latitudes, so as to occur at similar temperatures at all sites). *A. clausi*, also presented inverse multi-annual trends of abundance from SH (decrease) to U35 and B35 (increase) sites.

Annexes

Table A.1. Mean density ± standard error (individuals m^{-3}) and composition of the holoplankton groups considered in this study at B35, U35, L4 and SH. The value in parentheses is the contribution in percentage of each identified category to the total.

Taxa	**B35**	**U35**	**L4**	**SH**
Siphonophores	46.99 ± 6.74 *Muggiaea* (71.0) Siphonophores (23.8) *Muggiaea atlantica* (3.4) *Muggiaea kochii* (1.4) Sphaeronectidae (0.4)	13.72 ± 3.26 *Muggiaea* (74.4) Siphonophores (20.9) *Muggiaea kochii* (2.8) *Muggiaea atlantica* (1.8)	79.39 ± 10.89 *Muggiaea* (47.2) Siphonophores (41.8) *Muggiaea atlantica* (10.3) Other species (0.6)	8.87 ± 2.29 Diphyidae (51.8) *Muggiaea atlantica* (47.9) Other species (0.3)
Chaetognaths	21.69 ± 4.63 Sagittidae (90.4) *Parasagitta friderici* (9.6)	6.95 ± 1.63 Sagittidae (94.8) *Parasagitta friderici* (5.2)	40.38 ± 3.52 Chaetognathes (86.6) *Parasagitta setosa* (11.9) *Parasagitta elegans* (1.5)	20.54 ± 2.36 Sagittidae (81.6) *Parasagitta elegans* (17.7) Other chaetognaths (0.7)
Cladocerans	264.66 ± 39.61 *Evadne* (58.0) *Podon* (35.3) *Penilia avirostris* (6.7)	82.72 ± 17.52 *Evadne* (61.2) *Podon* (25.7) *Penilia avirostris* (13.1)	171.46 ± 28.33 *Evadne* (74.6) *Podon* (25.4) *Penilia avirostris* (>0.01)	37.19 ± 9.48 *Podon* (54.1) *Evadne* (45.9)
Copepods	2019.19 ± 174.23 *Acartia* (51.4) PCPC-calanus (25.8) *Oithona* (10.2) Other copepods (4.0) *Temora* (2.7) *Centropages* (2.5) *Oncaea* (2.3) Calanidae (1.0) Corycaeidae (0.2)	2205.03 ± 395.01 *Acartia* (55.2) PCPC-calanus (23.1) *Oithona* (9.0) Other copepods (5.3) *Oncaea* (3.4) *Centropages* (1.7) *Temora* (1.8) Corycaeidae (0.3) Calanidae (0.2)	2336.27 ± 110.17 PCPC-calanus (33.1) *Oithona* (17.6) *Oncaea* (15.4) *Temora* (9.3) Other copepods (9.2) *Acartia* (6.5) Calanidae (3.8) Corycaeidae (3.7) *Centropages* (1.2)	1356.88 ± 124.08 *Acartia* (41.0) PCPC-calanus (19.9) *Oithona* (19.1) *Temora* (10.9) Calanidae (5.1) *Centropages* (2.8 Other copepods (1.1) *Oncaea* (0.1) Corycaeidae (0.1)
Appendicularians	197.43 ± 24.61 *Oikopleura* (67.4) *Oikopleura dioica* (17.4) *Fritillaria pellucida* (4.4) *Fritillaria* (3.6) *Fritillaria borealis* (2.8) *Oikopleura fusiformis* (2.4) *Oikopleura longicauda* (1.9) *Fritillaria haplostoma* (0.02)	80.69 ± 13.21 *Oikopleura* (77.5) *Oikopleura dioica* (12.7) *Oikopleura fusiformis* (3.9) *Fritillaria* (2.6) *Fritillaria pellucida* (2.0) *Fritillaria borealis* (0.8) *Oikopleura longicauda* (0.5)	114.34 ± 12.49 Appendicularia	134.75 ± 18.62 Appendicularia
Doliolids	29.74 ± 6.65 *Doliolum nationalis* (67.0) *Doliolum* (33.0)	13.80 ± 5.02 *Doliolum nationalis* (61.0) *Doliolum* (39.0)	4.07 ± 1.67 Doliolidae	0.05 ± 0.03 Doliolidae

Table A.2. Mean density ± standard error (individuals m^{-3}) and composition of the meroplankton groups considered in this study at B35, U35, L4 and SH. The value in parentheses is the contribution in percentage of each identified category to the total.

Taxa	**B35**	**U35**	**L4**	**SH**
Hydromedusae	15.24 ± 1.94 *Obelia* (37.0) Medusae (22.2) Hydromedusae (10.3) *Lizzia blondina* (9.8) *Sarsia* (8.8) *Liriope tetraphylla* (7.8) Anthomedusae (3.3) Hydractiniidae (0.9)	8.10 ± 1.81 *Lizzia blondina* (24.9) *Obelia* (23.1) Medusae (20.5) *Sarsia* (12.8) Hydromedusae (12.1) *Liriope tetraphylla* (6.2)	41.73 ± 7.88 *Obelia* (24.6) Hydromedusae (21.9) *Lizzia blondina* (19.4) *Liriope tetraphylla* (16.2) *Solmaris corona* (13.7) *Aglantha digitale* (2.5) Other medusae (1.6)	2.91 ± 0.50 *Obelia* (43.6) *Hybocodon prolifer* (14.7) *Aglantha digitale* (13.4) *Rathkea octopunctata* (10.4) *Clytia hemisphaerica* (5.0) *Lizzia blondina* (4.18) Other medusae (3.5) *Sarsia* (2.4) *Corymorpha nutans* (1.7) *Leuckartiara octona* (1.2)
Bryozoan larvae	3.15 ± 0.58 Cyphonaute	4.28 ± 0.92 Cyphonaute	16.29 ± 1.51 Cyphonaute	43.31 ± 3.80 Cyphonaute
Gastropod larvae	74.10 ± 11.28 Gastropod veliger	254.11 ± 60.55 Gastropod veliger	63.57 ± 17.09 Gastropod veliger (81.2) *Limacina retroversa* (18.5) Other gastropods (0.3)	81.37 ± 28.64 *Limacina. retroversa* (70.8) Gastropod veliger (23.3), Gymnosomata (5.6) Other gastropds (0.3)
Bivalve larvae	52.37 ± 8.92 Bivalve veliger	45.72 ± 7.37 Bivalve veliger	54.50 ± 9.93 Bivalve veliger	86.67 ± 12.39 Bivalve veliger
Polychaete larvae	7.85 ± 1.29 Spionidae larvae (68.6) Polychaete larvae (15.7) Magelonidae larvae (5.6) Sabellariidae larvae (4.0) Polynoidae larvae (3.2) Aphroditidae larvae (2.7) Other polychaetes (0.2)	11.10 ± 1.61 Spionidae larvae (90.5) Polychaete larvae (2.6) Sabellariidae larvae (4.6) Polynoidae larvae (1.2) Other polychaetes (1.0)	20.96 ± 1.97 Polychaete larvae (98.8) *Tomopteris helgolandica* (1.2)	124.37 ± 33.04 Polychaete larvae (99.4) Other polychaetes (0.6)
Cirripede larvae	1061.93 ± 138.88 Cirriped nauplius (95.7) and cypris (4.3) larvae	314.41 ± 67.63 Cirriped nauplius (68.1) and cypris (31.9) larvae	356.89 ± 69.82 Cirriped nauplius (93.4), cypris (6.6) and rhizocephalan (0.02) larvae	55.55 ± 7.34 Cirriped nauplius (58.5) and cypris (41.5) larvae
Decapod larvae	26.06 ± 5.15 Brachiura (36.4) Decapod (15.9) Caridea (15.3) Crangonidae (5.5) Hyppolitidae (4.2) *Processa* (4.0) *Pisidia longicornis* (4.0) Paguridea (3.4) *Crangon crangon* (2.9) *Athanas* (2.5) Other decapods (2.3) *Porcellana* (2.0) Porcessidae (1.4)	9.03 ± 1.78 Brachiura (63.7) Decapod (11.5) Caridea (11.1) Crangonidae (6.8) Other decapods (3.6) *Phyllocheras* (1.1) Alpheidae (1.1) *Porcellana* (1.0)	16.68 ± 1.11 Decapod (55.9) Brachyura (30.6) Porcellanid (6.3) Other decapoda (1.9) *Necora* (1.8) Paguridae (1.7) *Upogebia* (1.7)	9.29 ± 1.01 Decapoda
Echinoderm larvae	4.80 ± 1.24 Ophiopluteus (61.5) Echinopluteus (32.7) Auricularia (5.8)	1.15 ± 0.34 Ophiopluteus (45.9) Echinopluteus (33.3) Auricularia (11.7) Brachiolaria (6.1) Bipinnaria (2.9)	106.26 ± 18.99 Echinoderm larvae (63.3) Ophiopluteus (29.7) Echinopluteus (4.0) Auricularia (1.9) Other echinodermata (1.1)	47.17 ± 7.63 Echinodermata larvae
Fish eggs and larvae	17.00 ± 4.12 *Engraulis encrasicolus* eggs and larvae (45.5) Fish eggs and larvae (36.0) Sardine eggs and larvae (18.5)	2.40 ± 0.56 Fish eggs and larvae (89.6) Sardine eggs and larvae (9.7) *Engraulis encrasicolus* eggs and larvae (0.7)	6.74 ± 0.57 Fish eggs and larvae (96.6) Clupeidae (sardine) eggs and larvae (3.4)	0.79 ± 0.12 Fish eggs and larvae (92.4) Ammodytidae larvae (7.5) Clupeidae (sardine) larvae (0.1)

Table A.3. Mean density ± standard error (individuals m^{-3}) and composition of cladoceran and copepod genera considered in this study at B35, U35, L4 and SH. The value in parentheses is the contribution in percentage of each identified category to the total.

Taxa	**B35**	**U35**	**L4**	**SH**
Evadne	153.49 ± 30.56 *Evadne nordmanii* (75.9) *Evadne spinifera* (12.8) *Evadne* (10.6) *Pseudoevadne tergestina* (0.7)	50.64 ± 13.10 *Evadne nordmanii* (89.1) *Evadne spinifera* (9.6) *Evadne* (1.0) *Pseudoevadne tergestina* (0.3)	127.98 ± 25.15 *Evadne*	17.06 ± 4.25 *Evadne nordmanni*
Podon	93.51 ± 18.35 *Podon* (52.2) *Podon intermedius* (47.8)	21.26 ± 6.14 *Podon* (80.7) *Podon intermedius* (19.3)	43.48 ± 6.31 *Podon*	20.14 ± 6.32 *Podon leuckartii* (57.3) *Podon intermedius* (20.6) *Pleopis polyphaemoides* (16.0) *Podon* (6.1)
Acartia	1036.97 ± 126.20 *Acartia clausi* (96.0) *Acartia tonsa* (3.7) Other *Acartia* (0.1)	1217.33 ± 325.22 *Acartia clausi* (99.0) Other *Acartia* (0.4)	152.48 ± 19.68 *Acartia clausi*	556.34 ± 82.28 *Acartia clausi* (99.8) *Acartia longiremis* (0.2)
Calanidae	20.02 ± 6.62 *Calanus* (93.7) *Eucalanus* (3.7) *Calanus helgolandicus* (2.5)	4.78 ± 0.96 *Calanus* (83.2) *Eucalanus* (10.5) *Calanus helgolandicus* (6.4)	89.48 ± 7.78 *Calanus helgolandicus* (96.8) *Calanus* (3.0) Other Calanidae (0.1)	69.02 ± 9.62 *Calanus* (55.9) *Calanus helgolandicus* (31.5) *Calanus finmarchicus* (7.1) Calanoida (5.6)
PCPC-calanus	520.24 ± 56.76 *Paracalanus parvus* (48.1) P-calanus (33.6) *Paracalanus* (12.2) *Clausocalanus* (5.6) Other PCPC (0.5)	509.06 ± 95.83 *Paracalanus parvus* (43.6) P-calanus (37.6) *Paracalanus* (12.4) *Clausocalanus* (5.8) Other PCPC (0.5)	774.23 ± 41.99 *Pseudocalanus elongatus* (36.4) *Paracalanus parvus* (33.4) Other PCPC (26.0) *Clausocalanus* (3.6) *Ctenocalanus vanus* (0.6)	269.46 ± 19.41 *Pseudocalanus elongatus* (79.6) *Paracalanus. parvus* (20.3) Other PCPC (0.1)
Centropages	50.50 ± 11.53 *Centropages typicus* (62.9) *Centropages* (37.1)	37.18 ± 10.70 *Centropages* (71.3) *Centropages typicus* (28.7)	29.00 ± 3.62 *Centropages typicus* (98.9) *Centropages hamatus* (1.0) *Centropages chierchiae* (0.02)	37.50 ± 5.30 *Centropages. hamatus* (73.6) *Centropages. typicus* (26.4)
Temora	55.10 ± 11.05 *Temora stylifera* (67.1), *Temora* (16.6) *Temora. longicornis* (16.3)	40.53 ± 9.85 *Temora stylifera* (53.8) *Temora longicornis* (29.4) *Temora* (16.8)	216.48 ± 30.04 *Temora longicornis* (99.9) *Temora stylifera* (0.1)	147.30 ± 16.36 *Temora longicornis*
Oithona	205.21 ± 26.39 *Oithona nana* (52.2) *Oithona. similis* (37.5) *Oithona. plumifera* (6.5) *Oithona* (2.2) *Oithona. davisae* (1.6)	199.18 ± 22.41 *Oithona similis* (62.0) *Oithona nana* (29.9) *Oithona plumifera* (5.9) *Oithona* (1.6) *Oithona davisae* (0.3)	411.22 ± 35.80 *Oithona*	259.21 ± 20.39 *Oithona*
Oncaea	47.74 ± 14.05 *Oncaea media* (87.6) *Oncaea* (12.3) *Monothula subtilis* (0.1)	74.87 ± 19.40 *Oncaea media* (89.7) *Oncaea* (10.2) *Monothul subtilis* (0.1)	361.05 ± 32.76 *Oncaea*	1.81 ± 0.23 *Oncaea*
Corycaeus	3.45 ± 0.57 *Ditrichocorycaeus anglicus*	5.99 ± 1.11 *Ditrichocorycaeus anglicus*	87.45 ± 8.65 *Ditrichocorycaeus anglicus*	1.25 ± 0.25 Corycaeidae

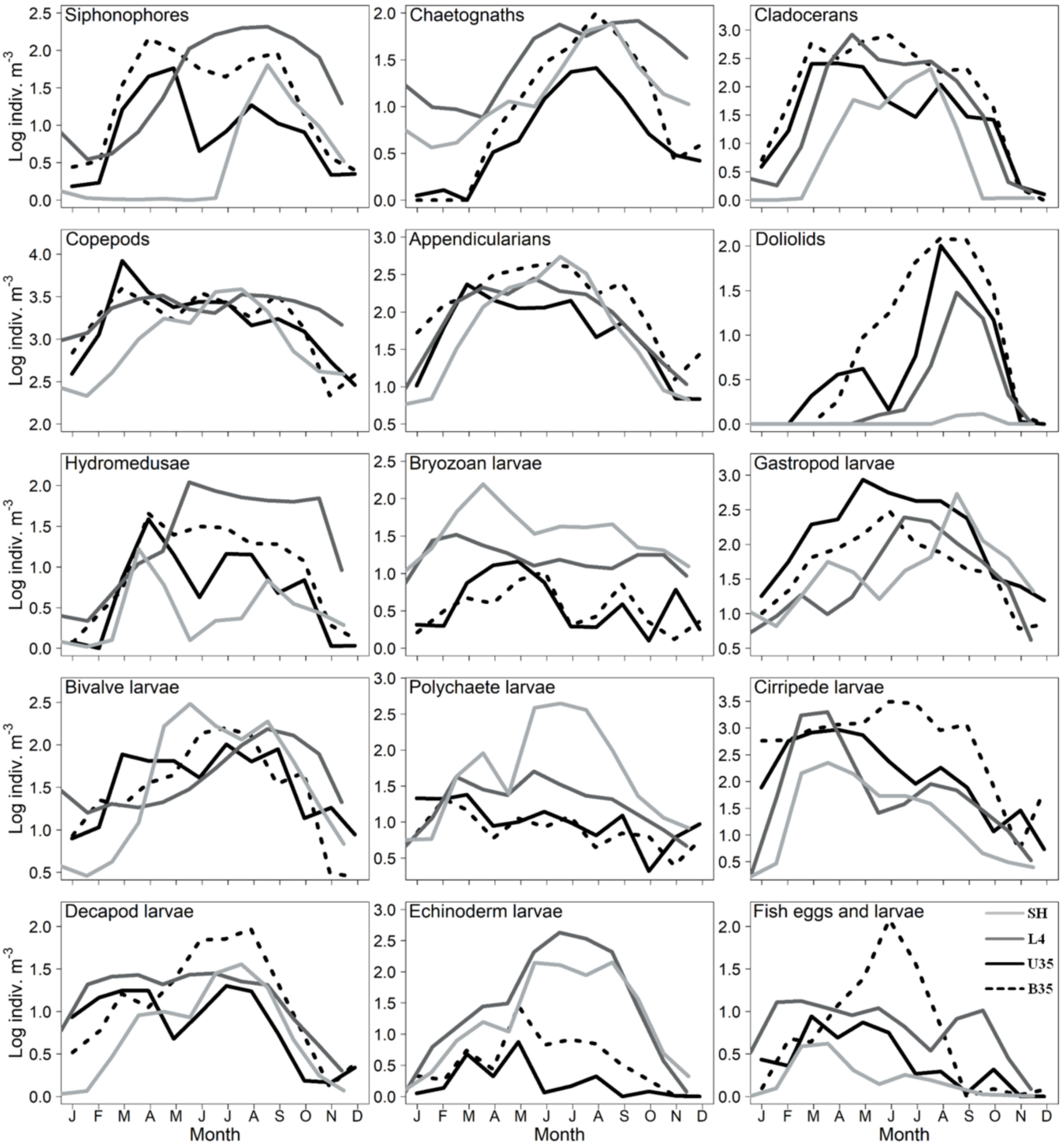

Figure 1S. Seasonal variation of monthly mean densities of ZG at B35 (dashed), U35 (black), L4 (dark grey) and SH (light grey).

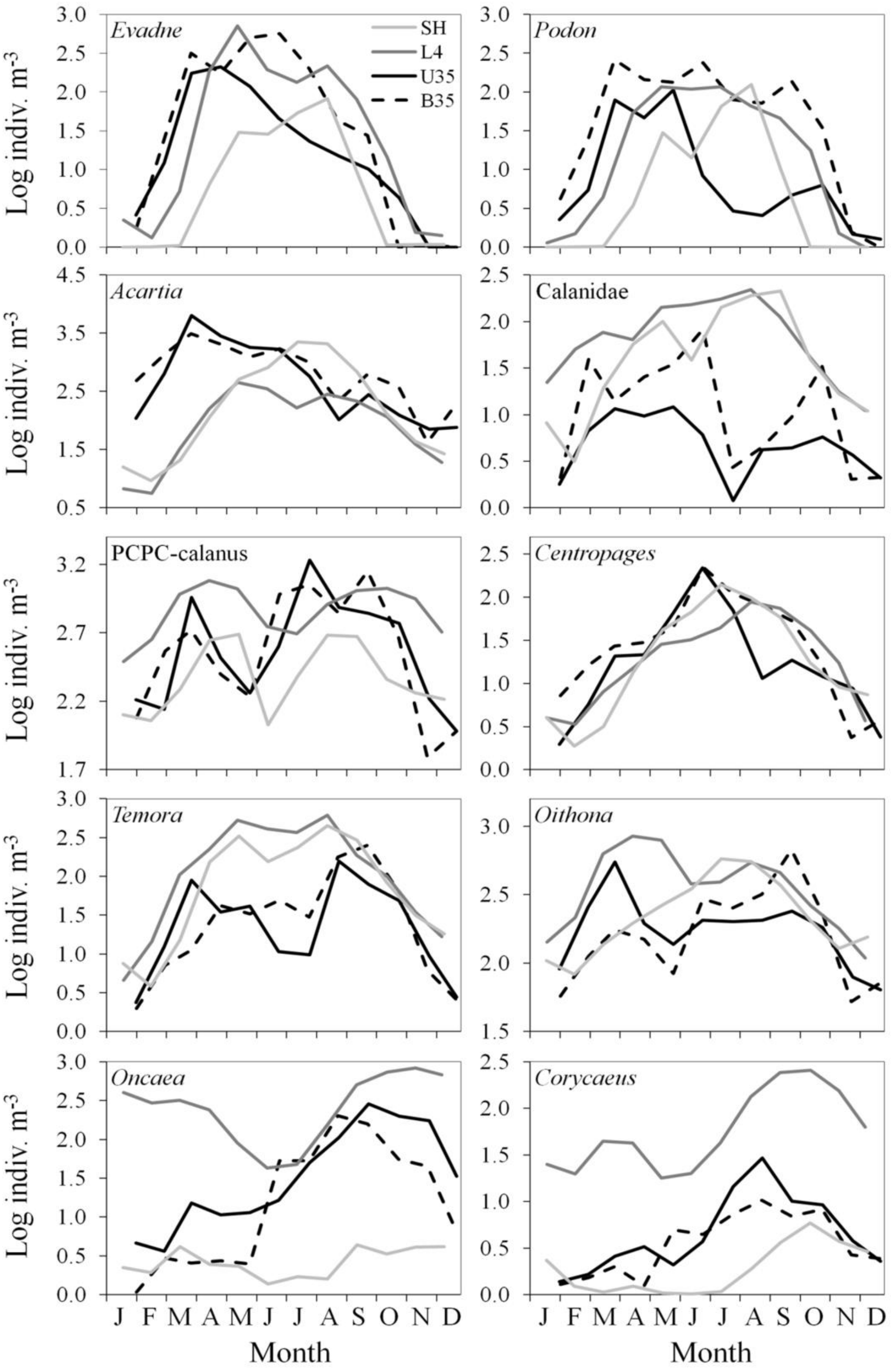

Figure 2S. Seasonal variation of monthly mean densities of CCGen at B35 (dashed), U35 (black), L4 (dark grey) and SH (light grey).