Universal ecological responses to climate change: understanding impacts on zooplankton

Thesis submitted in accordance with the requirements of the University of Liverpool for
the degree of Doctor in Philosophy by Stefano Corona

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ABSTRACT

Thesis author: Stefano Corona

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Copepods, typically dominate marine metazoan zooplankton both by numbers and biomass. They are a fundamental energetic link between the basal and the upper layers of the marine food web, and contribute substantially to the biogeochemical cycling of carbon. Copepods, being ectothermic and short-lived animals, are sensitive to temperature and thus represent good indicators of global warming effects. I use this group to investigate the three so-called “universal responses to climate change”: the temperature-size response (TSR), by which adult copepods present a reduced body size when reared at warmer thermal regimes; the phenological shift, which is investigated indirectly in copepod populations using the change in timing of seasonal abundance through the year; and the biogeographical shift, which is the change of latitudinal distribution of abundance to keep pace with the poleward shift in isotherms. I aim to investigate and compare each of these three responses in detail across seven ecologically important copepod species. First, I explore the body size change response by measuring the body size of copepods collected through the English Channel station L4 time-series (32 years). Besides calculating the percentage change of body mass per degree Celsius across multiple years and months, I also detected signs of long-term declines in body size with warming. On top of this, I further revealed a correlation between body size and copepod abundance at the beginning of their temperature-dependent phase (in April), and the correlations with the high zooplankton/phytoplankton ratio at this time of year suggested that body size reflected a trade-off with predator avoidance as well as food acquisition. Secondly, I used three multi-decal times-series of plankton abundance across the North-West European shelf (at L4, Stonehaven, and Helgoland Roads), to estimate the species-specific phenological and thermal niche change with temperature. Shifts in timing of seasonal abundance across years were inconsistent among species and stations and were explained more by time than temperature. Nevertheless, I detected signs of thermal niche conservation manifested through shifts in seasonal timing of abundance across different latitudes and across years. However, I suggest that the real phenological adjustment in some stations may have been obscured by the long-term decline in abundance at specific times of the year. Third, I investigated the thermal niche conservation that copepod populations demonstrate through biogeographical adjustments, by using the extensive Continuous Plankton Recorder (CPR) survey dataset. Latitudinal shifts with warming were detected, but these were stronger and

more consistent in the North-East Atlantic (where species conserved their thermal niche over time) than in the North Sea. In the end, I combined the above results to explore how the “universal” responses to climate warming were linked. I found that species that can adjust phenotypically by shifting in adult body size with warming tended to show the least degree of phenological shift to counteract warming. The intensity of shifts in seasonal timing and in latitude across species appear to be positively correlated: thus, species that shift most toward spring also shift northward more intensely. Moreover, in the North Sea, the phenological shift toward spring was found to be stronger at northern latitudes than at southern ones. Consequently, these three responses to warming need to be considered together in future studies that aim to better understand and possibly predict the long-term change in zooplankton spatio-temporal structure.

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CHAPTER 1:

General introduction

1.1 Global warming effects on the hydrosphere

Human health and wellbeing are fundamentally linked directly or indirectly, to my seas and oceans. Containing 97% of all Earth's water and covering 71% of Earth's surface, the oceans not only play a crucial role in the climate system, such as uptake and redistribution of carbon dioxide (CO_2) and heat, but they also provide food and water supply, renewable energy, along with positive effects for human health and quality of life through tourism, trade, transport, and culture (IPCC, 2019). The increase in concentration of greenhouse gases since around 1750, is unequivocally caused by human activities, which have led to an increase of ~1.09°C in the average global surface temperature over the course of around a hundred years (from 1850-1900 to 2011-2020; IPCC, 2021). Oceans have absorbed 80% of the total heat increase in the global climate system, but the pace of warming has been around two times slower in surface waters (0.88°C increase), compared to air temperatures over land (1.59°C increase). Despite this, the rate of latitudinal shift of annual sea surface temperature (SST) isotherms in the oceans was faster than or similar to that on land (Burrows et al., 2011).

For marine organisms, either a continuation in the current rate of warming or any increase poses a major threat. Most ectothermic aquatic organisms experience a shorter thermal safety margin (they live closer to their upper thermal limit) than terrestrial ones, whose local extirpations were found to be half of the ones in the ocean (Pinsky et al., 2019). For these reasons, keeping pace with the changing of biotic dynamics in the marine environment as the global climate keeps warming is of utmost importance. Global average temperature is predicted to increase by ~4°C during the 21st century (IPCC, 2021), along with extreme climatic events (Schär et al., 2004; Diffenbaugh and Ashfaq, 2010) unless serious counter-measures at a large scale are taken.

Temperatures over the thermal tolerance range of an ectothermic marine organism can affect its metabolism, growth and reproduction (Schulte, 2015; Pörtner et al., 2017; Somero et al., 2017) which can lead to ecological disruptions, such as shifts in phenology and geographical range (Parmesan and Yohe, 2003; Perry et al., 2005; Burrows et al., 2011; Poloczanska et al., 2016), local extinction and biodiversity change (Cheung et al., 2009). Studies are increasingly emphasising multi-stressors, so for example the effects of acidification (caused by increase of anthropogenic CO_2 in water) on organisms with calcifying body structures (Guinotte and Fabry, 2008) can be exacerbated by higher temperatures (Kroeker et al., 2013; Mackenzie et al., 2014; Rosas-Navarro et al., 2016). Moreover, warmer thermal regimes can also affect the delicate balance of biogeochemical nutrient cycles, on which most marine ecosystems depend (Henley et al., 2020).

1.2 Copepods

Copepods, a subclass of Hexanauplia (Subphylum Crustacea), are the most representative taxonomic group among zooplankton (Humes, 1994; Hopcroft et al., 2005). They are mainly holoplanktonic, spending their whole life cycle in the plankton (unlike meroplanktonic organisms, which have at least one benthic phase in their life). Because of their intermediate size between ecologically important unicellular microplankton (primary producers and heterotrophic protists) and major pelagic animals such as planktivorous fish and predatory invertebrates, copepods play an essential role in transferring energy from lower to higher trophic levels (Steele, 1974; Atkinson, 1996; Calbet and Saiz, 2005). They contribute substantially to biogeochemical cycles (Sadaiappan et al, 2021), mainly through the release of faecal pellets that transfer organic matter through the water column (Turner et al., 2015), and through grazing (Le Quéré et al., 2016). Therefore, they are crucial to maintaining the function of oceans and seas, as well as freshwater bodies such as lakes, rivers, and ponds. Furthermore, copepods play a major role in commercially-valuable food chains, since they feed most of the important fish species for human use (Hjort, 1914; Cushing, 1990; Beaugrand et al., 2003). Copepods (and zooplankton in general) are excellent indicators of hydrometeorological changes in the environment (Richardson, 2008) for several reasons: first, they are ectothermic, which means that their physiological processes, such as growth, respiration, ingestion and development are greatly dependent on the environmental temperature, typically showing about two- to three-fold increases with 10°C warming (Mauchline, 1998). Moreover, having a short life (hence multiple generations within a year), being free drifters across different marine areas, and not being commercially exploited, make them excellent candidates for studying global warming effects on ecosystems. The copepod species chosen for this work were: *Acartia clausi, Calanus helgolandicus, Centropages typicus, Oithona similis, Paracalanus parvus, Pseudocalanus elongatus, Temora longicornis* (**Figure 1.1**). These species are some of the most abundant across the North-West European shelf (Eloire et al., 2010), hence they represent some of the most ecologically and commercially important taxa of the local marine ecosystems. All these species are part of the Order Calanoida, with the only exception of *O. similis*, which belongs to the Cyclopoid Order. Calanoid copepods are the most successful group (in terms of individual abundance) of the pelagic realm, comprising around 2000 species, possessing generally larger body sizes compared to other orders, releasing eggs directly in the water column (broadcast spawning strategy; although some calanoids, like *P. elongatus,* are sac spawner), and largely utilizing feeding currents to entrain and capture prey (Eloire et al., 2010; Kiørboe, 2011; Brun et al., 2017). Conversely, cyclopoid copepods are generally much smaller in body size, carry the eggs in either a single or pair of egg sacs until hatching (sac-spawning strategy), and do not rely on active filtration through feeding currents, but on passive ambush feeding (Paffenhofer, 1993; Brun et al., 2017). In fact, cyclopoids exhibit a lower metabolic demand compared to calanoids and the reason for this could be related to the different feeding modes (ambush in cyclopoids vs. continuous water filtration in calanoids, Kiørboe, 2011).

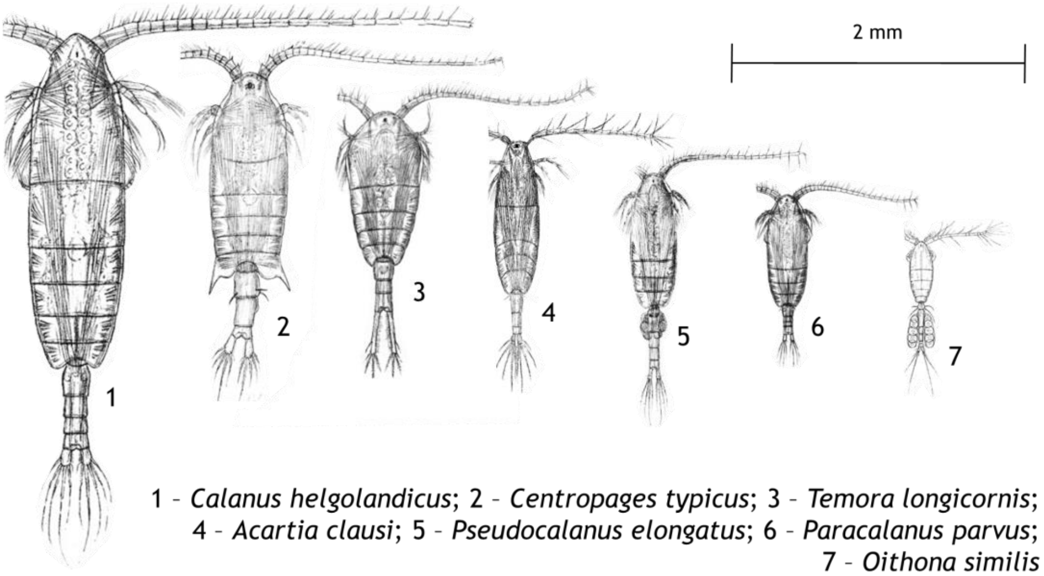


Figure 1.1: Accurate drawings of the seven copepod species considered in this thesis. Sizes are to scale (see scale bar at the top right). Source of the drawings: Sars, 1837-1927 (Vol. 4 and 6).

1.3 The effects of warming on the physiology and ecology of zooplankton

An important concept to consider when exploring the effects of warming on ectothermic organisms is the thermal tolerance range. This is described by the response curve for the performance value (such as growth, respiration, egg production, etc.) for a given species against the experienced temperature, with the peak of the bell-shaped distribution indicating the species' thermal optimum (**Figure 1.2**). In this model, the critical measure of performance for population dynamics is fitness, or the life-history traits that correlate to it. Being outside the thermal optimum does not mean immediate physiological stress, because, depending on the species, an animal can undergo a certain set of plastic behavioural adjustments. When the animal is found outside its thermal tolerance limits, phenotypic plasticity cannot compensate anymore and thus physiological stress becomes inevitable, leading to immunodepression, anaerobiosis, torpor and ultimately death (Beaugrand and Kirby, 2018). Therefore, phenotypic adaptations stand between the species and its extinction. However, these adjustments have to take place with respect to other environmental parameters (such as food availability, competition, predation, parasites, salinity, weather, etc.), which means that trade-offs are likely to occur (McQuinn, 1997; Guilford et al., 2009; Jònasdòttir and Koski, 2011). The main interest of this thesis is in fact the phenotypic plastic responses to warming that copepods can display at the individual and population levels. The main responses are three and are generally referred to as 'the three universal responses' to global warming: the change in adult body size (temperature-size response (TSR), Gardner et al., 2011), the shift in phenology (Richardson, 2008), and the shift in biogeographical range (Beaugrand et al., 2002). Responses to warming may be observed as phenotypically plastic responses exhibited by individuals (e.g.: the TSR, temperature-induced seasonal advance of maturity (advanced phenology), temperature-related increases in poleward abundance shift) or may result from selection (e.g.: elimination of genotypes that mature at large sizes, that breed late in the season or that do not survive latitudinal shifts in distribution).

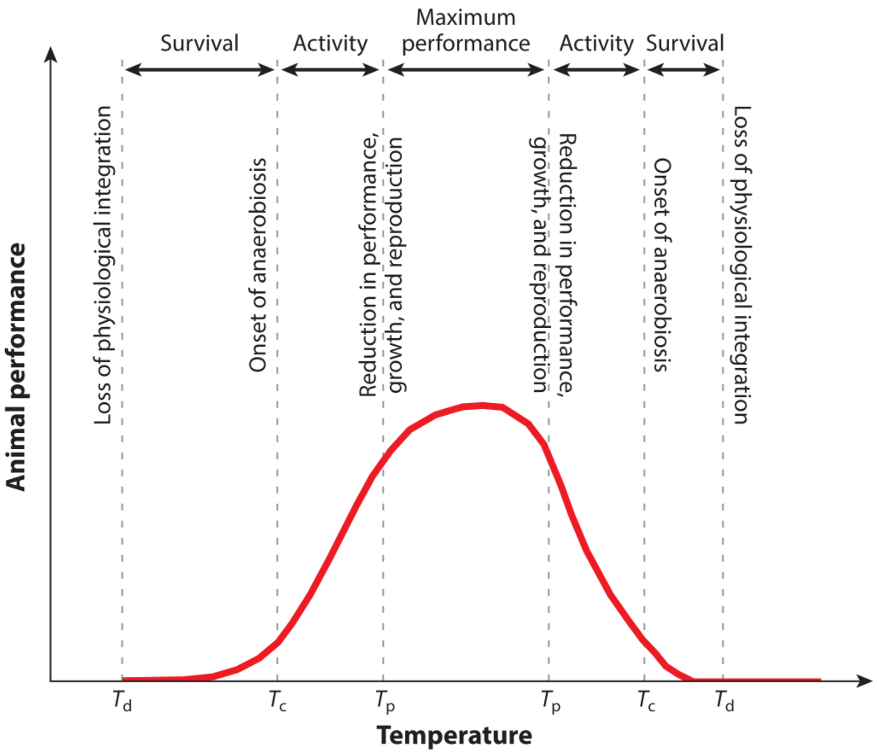


Figure 1.2: Figure reproduced from Beaugrand and Kirby (2018). *Tp* is the pejus thermal threshold, at which aerobic metabolism begins to diminish; *Tc* is the critical thermal threshold, at which activity ceases; and *Td* is the denaturation thermal threshold, at which denaturation begins.

1.4 The temperature-size response

The change in adult body size with warming is a phenotypic physiological response of ectotherms at the individual-level: at warmer temperatures, development rate increases more than growth rate (van der Have and de Jong, 1996, Forster et al., 2011a, b), so that copepods reared in warm seasons will reach maturity in a shorter time with a faster growth rate, but with a smaller body sizes, than the ones reared in colder conditions (Atkinson, 1994). Consequently, it is a response detectable only in species that produce several generations across a thermal gradient (across different seasons), such as copepods. This process is defined as the “temperature-size response” and it is typically stronger for aquatic ectothermic organisms than terrestrial ones, most likely due to the greater oxygen limitation in water (Forster et al., 2012; Horne et al., 2015). In zooplankton, this phenomenon occurs during the warming part of the year, generally called the “temperature-dependent phase” (TDP, Hirche et al., 2019), roughly from April to October. Adults of the last generation at the end of this period, when thermal conditions start to become unsuitable (colder) again, enter the “resting phase”, in which they stop growing and reproducing, until they encounter warmer conditions in the following year. A global synthesis has already assessed that temperature, rather than food, is the dominant explanatory variable affecting seasonal changes in adult copepod body size, and that calanoids copepods, which generate currents to feed, exhibit a greater reduction in adult body mass per °C (-3.66%) than cyclopoids (-0.91%), which are ambush feeders (Horne et al., 2016). Furthermore, that synthesis found no influence of average body size and reproductive strategy on the strength of the seasonal T-S response (Horne et al., 2016).

The scaling of body size with temperature and the effects of body size and temperature on metabolic rates are found to be different among populations of the same community. Therefore, a warmer environment may alter size-dependent interactions such as the predator-prey size ratio (Twomey et al., 2012; Gibert and DeLong, 2014), cause shifts in biological rates (growth and respiration, Twomey et al., 2012) and behavioural processes such as diel vertical migration (Ohman and Romagnan, 2016; Bandara et al., 2021), potentially inducing trophic cascades (Jochum et al., 2012). Therefore, it is crucial to investigate the possible warming-related long-term adult body size change in the copepod community and how this could affect the plankton food web.

1.5 Phenological shift

Phenology is the timing of periodical seasonal life cycles of organisms that occur each year, such as reproduction, egg spawning, blossoming, migration, etc. Because phenology can shift in time based on environmental drivers, this can induce major changes at the population and community levels rather than at the individual one. This response is fundamentally related to the concept of the thermal niche conservation principle (ter Braak, 1996).

Ectothermic organisms, not being able to adjust their internal temperature to the external ones, must remain within their thermal range in order to perform normally and not lose fitness. Copepod populations track their thermal niche either by changing their phenology such that they appear at a more suitable time of the year thermally, or by shifting their geographical range (usually with a poleward movement/expansion, Poloczanska et al., 2013) and also by spending more time at depth (Bandara et al., 2021). Plankton phenology is often defined differently from the above definition -as the seasonal distribution of the abundance of a species- and can be indexed in several ways: julian day of maximum annual abundance, Julian day at which occurs a certain quantile of cumulative abundance (25th, 50th or 75th), Julian day at which occurs the "center of gravity" of abundance, (Edwards and Richardson, 2004; Mackas et al., 2012; Thackeray, 2012; Atkinson et al., 2015). Seasonal timings are driven by environmental conditions such as temperature, photoperiod, nutrient availability, and the structure of the water column (Ji et al. 2010, Mackas et al., 2012, Thackeray et al., 2016) and only secondarily by prey availability (Visser and Both, 2005, Winder et al., 2009). However, seasonal timing of marine zooplankton remains highly variable, and often difficult and challenging to interpret and predict. Ji et al. (2010) suggest, possible explanations for this high variability: 1) great reliance on dormant life stages; 2) having multiple short generations within a year that may prevent any fitness advantage of fixed-calendar response (because reproductive success cannot be passed on by individuals of the previous year); 3) individuals of one generation are not able to perform horizontal geographical shifts at large scale in order to find better environmental conditions in another area; and 4) the controls upon which phenology rely (such as thresholds of temperature, food availability, physiology) also show great interannual variability. Indeed, sea surface temperature has risen with a strong interannual variability, so much so that climate change effects, such as the response to signals of increasing sea surface temperatures, are often only clearly and statistically demonstrable using multi-decadal time-series datasets (Giron-Nava et al., 2017; Henson et al., 2010).

Nevertheless, shifts in seasonal timing in response to warming have been reported for most planktonic groups. Examples of shifts in timing of annual maximum abundance, include *Neocalanus plumchrus,* which has shifted 60 days earlier in warm years than in cold ones (Mackas et al., 1998), and *Calanus helgolandicus* by one month since 1960 (Beaugrand, 2009). Between 1992 to 2012, in the English Channel, shifts in timing of population peak of abundance per °C in sea surface temperature were ~ 15 days earlier in *Acartia clausi* and *Pseudocalanus elongatus* and ~ 17 days later in *Centropages typicus*, whereas the timing of occurrence of other taxa such as *C. helgolandicus* and *Oithona spp.* has remained relatively stable (Atkinson et al., 2015). Also the appearance of meroplankton (larvae of cirripedes, cyphonautes, decapods, echinoderms, fish, and lamellibranchs) has advanced by 27 days in the last 45 years (Edwards and Richardson, 2004). Even fish larvae have shown signs of an earlier seasonal peak of abundance (Greve et al., 2005).

The most likely and severe consequence of this kind of temporal shift is the mismatch of the synchrony between two adjacent trophic levels (Cushing, 1990). For instance, over the past 45 years, primary producers such as dinoflagellates and diatoms advanced the timing of their peak of abundance by 22-23 days, whereas their primary consumers, copepods and other holoplankton, advanced by only 10 days (Edwards and Richardson, 2004). However, evidence for fitness losses in the planktonic primary consumers as a consequence of trophic mismatch are difficult to find (Thackeray 2012; Atkinson et al. 2015)

1.6 The biogeographical range shift

This universal response is similar to the phenological one, but instead of tracking the thermal niche by occupying new seasons, copepod abundance shifts toward the poles, following the same direction as the movement of isotherms (Parmesan and Yohe, 2003; Poloczanska et al., 2016; Chivers et al., 2017). In zooplankton specifically, the mean body size of the population has decreased over time in the North Sea, due to the retreat of larger species from the south into the north (Pitois and Fox, 2006). The most renowned case of copepod biogeographical shift is the expansion of *Calanus helgolandicus* range northward, along with other temperate species such as *Centropages typicus* and *Pseudocalanus elongatus*, simultaneous with the contraction of the cold-adapted species *Calanus finmarchicus* (Chust et al., 2014; Hinder et al., 2014; Chivers et al., 2017), whose abundance has declined substantially across the North-West European shelf (Beaugrand et al., 2002; Pitois and Fox, 2006). Both the intensity and direction (northward or southward) of these range shifts are not always consistent among different populations. In fact, cases of southward shifts in the northern hemisphere have also been found, like in *Metridia longa* and *Metridia lucens* (Chivers et al., 2017), which notably are species with a larger diurnal vertical migration ambit, along with the Arctic and Subarctic species *Calanus glacialis* and *Calanus hyperboreus* (likely due to the movement of cold water from north to south, Beaugrand et al., 2002; Chust et al., 2014). This drastic spatial rearrangement of marine communities may lead to cascade disruptions in energy transfer, trophic interactions and biogeochemical cycles (Doney et al., 2012; Chivers et al., 2017). For instance, negative effects on the Arctic food web structure, as a consequence of its “Atlantification”, have been detected (Kortsch et al., 2015; Eriksens et al., 2017; Frainer et al., 2017; Vihtakari et al., 2018). The latitudinal shifts of copepods may even be partly responsible for the spatiotemporal modifications of the carbon export in the North Atlantic (Brun et al., 2019).

1.7 Thesis outline

Within this thesis, I investigate all three responses to global warming across the same seven copepod species, through the analysis of different time-series of plankton samples in the North-West European shelf: three sampling stations (L4 – Plymouth, Stonehaven and Helgoland Roads) and the Continuous Plankton Recorder (CPR) survey (**Figure 1.3**). The stations have been sampling the whole water column every day/week for multiple decades, the CPR activity has been sampling also for multiple decades across a much wider area, but only the near surface water layer, using commercial ships of opportunity.

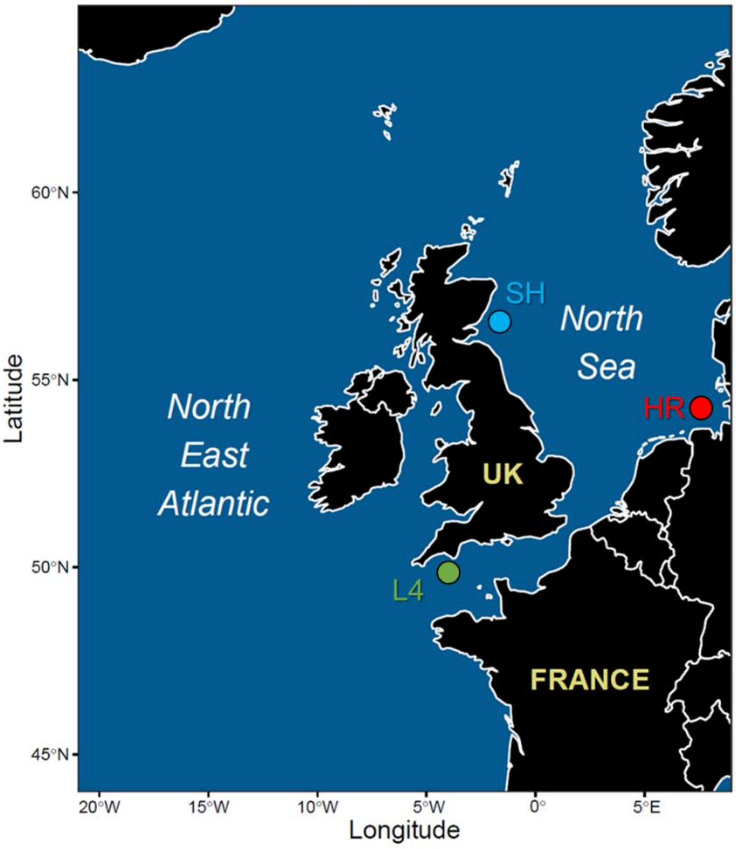


Figure 1.3: A map showing the location of the three sampling stations: L4-Plymouth (green), Helgoland Roads (HR, red), Stonehaven (SH, light blue). The CPR areas that I considered were the North-East Atlantic and the North Sea (see Chapter 4 for details).

Chapter 2 examines the effect of temperature, food, and holoplankton abundance on individual copepod body size. I measured the body mass of 7089 female adult copepods across seven species, over seven months (from April to October), and in 13 years spanning a total period of 27 years. I found that temperature greatly (and more than food) explained seasonal body size variation, but holoplankton abundance negatively influenced body size in the month of April only. Long-term signs of body size decrease were also found.

- Chapter published: Corona, S., Hirst, A.G., Atkinson, D., Atkinson, A., 2021. Density-dependent modulation of copepod body size and temperature–size responses in a shelf sea. Limnology and Oceanography, **66**: 3916–3927. https://doi.org/10.1002/lno.11930

Chapter 3 investigates if and how copepods manage to conserve their thermal niche by shifting in seasonality (phenological shift) with warming over time. I used three different copepod abundance time series of data (from three different stations in the North-West European shelf) to index copepod phenology and copepod realized thermal niches. Great inter-annual variability of both biotic and abiotic parameters and different temporal abundance distribution trends among different species and stations made the interpretation of phenological shift response quite challenging. Nevertheless, I detected signs of thermal niche conservation manifested through shifts in seasonal timing of abundance across different latitudes and across years.

- Chapter under review: Corona, S., Hirst, A.G., Atkinson, D., Renz, J., Boersma, M., Atkinson, A., 2023. Do zooplankton conserve their thermal niche by changing their phenology? A long-term study of declining copepod populations on the NW European continental shelf. Limnology and Oceanography.

Chapter 4 identifies the biogeographical range shift of 8 copepod taxa over 60 years in two different adjacent marine areas: the North-East Atlantic and the North Sea, through the continuous plankton recorder (CPR) survey dataset. Similarly to the previous chapter, I tested if and how copepods manage to maintain the same thermal niche by shifting in latitude. Latitudinal shifts with warming were detected, but these were stronger and more consistent in the North-East Atlantic (where species conserved their thermal niche over time) than in the North Sea.

- Chapter close to submission: Corona, S., Hirst, A.G., Atkinson, D., Atkinson, A. Thermal niche conservation and latitudinal shift: contrasting trends of adjacent copepod populations in the North Sea and Northeast Atlantic.

Chapter 5 explores the relationships among the three different responses to climate across these copepod taxa from previous chapters. Here I discuss new findings from linking the responses that have been described earlier in the thesis. Some recommendations for future research are proposed, in order to improve understanding of how these population responses to climate change are inter-related and to possibly solve the new emerging questions that have arisen from this work.

CHAPTER 2:

Density-dependent modulation of copepod body size and temperature-size responses in a shelf sea

2.1 ABSTRACT

Body size is a fundamental trait in ecology, and body size reduction with increasing temperature has been termed the third universal response to climate warming. Whereas effects of temperature and food on phenotypic plasticity of zooplankton adult body size have been investigated, density-dependent effects have been neglected. I measured seasonal changes in the prosome length of 7098 adult females of seven dominant copepod species in 13 years spanning a 27-year period of warming at the L4 time series off Plymouth, UK. The seasonal Temperature-Size (T-S) response varied greatly among species, from reductions of 2.93% of carbon mass $°C^{-1}$ for *Paracalanus parvus* to 10.15% of carbon mass $°C^{-1}$ for *Temora longicornis*. Evidence for a longterm T-S response was detected in at least two species, supporting the hypothesis that climatic warming leads to smaller adult sizes. April was a crucial month for determining the strength of the T-S response. During this month, body size related negatively to total zooplankton abundance. I suggest that the mechanism for this density-dependence is via competition for food and/or intraguild predation, since spring was also the period when the ratio of food biomass to zooplankton biomass was at its lowest. Our study is among the first *in situ* demonstrations of density-dependent effects on the body size of marine zooplankton and shows the need to consider the effect of top-down as well as bottom-up factors on body size in a warming climate.

2.2 INTRODUCTION

Body size is a ‘master trait’, influencing a multitude of physiological and ecological processes and rates (Kleiber, 1947; Hirst et al., 2014; Andersen et al., 2016). Understanding how body size is influenced by environmental conditions, including warming, is particularly important, given that trophic interactions and vital rates are highly size-dependent in many pelagic ecosystems (Hansen et al., 1994; Cuthbert et al., 2020). The commonly observed plastic phenotypic response, in which body size at maturity of an ectothermic species declines with increasing developmental temperature, is called the Temperature-Size Rule (Atkinson, 1994). Indeed, smaller adult body size following growth in warmer conditions (a negative temperature-size (T-S) response) is evident in many multivoltine aquatic invertebrates in laboratory experiments and across thermally varying seasons and latitudes (Lock and McLaren, 1970; Forster et al., 2012; Horne et al., 2017). More broadly, a warming-induced reduction in body size within a community is regarded as one of the three universal responses to climate change, along with responses of phenology (seasonal timing of life cycles) and geographical redistribution (Gardner et al., 2011).

Body size reductions with warming are not only observed under controlled experimental temperatures with food satiation (Lock and McLaren 1970; Lee et al., 2003; Forster et al., 2012), but have been widely documented in thermally variable seasonal environments, often at temperate latitudes (Horne et al., 2016, 2017) and with latitude (Horne et al., 2015). Such correlations between seasonally-varying temperature and the body size of adults are largely dependent upon periods in which adult recruitment is significant, and therefore the measured environmental conditions relate closely to those experienced during the juvenile growth phase of the recruiting adults. In many copepod species, in temperate and high latitude environments, recruitment of adults is especially marked between spring and late summer, which has been termed the ‘Temperature Dependent Phase’ (TDP), followed by a ‘Resting Phase’ (RP) in autumn-winter (Hirche et al., 2019).

Shifts in copepod body size have the potential to impact upon both their prey and their predators. Besides temperature, food concentration and predation pressure can also impact growth and development rates, and the body size achieved by copepod adults (Hall et al., 1976; Abrams and Rowe, 1996; Bonecker et al., 2011). Indeed, seasonal body size variation is influenced by food availability not only in copepods (Durbin et al., 1983) but other invertebrates too (Berrigan and Charnov, 1994). Other factors that relate to the strength of T-S response in copepods include the feeding mode and taxonomic affiliation of the species (Horne et al., 2016).

Pelagic copepod species play a pivotal role as grazers of microplankton, and as conduits of energy and material to higher trophic levels, including fish and predatory invertebrates (Banse, 1995; Beaugrand et al., 2003). It is still unclear how their seasonal body size distribution varies from year to year, and among species, and how this may change with respect to climate warming. Sea surface temperature has increased significantly worldwide in the last 100 years (IPCC report, 2014). However, climate warming occurs with great inter-annual fluctuations, yet few previous studies have investigated the effects of copepod body size over multiple years (although see e.g.: Evans, 1981) and no study has simultaneously considered the T-S response of many species over several years at a single location. To achieve such multi-year, within-site synthesis of species body size responses across a copepod community I investigated seven contrasting copepod species (*Acartia clausi*, *Oithona similis, Paracalanus parvus, Pseudocalanus elongatus, Calanus helgolandicus, Centropages typicus, Temora longicornis*) in 13 years sampled from a 27-year record at the Plymouth L4 site, an inshore site in the Western English Channel. To have several species measured over so many years allows us to explore both inter-annual changes in body size and whether such patterns could be related to drivers related to climate change.

Previous interpretations of size responses of different species based on meta-analyses across different geographical locations and different decades (e.g. Horne et al., 2016) are potentially complicated by the confounding effects of location-specific environmental factors. These confounding effects make it hard to tease out the various biotic and abiotic factors that shape the T-S response. For example, in addition to bottom-up factors, zooplankton community density has already been shown to be related to increased mortality of copepod early-stages and to decreased population growth, egg production and hatching success rate (Ohman and Hirche, 2001; Franco et al., 2017). However, very little is known about the influence of community abundance *in situ* on individual copepod body size. I used, for the first time, an extensive dataset from a single location across multiple years to address the following questions: 1) To what degree do the seasonal temperature-size responses of the seven copepod species differ? 2) Do food concentration, long-term temperature anomalies, or both, regulate copepod size changes across years? 3) Are copepod body size and strength of T-S response also governed by factors that depend on zooplankton community density?

2.3 METHODS

All the samples used for this study were taken from the zooplankton time series at Plymouth Station L4 (50°15’N 4°13’W), 13 km SSW of Plymouth, which has been sampled at weekly resolution, weather permitting, since March 1988 by Plymouth Marine Laboratory (Harris, 2010; http://www.westernchannelobservatory.org.uk/). The water is ~54 m deep, which stratifies seasonally, with a thermocline forming typically from about May to September. Surface water temperature typically ranges from about 9°C in winter to 17°C in summer. The study location is affected periodically by freshwater outflow from the rivers Plym and Tamar at Plymouth (Rees et al., 2009). Further details about the Plymouth L4 station and microplankton and zooplankton sampling are reported respectively in Widdicombe et al., (2010) and Atkinson et al. (2015).

2.3.1 Environmental data

An electronic probe for conductivity, temperature and depth analysis (CTD) is currently used at L4, but sea surface temperature (SST) has been measured consistently throughout the whole time series using a mercury-in-glass thermometer inside a stainless steel bucket of water collected at the surface. For consistency across all my sampling years, I used these latter measurements, which showed close agreements with surface values from the CTD (Atkinson et al. 2015).

2.3.2 Food data

Microplankton, which are used to represent the copepod food variable in my study, have been routinely collected and identified on a near-weekly basis since October 1992. Paired water samples collected from 10m depth were preserved at sea with acid Lugol's iodine and neutral formaldehyde. Samples were analysed at Plymouth Marine Laboratory following the British and European Standard EN 15204:2006 "Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy" (Utermöhl technique, more microplankton sampling details in Widdicombe et al., 2010). For this study I used the carbon mass density values (measured as mg C m^{-3}) of diatoms, dinoflagellates, coccolithophorids and *Phaeocystis*, as well as ciliates and flagellates, from the following copepod data years that had microplankton data availability: 1993, 1997, 2000, 2003, 2005, 2008, 2010, 2012, 2013 and 2014. The flagellate group includes taxa that are nanoplanktonic in size, although only the larger nanoflagellates were counted using the above method. However for simplicity I term my copepod food variable "microplankton" since this group encompasses the size range which the copepods can eat.

2.3.3 Zooplankton data

Zooplankton have been sampled since 1988. Each sample consisted of two replicate tows made with a WP-2 net (56 cm diameter, 200 µm mesh) towed vertically from a depth of 50 m to the surface. Each plankton sample was fixed in 4% buffered formalin. Laboratory analysis involved counting and identifying large and/or rare taxa in a fraction of the original sample (a half, a quarter or an eighth, depending on the abundance of the original), sub-sampled using a Folsom plankton splitter (Atkinson et al., 2015; Highfield et al., 2010). Each replicate was given an estimated abundance average, measured as individuals m^{-3} (John et al., 2001). The source of the dataset used here can be found in Atkinson et al. (2019).

For this study, one sample per month from April to October was considered from each of the years 1989, 1991, 1993, 1995, 1997, 2000, 2003, 2005, 2008, 2010, 2013, 2014, 2016. These years were chosen to cover a wide range of temperature conditions between years at L4, with 1991 being the coldest, and 2014 the warmest year. Other years, with intermediate temperature, were chosen in order to provide a wide range of coverage and conditions.

2.3.4 Body size measurements

One quarter of each plankton sample was removed and observed under the stereomicroscope in order to identify the first 30, when possible, adult females of each of the species: *Acartia clausi, Oithona similis, Paracalanus parvus, Pseudocalanus elongatus, Calanus helgolandicus, Centropages typicus and Temora longicornis*. In the case of *C. helgolandicus* a small number of the congener *Calanus finmarchicus* may have been inadvertently included, as these occur rarely at the site (a median composition of 4% of *C. finmarchicus*, according to Maud et al., 2015). Each individual was photographed with a stereomicroscope digital camera and then processed using the software ImageJ to determine prosome lengths, by measuring from the tip of the cephalothorax to the end of the last pedigerous somite. To avoid erroneous size estimations of *C. typicus* resulting from broken spines, the end of the prosome was considered not as the tips of its two most posterior lateral spines, but as the mid-point where the prosome articulates with the urosome. In *O. similis* the end of the prosome is very hard to identify because of its complex structure and reduced size, so, instead, I measured to the connection between the first and the second segment of the urosome, as this is more visible (as done in Sabatini and Kiørboe 1994). Prosome lengths were converted into estimated carbon mass (µg) using appropriate species-specific equations from the published literature (See **Table S 2.1**). These equations were obtained from copepods commonly sampled in the North Sea**.** However, for all analyses I tested both estimated carbon mass and prosome length, in order to see if different patterns would emerge.

2.3.5 Determination of temperature-dependence of body size reduction

I defined the TDP for each species as between the month with the largest average size (1st generation of copepods of the year) and the month with the smallest average size (last generation of the year). To quantify the relationship within the TDP between mean adult body size in each month and temperature in the previous 30 days, the mean carbon mass values for each species in each month in each year was regressed against mean temperature recorded in the 30 days prior to and including the sample day. I chose 30 days partly to correspond broadly with previous studies on the generation times of the same seven species (Sabatini and Kiørboe, 1994; Irigoien and Harris, 2003; Leandro et al., 2006; Carlotti et al., 2007; Stegert et al., 2007; Dzierzbicka-Głowacka et al., 2011). For fitting the body size-temperature regressions (µg C individual^{-1} vs. °C) I used exponential models as in Horne et al. (2016). The slopes from these exponential regressions were transformed into percentage change in carbon mass per ^{o}C as: ($\exp^{slope}$ -1) ×100 (Forster et al., 2012). These values have a negative value when mass decreases with warming, and a positive value when body mass increases with increasing temperature.

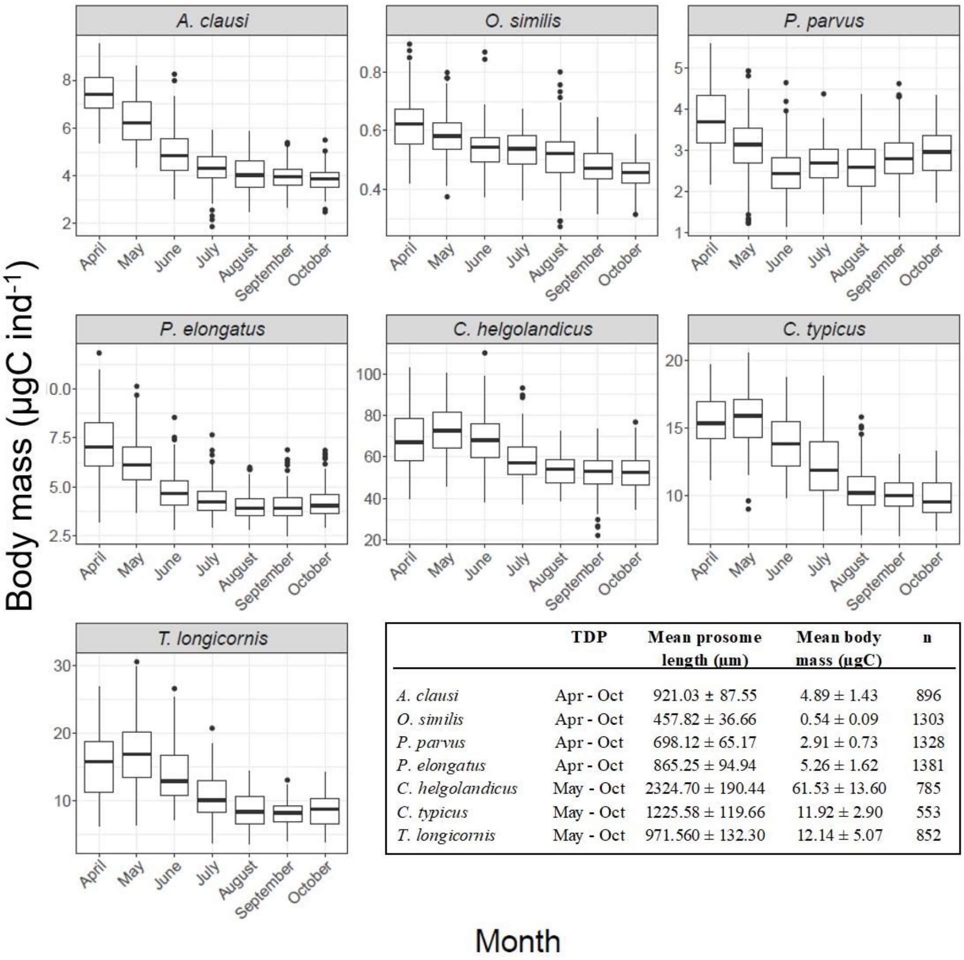
To assess interannual change in body mass, carbon mass values were transformed into anomaly values i.e., the observed value for a specific month in a given year, minus the mean value for that month across all 13 years. Anomalies of temperature and food biomass concentrations were also determined, but to ascribe these values to the copepod juvenile developmental period they were calculated as mean values over the 30 days prior to the date at which the copepod body sizes were determined. To assess the seasonal effect of food, estimated as the sum of diatoms, dinoflagellates, coccolithophorids, *Phaeocystis*, flagellates and ciliates biomass (mg C m^{-3}), on body size, the body mass values (µg C) were plotted against the average concentration of food in the 30 days prior to the sample day. A multiple linear regression model was used to test the effect of food and temperature together ($\log_{10}$(Carbon mass) ~ Temperature + Food). Food data were derived from fewer years (nine years), so the relationships among food, temperature and copepod body size were examined using the same nine sample years across all months.

To test the relationship between zooplankton density and adult female copepod body size, I first tested for each month the effect of total zooplankton density on carbon mass values of all the 7 copepod species (scaled, centred and pooled together with the z-score method: (value – mean) / standard deviation). The only significant size-density correlation was in April, which had the strongest beta coefficient ($\beta = -0.52$, $p < 0.01$, n = 81). I therefore regressed body mass values of all copepod species in April against the density (ind m^{-3}) of the different groups of zooplankton: 1) gelatinous zooplankton (i.e. cnidarians and ctenophores), 2) holoplanktonic crustaceans, 3) fish larvae, 4) non-gelatinous/non-crustacean holoplankton, and 5) meroplankton. These were all estimated as the mean density in the 30 days prior to the date on which body size was determined. The second variable (holoplanktonic crustacean density) was the strongest and only significant one. I therefore performed species-specific linear regression between carbon mass and holoplanktonic crustacean density, for each month. Another multiple regression was performed to assess the effect of holoplanktonic crustacean density, temperature and food on copepod body size in April. In all multiple regressions I checked the collinearity of each independent variable (those with a Variance Inflation Factor greater than 5 were discarded, as suggested in James et al., 2017).

2.4 RESULTS

2.4.1 Seasonal variation in adult body size

A total of 7098 adult females were measured from the seven copepod species studied. The duration of the Temperature Dependent Phase (TDP) differed slightly among the species: this period was from April to October for *Acartia clausi*, *Oithona similis*, *Paracalanus parvus*, *Pseudocalanus elongatus*, and from May to October for *Calanus helgolandicus*, *Centropages typicus*, *Temora longicornis* (**Figure 2.1**). In each species, body mass showed a strong significant negative correlation with temperature, being weakest in *Paracalanus parvus* ($R^2 = 0.25$, compared to 0.48-0.82 for the other species; **Figure 2.2**). Copepod carbon mass variation was always better explained by temperature than by food biomass, as judged by the outcomes of the models in **Table 2.1**.



	TDP	**Mean prosome length (µm)**	**Mean body mass (µgC)**	**n**
A. clausi	Apr - Oct	921.03 ± 87.55	4.89 ± 1.43	896
O. similis	Apr - Oct	457.82 ± 36.66	0.54 ± 0.09	1303
P. parvus	Apr - Oct	698.12 ± 65.17	2.91 ± 0.73	1328
P. elongatus	Apr - Oct	865.25 ± 94.94	5.26 ± 1.62	1381
C. helgolandicus	May - Oct	2324.70 ± 190.44	61.53 ± 13.60	785
C. typicus	May - Oct	1225.58 ± 119.66	11.92 ± 2.90	553
T. longicornis	May - Oct	971.560 ± 132.30	12.14 ± 5.07	852

Figure 2.1: Seasonal body mass distribution (µg C ind^{-1}) of adult females for each of the seven pelagic copepod species at the L4 site. The TDP of each species is reported in the box on the bottom-right, along with species-specific mean prosome length and mean body mass values (both with corresponding ± standard deviations) across all samples.

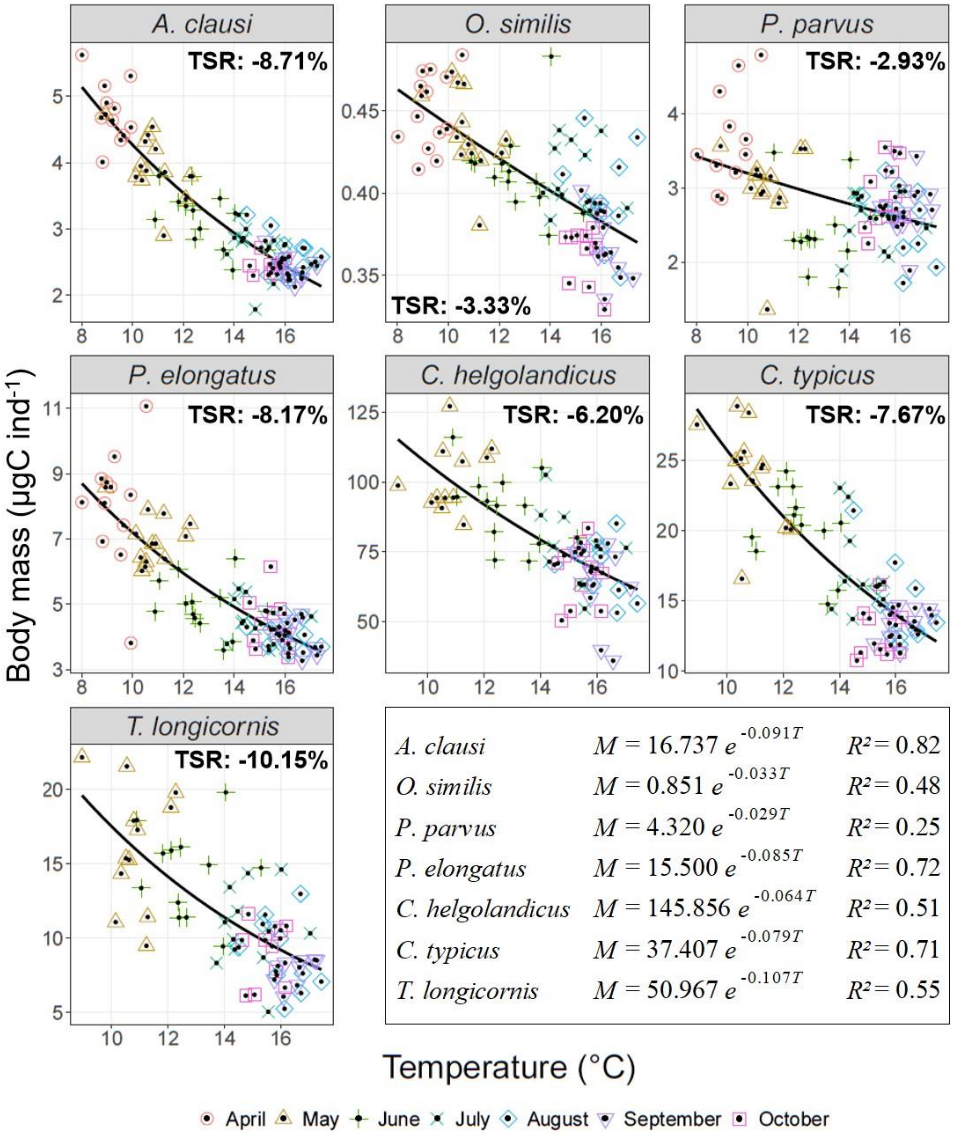


Figure 2.2: Exponential regressions (solid lines) between monthly average body mass (µg C ind^{-1}) values and temperature (°C), across all 13 years studies. On each plot the TSR is also reported (expressed as % change in µg C °C^{-1}). Values for each month are indicated by the different symbols, see key in the lowest part of the figure. In the box on the bottom-right, each exponential TSR regression is reported along with its corresponding R^2 value (note: M = body mass, T = temperature).

Table 2.1: Multiple linear regressions coefficients: Standardized beta coefficients (β_T refers to temperature coefficient and β_F refers to food biomass coefficient, in bold when significant) R^2 and p values of each model. The regression equations with actual units for each species are here reported: *A. clausi:* $\log_{10}M = 2.75 - 0.08T + 3 \times 10^{-4}F$; *P. parvus:* $\log_{10}M = 1.74 - 0.02T - 0.07F$; *P. elongatus:* $\log_{10}M = 2.83 - 0.07T - 0.05F$; *O. similis:* $\log_{10}M = -0.30 - 0.04T + 0.05F$; *C. helgolandicus:* $\log_{10}M = 4.61 - 0.05T + 0.06F$; *C. typicus:* $\log_{10}M = 3.26 - 0.07T + 0.07F$; *T. longicornis:* $\log_{10}M = 3.70 - 0.09T + 0.02F$ (note that M refers to body mass measured as µg C, T refers to temperature measured as the average °C of sea surface in the 30 days before sampling day, and F refers to food biomass measured as the average mg C m^{-3} in the 30 days before sampling day).

RESPONSE	**Log$_{10}$(Body mass)**			
PREDICTOR	**Temperature + Food biomass**			
	β_T	β_F	R^2	p
Acartia clausi	**-0.90**	0.00	0.81	< 0.01
Paracalanus parvus	**-0.41**	**-0.26**	0.30	< 0.01
Pseudocalanus elongatus	**-0.79**	-0.14	0.73	< 0.01
Oithona similis	**-0.82**	**0.27**	0.54	< 0.01
Calanus helgolandicus	**-0.77**	0.18	0.48	< 0.01
Centropages typicus	**-0.90**	**0.18**	0.74	< 0.01
Temora longicornis	**-0.76**	0.04	0.54	< 0.01
Average	-0.76	0.04		

2.4.2 Inter-annual variation in copepod adult body size

Simple linear regressions between monthly adult carbon mass and year were never significant (no evident increase or decrease overall over years) for any species. However, any potential body size reduction driven by climate change is likely to be obscured by great inter-annual fluctuations in warming at the L4 site over time (Atkinson et al., 2015), including a lack of correlation between warming anomalies in one season versus another season. For instance, temperature during summer at L4 is not significantly correlated with the temperature in the preceding spring ($R^2 = 0.09$; $p = 0.08$, $n = 31$). Therefore, relatively colder summers may sometimes follow relatively warmer springs and *vice versa*. For this reason, monthly anomaly values of carbon mass, temperature and food biomass across years were used to assess long-term changes in these variables and their co-dependence in each of the copepod species. Monthly carbon mass anomalies were negatively related to monthly temperature anomalies for: *Acartia clausi* and *Centropages typicus* as shown in **Figure S 2.1**, revealing the existence of an inter-annual T-S response along with the seasonal one. No species showed significant regressions between their body mass anomaly and food biomass anomaly.

In April, the only month that showed a significant relationship between body size of all species pooled and total zooplankton density, effects of densities of different groups of zooplankton on the overall copepod carbon mass (z-scores) showed that holoplanktonic crustacean density was the strongest and only significant explanatory variable ($\beta = -0.69$, $p < 0.05$). Across the seven copepod species and seven months (49 combinations) examined, significant correlations were found between $\log_{10}$ of adult body carbon mass of a given copepod species and $\log_{10}$ crustacean holoplankton density in eight cases: six were confined to April (all species except *A. clausi*) and one in October (*Calanus helgolandicus*). All these seven body mass-holoplankton density relationships were negative, accounting for between 47 and 80% of the variance in log adult copepod body carbon mass in April, and 38% in October (**Figure 2.3**).

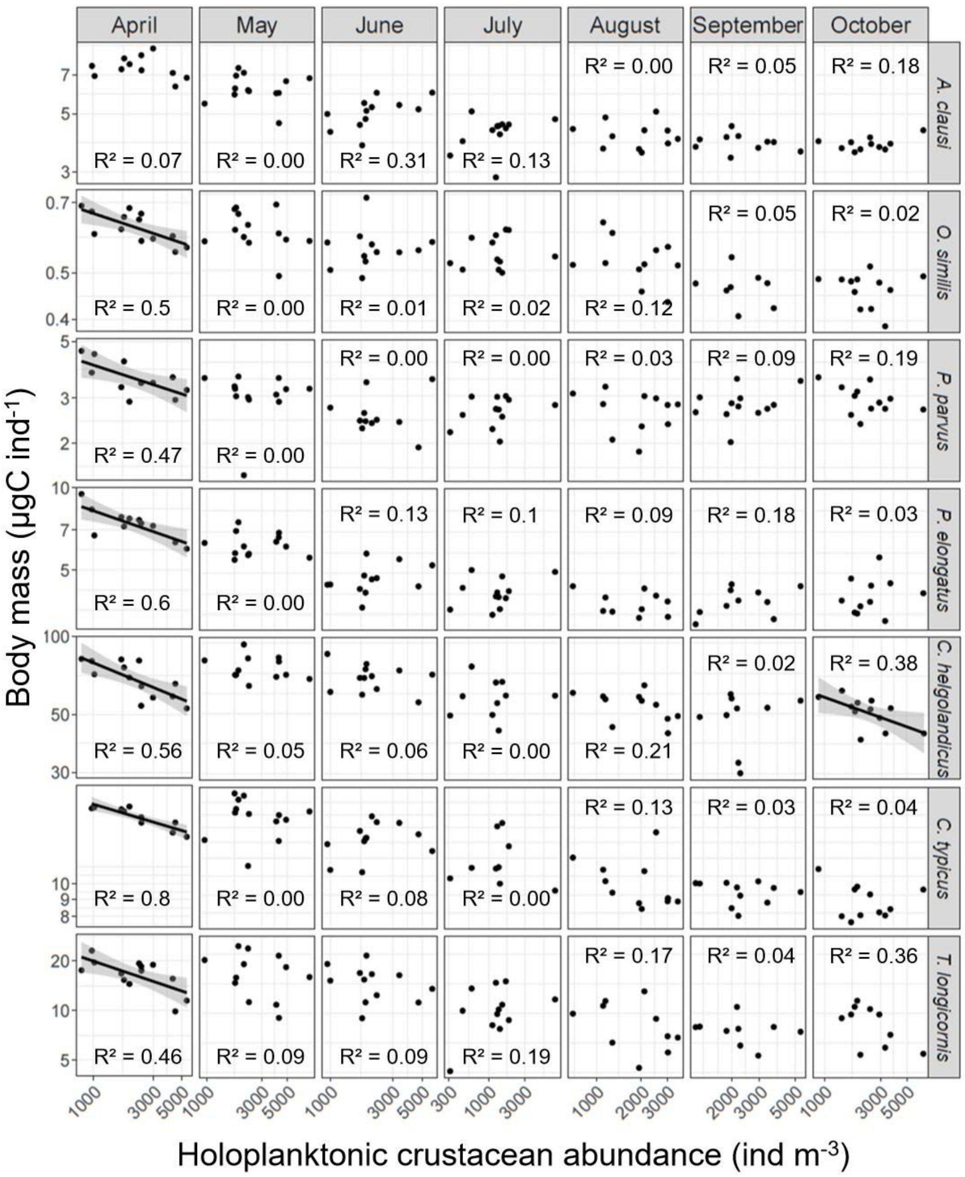


Figure 2.3: Monthly mean body mass of the adult females of the 7 copepod species (µg C ind^{-1}) plotted against holoplanktonic crustacean abundance (ind m^{-3}). Associated R² values are reported, solid black lines represent significant regressions. Note that both the x and y scales are on a log$_{10}$ scale.

2.5 DISCUSSION

2.5.1 Seasonal T-S response and taxonomic differences

This study represents one of the most comprehensive comparisons of seasonal and inter-annual shifts in body size of multiple copepod species at a single site, based on 7098 individuals measured. Indeed, this work allows the assessment of pelagic copepod body sizes from a single location and hence with similar environmental conditions and annual temperature range (~ 10 °C), which makes the comparison across species much easier than in previous meta-analyses, in which different species had experienced different environments (Horne et al., 2016). Firstly, I assessed that the TDP of my species started around April or May (depending on the species) and generally terminated around October, similar to what was shown in previous *in situ* analyses (Hirst et al., 1999; Horne et al., 2016; Cornwell et al., 2018). During the rest of the year (the so-called 'Resting Phase', set roughly between November and March) there appears to be little or no recruitment to the adult stage (Djeghri et al., 2018), hence, adult size in the resting phase is a legacy of conditions at the end of the previous TDP, possibly coupled with any size-dependent adult mortality during the resting phase. The 'Transition Phase' then follows, usually in the first months of Spring (March, April, May), when a mix of older but smaller individuals (from the Resting Phase), and newly recruited, larger, individuals are found (Hirche et al., 2019).

Among my seven species, the range in T-S strength, based on monthly size and temperature values, across the TDP over 13 years, was wide: strongest values were ~-8% to ~-10% change in C mass $^{o}C^{-1}$ for *Acartia clausi, Centropages typicus, Pseudocalanus elongatus* and *Temora longicornis*. This last species had the most intense T-S response (-10.15% change in C mass $^{o}C^{1}$), with body size in October roughly half that at the start of the TDP. This reduction of body mass may be associated with a change in copepod fitness, feeding rates and food web connectivity (Hansen et al., 1994; Rice et al., 2014). The weakest T-S response (-2.93% change in C mass $^{o}C^{-1}$), demonstrated in *Paracalanus parvus* (Order Calanoida), was considerably weaker than that of the other Calanoida, while *Oithona similis*, the only non-calanoid, had the second weakest T-S response (-3.33% change in C mass $^{o}C^{-1}$). These marked differences concur with the weak T-S response in both *O. similis* and *P. parvus* observed by Horne et al., (2016). This latter species also showed the most inconsistent T-S relationship, with the lowest R^2 (0.25, see **Table 2.1**). However, the seasonal body length distribution of *P. parvus* observed by Riccardi and Mariotto (2000) and Hirche et al. (2019) was similar to the one I have found. A difference in the T-S response strengths between Cyclopoida and Calanoida species, although not statistically supported here (possibly because of limited number of taxa), has previously been observed by Horne et al. (2016), who suggested this may relate to their different feeding strategies (active filtering in calanoids vs. ambush-feeding in cyclopoids) and the difference in body mass scaling of physiological rates between these different feeding types.

2.5.2 Effects of food concentration on copepod body size

The models showed that, seasonally, temperature was always a stronger predictor of copepod body size than was food biomass (**Table 2.1**). It is possible, however, that my use of measured body lengths rather than actual masses or biovolumes may have led to my overlooking effects of food concentration on changes in body condition, hence actual carbon mass values. As Klein Breteler and Gonzalez (1988) found in laboratory experiments, food is likely to affect more the "fatness" of a copepod than its prosome length. However, the direct C mass determinations made from L4 (Fig. 6 in Pond et al. 1996) show that even for the larger lipid-storing species *Calanus helgolandicus* females have a -8.9% change in µg C $°C^{-1}$ (95% C.I.: -11, -6.8) during the TDP. This does not differ significantly from the estimates in my study based on prosome length-mass conversion (-6.2%; 95% C.I.: -7.5, -4.9).

2.5.3 Inter-annual body mass variation

I tested species-specific size anomaly against temperature anomaly from each individual month and year, to see how the copepod body size would react in the same month of another year with anomalous temperature (**Figure S 2.1**). Only two species (*Acartia clausi* and *Centropages typicus*) showed a significant negative correlation between carbon mass anomaly and temperature anomaly. This shows the existence of a long-term T-S response in at least these two species. Although R^2 values were ≤ 0.2, in evolutionary terms, even low effect size values may still be relevant and significant. High scatter may also have been caused by the low number of years (13), lag effects, food seasonality, and the high scatter of the SST - time relationship at L4 itself ($R^2 = 0.17$, Atkinson et al., 2015). Temperature increases are apparent in the English Channel, although with a shallow slope (0.02° C increase per year, Atkinson et al., 2015) and great inter-annual variability, making climate-change impacts on the plankton community more challenging to detect. Moreover, body size in April was not modulated by temperature or food, and as described in the next section, density-dependent effects became more important then. When April samples were removed, two further species showed significant and negative long-term T-S responses: *Pseudocalanus elongatus* and *Oithona similis*. Therefore, it is reasonable to hypothesize a generalized long-term T-S response in most of the copepod species, consistent with similar findings in *Acartia tonsa* and *A. hudsonica* by Rice et al., (2014) and in fish by Daufresne et al. (2009). A long-term decrease in body size, driven by global warming, would have major implications for food web structure and function, given the strong size-structuring (Webb, 2012) and importance of copepods in these food webs (Ware and Thomson, 2005).

2.5.4 Copepod T-S response strength relates to zooplankton density

To date, very few studies (e.g. Cyr and Pace 1993) have explored how body size of copepods may be impacted by the abundance of their own species or of the whole community, and this topic has been historically underexamined in the marine environment. Our analysis of large numbers of individuals, sampled through multiple seasons at a single site, showed a strong relationship between copepod size and holoplankton density (in April), providing new evidence that density-dependent controls on copepod body size can operate *in situ*.

Are the changes in copepod body size reflecting density-dependent changes in competition or predation? The timing of the strong relationship with zooplankton density, being found only in April, may provide some clues to the cause. In the spring months of March and April, food levels were still low and increasing, whereas their grazers had already reached fairly high biomasses (Fig. 4; Atkinson et al. 2015). The period preceding and including April thus experienced a low food concentration to grazer biomass ratio (**Figure 2.4**), pointing to the potential for increased competition for food at this time. The ensuing competition for food may provide one explanation for the reduced copepod size with increased community density in April.

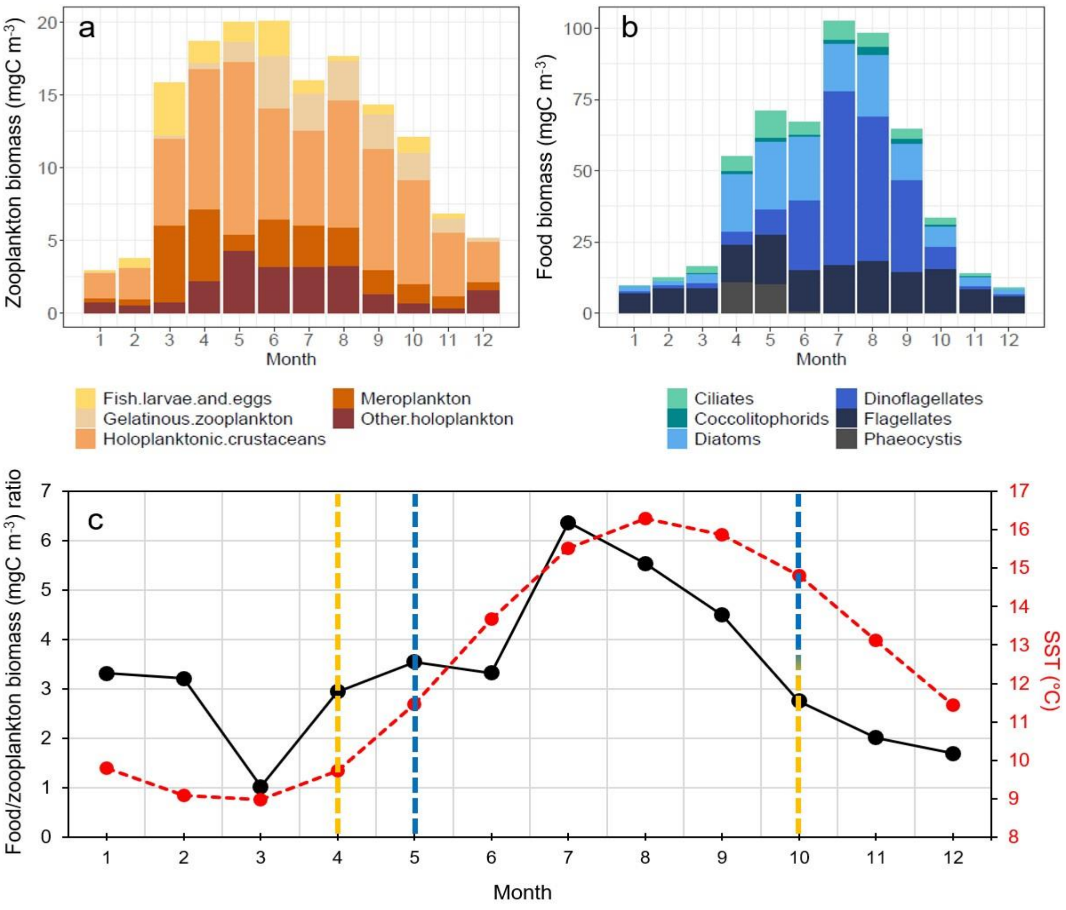


Figure 2.4: a) Zooplankton biomass (mg C m^{-3}) distribution across months for five major functional groups (see key; note that "holoplanktonic crustaceans" is the group whose density in April is inversely correlated with copepod body mass in April (Figure 3); "other holoplankton" refers to non-crustacean and non-gelatinous holoplankton); b) food (microplankton) biomass (mg C m^{-3}) across months for six major functional groups (see key); c) food/zooplankton biomass ratio (black solid lines) and sea surface temperature (red dashed lines) across months, vertical dashed lines indicate the boundaries of the TDP April- October (yellow vertical line) and the TDP May-October (blue vertical line). Plots are based on the whole L4 time-series (1988-2019).

An alternative or additional explanation for the density-dependent effects observed in April may relate to direct predation. Work at L4 has increasingly emphasized the role of predation in regulating copepod abundance and phenology (e.g.: Bonnet et al., 2005; Atkinson et al. 2015; Cornwell et al. 2018). Some of the food consumed by adult copepods is in fact often early stages of copepods (Irigoien and Harris, 2006; Boersma et al., 2014; Djeghri et al., 2018), as well as eggs (Hirst et al., 2007; Maud, 2017; Cornwell et al., 2018). Ohman and Hirche (2001) suggested that cannibalism could even be a form of population self-limitation from their study on *Calanus finmarchicus*, and likewise Maud (2018) concluded the same for *C. helgolandicus* at L4. This intraguild/cannibalistic predation on young stages would explain a reduced size at adulthood according to Abrams and Rowe (1996), who affirm that high mortality rates on early stages favour earlier maturity (hence smaller size at adult). The same phenomenon occurs for fish when exposed to intense fishery activity (Olsen et al., 2004). However, this would imply that juvenile copepods must produce an adaptive plastic response (developmental acceleration) to predation cues during the Transition Phase (April), when exposed to high predation risk. Hence, the intraguild predation hypothesis seems to need several assumptions in order to be sustained, whereas the competition for food appears a simpler explanation. As is apparent in **Figure 2.4**, increases in zooplankton abundance at L4 tend to occur well before the spring bloom, a phenological effect seen across a suite of the component taxa including copepods (Atkinson et al. 2015). It is possible that the first cohorts of copepods of the TDP (around April) that are highly abundant, when food is relatively scarce, will suffer from a poor diet and reach adulthood at a more reduced size than usual (Durbin and Durbin, 1978; Klein Breteler and Gonzalez, 1988).

In this study I observed that the T-S response strength varies substantially among different copepod species. This variation may relate to different thermal sensitivities of mechanisms generating thermal reaction norms for growth and development rates (Verberk et al. 2020). Intriguingly, large differences between species also exist in the two other "universal" responses of copepods to climate change: the phenological response (Atkinson et al., 2015; Mackas et al., 2012) and the distributional change (Chivers et al., 2017). These contrasting responses are also manifested in their long-term population trajectories of the major copepod species. Most have undergone substantial declines in summertime abundance across the NE Atlantic, whereas a few species have increased (Schmidt et al. 2020). It is therefore an urgent priority to explore whether these responses to climate change are inter-related, paying particular attention not just to bottom-up controls, but also to top-down factors.

CHAPTER 3:

Do zooplankton conserve their thermal niche by changing their phenology? A long-term study of declining copepod populations on the NW European continental shelf

3.1 ABSTRACT:

Under climatic warming many species shift their seasonal timing of life events (phenology) and seasonal abundance distribution, but whether they maintain the same thermal niche is still poorly understood. Here I studied multi-decadal trends in abundance and phenology of seven major copepod species across three stations (Stonehaven, Helgoland Roads and Plymouth L4) on the NW European shelf, spanning a ~6.5 degree latitudinal gradient. All seven species consistently occupied colder temperatures at the northern station compared to the southerly station, but they kept the same realized thermal niche over years. Expected phenological shifts (i.e.: earlier when warmer) were obscured by varying balances between food and predation controls that produced bimodal (often spring and autumn) peaks at some stations in some years. These controls also shifted over time, leading to large (~50%) spring-summer declines in abundance of many North Atlantic pelagic species over the last 5 decades, which also influenced the metrics of seasonal timing. To separate the seasonal timing of life events from that of seasonal abundance distribution, I used a time series of egg production rate of *Calanus helgolandicus* at L4, and found that this shifted later into the summer-autumn over the last 30 years of warming, coincident with declining spring-summer food and increasing predator abundance. Overall, direct temperature effects do appear to influence the seasonal timing of the copepods, but to explain impacts at individual stations or long-term trends in population size or phenology, understanding the changing balance of food and predators is critical.

3.2 INTRODUCTION

Climatic warming is affecting many aquatic and terrestrial species and has led to varying degrees of ecological disruption (Walther et al., 2002; Burrows et al., 2011; Poloczanska et al., 2013). Particularly for plankton, three “universal” responses to climate change have been described: body size response to warming (Atkinson, 1994, Daufresne et al., 2009; Horne et al., 2017), phenological response (Parmesan and Yohe, 2003; Edwards and Richardson, 2004), and geographical redistribution (Beaugrand et al., 2002; Chivers et al., 2017). The key concept behind the distributional responses in space and time can be found in the conservation of the thermal niche (or bioclimatic envelope, ter Braak and Barendregt, 1986). Thus, a shift in phenology or geographical distribution can be seen as a response that conserves the same thermal niche by changing timing in the year, or through a shift in latitude (Socolar et al., 2017, Beaugrand and Kirby, 2018). The distribution of abundance of a species, both in space and time, can be affected by other physical and biological factors besides temperature, such as currents, food availability and predation (see for example Peterson et al., 2011). Therefore, the thermal niche detected *in situ* can be defined as the "realized" thermal niche (Grüner et al., 2011), which is the focus of my study. Likewise, following common convention (see Thackeray, 2012), I use the term "phenology" to denote seasonal timing of metrics of copepod abundance, even though this is a balance between birth, death rates and advection at a given location, and thus not strictly comparable to classic phenological indices such as dates of first egg laying, bud burst or flowering (Ji et al., 2010).

Phenological adjustment is one way to conserve the same realized thermal niche in a changing environment. In fact, temperature can often explain up to ~25-30% of the copepod variability in timing of peak abundance from year-to-year (Mackas et al., 2012; Atkinson et al., 2015), with different intensities and directions of shifts observed in different species and at different latitudes (Edwards and Richardson, 2004; Mackas et al., 2012, Usov et al., 2013; Reygondeau et al., 2015; Uriarte et al., 2021). One of the possible implications of shifts in the timing of seasonal copepod abundance is the trophic mismatch between the timing of consumer and resource abundances (Cushing, 1990). However, evidence for penalties incurred from a trophic mismatch remains scarce, and mainly apply to specialized taxa that show an abrupt increase or pulse of abundance (Thackeray, 2012; Atkinson et al., 2015, Samplonius et al., 2020).

Copepods make up roughly 80% of the mesozooplankton abundance and have a crucial role in the marine ecosystems as the primary trophic link between unicellular primary producers and upper trophic levels, such as many commercially important fish species (Sundby, 2000, Orlova et al., 2005). Copepods are also excellent candidates to explore responses to climate change since they are ectothermic and generally (in temperate and tropical regions) multivoltine such that their abundance is quickly responsive to thermal changes.

Many planktonic copepod species are also widely dispersed by current and tidal movements, making shifts in their success between regions less limited by physical barriers than many non-planktonic species. Multi-decadal time series are essential to explore long-term patterns and year-to-year changes, while the high-frequency (weekly) sampling is valuable to detect shifts in timing. Several long time series (> 10 years) are now available, mainly from the North Atlantic and European shelf seas (Mackas and Beaugrand, 2010). Copepods are good exemplars of all three “universal” climate warming responses: shifts in range (poleward movement/expansion, Beaugrand et al., 2002; Chivers et al., 2017), in phenology (earlier peak of abundance, Mackas et al., 2012; Atkinson et al., 2015) and in body size (reduced adult body mass, Rice et al., 2014; Horne et al., 2016; Corona et al., 2021). There is also increasing evidence for major and extensive declines in copepod abundance across the North-West European shelf (Boersma et al., 2015; Capuzzo et al., 2017; Schmidt et al., 2020). How these declines relate to shifts in phenology, range and size has not yet been examined.

In this study, I focussed on the extent to which shifts in phenology helps to conserve the thermal niche. I benefitted from 3 long time-series of stations that spanned a latitudinal gradient across the warming NW European shelf, comparing indices of phenology across seven major copepod species that were sampled consistently at each site. The questions I address in this paper are first: do species conserve their thermal niche in response to climate warming, for example by adjusting their timing so that they increase at a more thermally suitable time of year? The second question is whether these phenological adjustments are consistently greater for some species than others, or whether they differ more across the range of any given species? The third question was how do these timing shifts relate to the observed long-term decrease in copepod abundance? In addition to the metrics of species abundance, I examined the egg production rate of *Calanus helgolandicus* at one of the sites to obtain a more mechanistic understanding of the processes that modulate the seasonal dynamics of copepods.

3.3 METHODS

3.3.1 Sample data

The sampling station chosen for this study were Helgoland Roads (HR) (Helgoland, Germany), L4 (Plymouth, UK), and Stonehaven (SH) (Stonehaven, UK) (**Figure 3.1**). These sampling stations were chosen for their similar high temporal sampling resolution (weekly and three times per week), for their long time-series of data (from 20 up to 43 years in duration) and because they span a latitudinal gradient without being too far apart to have different species compositions. The seven copepod species chosen for this study were: *Acartia clausi*, *Calanus helgolandicus*, *Centropages typicus*, *Oithona* spp., *Paracalanus parvus*, *Pseudocalanus elongatus*, *Temora longicornis*. These are major species with contrasting traits such as size, feeding- and spawning mode but are encountered across all the three of my selected stations. In the case of *C. helgolandicus* a small number of the congener *Calanus finmarchicus* may have been inadvertently included, as these occur rarely at the L4 station (a median composition of 4% of *C. finmarchicus*, according to Maud et al., 2015), as in HR and SH (unpublished data). Some key descriptors of the locations, together with methods of sampling and comparable data collected across the three stations are summarized in **Table 3.1**.

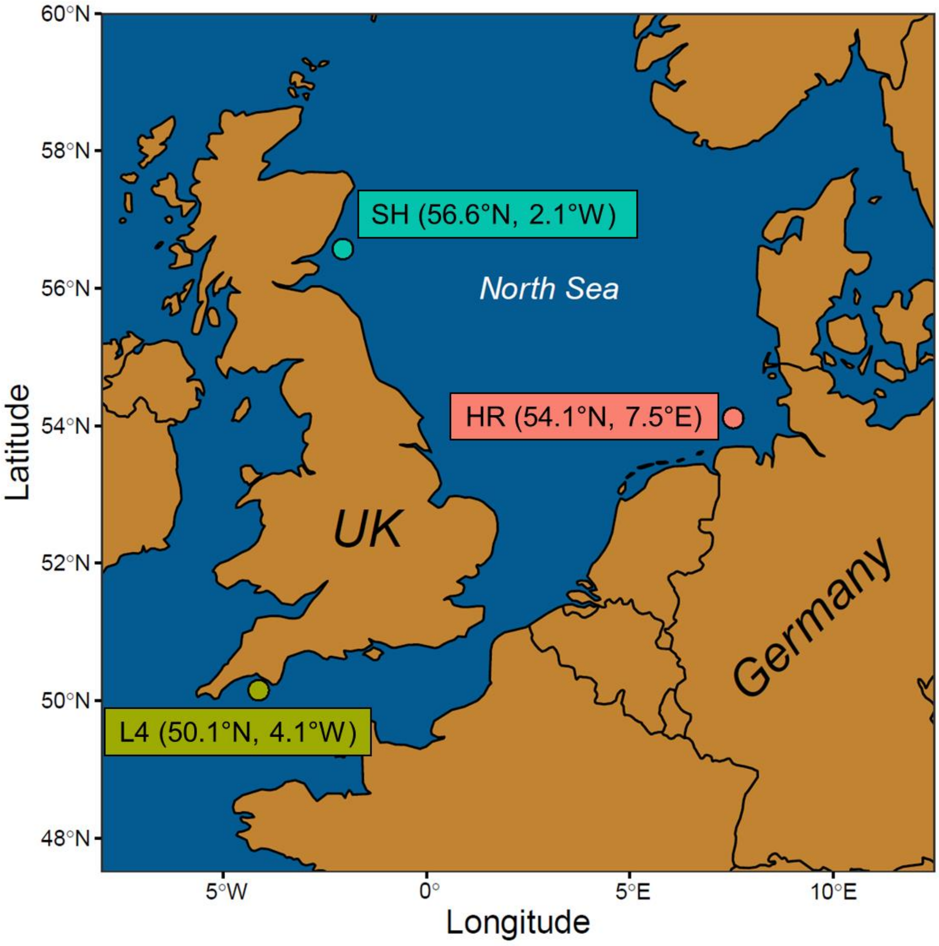


Figure 3.1: Location of the three stations in the North Sea and English Channel. See Table 3.1 for environmental and sampling summaries for the three stations.

Table 3.1: Environmental characteristics of each station and corresponding sampling methods used

	HR	L4	SH
Full name and location	Helgoland Roads (Germany)	L4 - Plymouth (UK)	Stonehaven (UK)
Average depth	between ~ 6 and ~ 10 m	~ 54 m	~ 50 m
Average sea			
surface temperature (SST) Average annual	10.3° C	12.6 °C	9.7 °C
minimum and maximum SST	3.12°C - 18.13°C	8.42° C -17.46°	5.71° C -13.94°
Years of samples used	43 years (from 1975 to 2017)	32 years (from 1988 to 2020)	20 years (from 1998 to 2018)
Sampling net mesh size	150 µm	200 µm	200 µm
Sampling activity resolution	Daily/3 times week^{-1}	Weekly	Weekly
SST difference between warmest and coldest year	2.96° C	2.01° C	1.84° C
SST difference between the mean of the 10 warmest and coldest years	2.13° C	1.21° C	0.83° C

For the L4 site (http://www.westernchannelobservatory.org.uk), sampling methods are reported in detail by Atkinson et al. (2015), but in summary, sampling has occurred every week, subject to weather conditions, since 1988. For weekly zooplankton sampling, two vertical (50-0 m depth) replicate tows of a WP-2 net (56 cm ring diameter, 200 µm mesh) are made within the ~54 m deep water column. Each sample is then fixed in 4% buffered formalin, from which sub-samples are obtained for counting and identification of taxa. I used the most recent dataset available (McEvoy et al. 2022). *Calanus helgolandicus* female egg production experiments have been conducted since October 1992, and are based on live material collected weekly with a 710 µm mesh in the surface layers at L4 and returned to the laboratory in a cool container. Further method details are provided in Atkinson et al. (2015) and in Maud et al. (2015). For consistency of measurement across the whole time series I used SST values measured with a mercury-in-glass thermometer in a stainless-steel bucket of freshly collected surface seawater. These values corresponded well to values measured in more recent years with an electronic probe for conductivity, temperature and depth analysis (CTD; Atkinson et al., 2015).

At the SH station (https://data.marine.gov.scot), zooplankton have been sampled weekly since 1997 by vertical hauls from 45m to the surface with a 200 µm mesh Bongo nets, 40 cm ring diameter. Fixation, counting and identification of the samples were similar to the those at L4. More details on the methodology used for data acquisition can be found in Bresnan et al. (2015), zooplankton data source: Marine Scotland Science. 2018. Scottish Coastal Observatory - Stonehaven station data. doi:10.7489/610-1. SST at SH was measured using a digital reversing thermometer fitted to the Niskin sampling bottle.

At the HR station (https://deims.org/1e96ef9b-0915-4661-849f-b3a72f5aa9b1) zooplankton samples have been taken three times per week, weather permitting, by two oblique tows (net mesh size: 150 µm, diameter: 17 cm) since 1975. Temperature was measured on a work-daily basis. More information on characteristics and methods of sampling can be found in Wiltshire et al. (2010). Unlike the other two stations, the abundance data of *P. elongatus* and *P. parvus* were not available, since these two species are summed up into a single taxon in HR (source of the dataset: Boersma et al., 2017). Further details of the copepod sampling, analysis and trends are provided in Greve et al. (2004) and Boersma et al. (2015), including a phenological analysis in Wiltshire and Boersma (2016).

3.3.2 Data analysis

I used the abundance values, of all copepodite stages, from each time-series to obtain the phenological timing indices of each species at each station using the method of Greve et al. (2005). This method uses the Julian day at which a certain cumulative percentile of abundance is reached within a year. I used the Julian days corresponding to the 25th and the 50th cumulative percentiles of abundance (CPA) of the given year as a timing index. These indices approximate respectively to the early and the middle of the copepod productive season and are often used in plankton phenology studies (Greve et al., 2005; Castellani et al., 2015). I also estimated the duration of the density peak as the number of days between the 25th and the 75th CPA. When looking for timing shifts related to climatic warming, I linearly regressed each individual timing index and the amplitude value against spring (April, May, June), summer (July, August, September) and combined spring+summer SST. This was done because spring temperature does not strongly correlate to summer temperature across years ($R^2 = 0.09$; $p = 0.08$, $n = 31$), measured as a mean value across the months. From these regressions I obtained slope values that indicate the number of days shifted per 1 °C increase in temperature, where positive slopes mean a later timing with warming, negative slopes mean an earlier timing with warming. I also linearly regressed each individual timing index against "year" in order to assess any long-term trends in copepod phenology. I calculated a 'realized thermal niche' index of each species at each year and station in order to detect the temperature around which the whole annual species-specific abundance is centred (see "centre of gravity" method: Colebrook and Robinson, 1965; Edwards and Richardson, 2004; Atkinson et al., 2021), using the following formula:

$$R = \sum(N_T * T) / \sum N_T$$

Where R is the realized thermal niche, expressed in Celsius degree (°C); N_T is the abundance of each species at each sampling time point with sea surface temperature T.

I performed an ANOVA on measures of thermal niche index of all species, years, and stations (aov function in R, Chambers et al., 1992) followed by post-hoc pairwise comparisons (TukeyHSD function in R, Yandell, 1997) to tests the effects of the species and station variables. I divided each time series into two pairs of decades: the 10 warmest and 10 coldest years and the first 10 and last 10 years, in order to compare copepod abundance distributions between distant chronological phases and between distinct thermal regimes (these were based on the average monthly temperature between June and September inclusive). We chose to use the maximum sample size possible (this was 20 since the shortest time series of any of the stations was 20 years in length) for all stations given the high interannual variability of both temperature increase and copepod density change. These abundance distributions and their relative timing indices were plotted for each species at each station. Finally, in order to calculate the seasonal timing difference between the first and final decades and between temperature regimes, I also performed an ANOVA test on the overall of the timing indices of each year and stations, with all species scaled, centred and pooled together (z-scores).

3.3.3 *Calanus helgolandicus* egg production - case study

At L4, *Calanus helgolandicus* egg production rate (EPR) has been measured at weekly resolution, albeit with some data gaps, since 1992 (see Maud et al., 2015). Live net samples are transported to the laboratory inside a cool box and 5 replicates of five healthy adult females (25 in total, where available) are picked within 2-3 hours of collection. These are incubated in a 500 μm mesh-bottom Plexigas chamber inside a 2 L plastic beaker filled with 1.5 L of 0.2 μm filtered seawater, at ambient L4 surface temperature and constant darkness for 24 h. Total numbers of eggs are counted and EPR calculated (eggs female^{-1} day^{-1}). I calculated the timing indices of EPR in the same way I did for abundance (the Julian days of the 25th and the 50th percentile of EPR of that given year), and linearly regressed these against the phenological indices of *C. helgolandicus.* I also calculated the relative change over years by each month of *C. helgolandicus* EPR, along with total zooplanktonic predator density (calculated as the total ind. m^{-3} sum of fish larvae, cnidarians and chaetognaths) and total microplankton biomass (calculated as the total mgC m^{-3} sum of diatoms, dinoflagellates, flagellates, ciliates, coccolithophorids and *Phaeocystis*). These microplankton biomass values are based on Lugol's preserved water samples from 10m depth, with inverted microscopy used to determine taxonomic groups and compute carbon from biovolume estimates specific to the station (Widdicombe et al. 2010; see Widdicombe and Habour, 2021 for source data used here). To examine seasonality among these variables, I $\log_{10}$ transformed *C. helgolandicus* abundance, *C. helgolandicus* EPR, total zooplanktonic predator density and total microplankton biomass. To further provide comparability across variables with different units I transformed them into z-scores.

3.4 RESULTS

3.4.1 Seasonality

The average seasonal copepod density at HR is characterized by one single main peak (between spring and summer), whereas at L4 and SH, a bimodal density distribution is more common (**Figure 3.2**). *Acartia clausi, Calanus helgolandicus, Centropages typicus* and *Oithona spp.,* at SH, present on average a more pronounced peak in summer than in spring when compared to their southern counterpart (L4). *Pseudocalanus elongatus* abundance generally peaks in both spring and summer at the northerly station (SH), but only in spring in the south (L4). This summer preference at SH was also detected statistically by the *post-hoc* pair-wise test on species-specific timing indices between stations: five out of seven species had an abundance distribution significantly later in the year in SH compared to L4 (**Figure S 3.1**). Species-specific timing indices at SH significantly correlated with the ones in L4 (**Figure 3.3b**), indicating a consistent difference in timing among species. This correlation was not present when comparing the species-specific timing indices between L4 or SH with the ones in HR. The 50th CPA of all species (pooled together) across years differed significantly between each pair of stations, but the higher difference was detected between SH and L4 (difference in means: 27.38 Julian days; 95% C.I.: 14.86, 39.89, $p < 0.01$, **Figure 3.3d**).

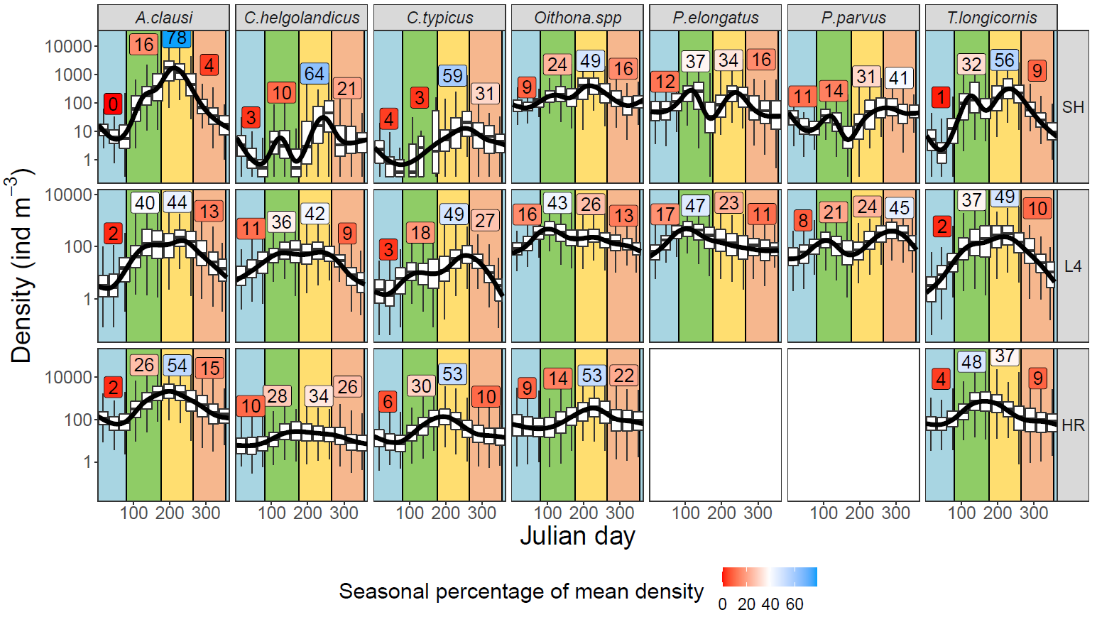


Figure 3.2: Average yearly copepod density distributions are indicated by black lines (smoothed average) for each species at each station, density values are reported on the y axis in a log scale. The background colours of each vertical band refer to the four seasons, blue: winter, green: spring; yellow: summer; orange: autumn. White boxplots indicate median and distribution of monthly density values, white boxes indicate the 50% quartile and whiskers indicate the 95%. The numbers reported in the boxes in each season above the curve indicate the seasonal percentage of mean density relative to the whole year mean (the colour of the boxes correlate to the value of these percentages, as indicated by the colour scale at the bottom), thus, the values in the boxes show how successful, in terms of density, copepods are in a given season compared to other seasons. Thermal niche conservation is demonstrated in the always higher summer relative abundance in Stonehaven (SH) than in L4, and the opposite relationship for the spring relative abundance.

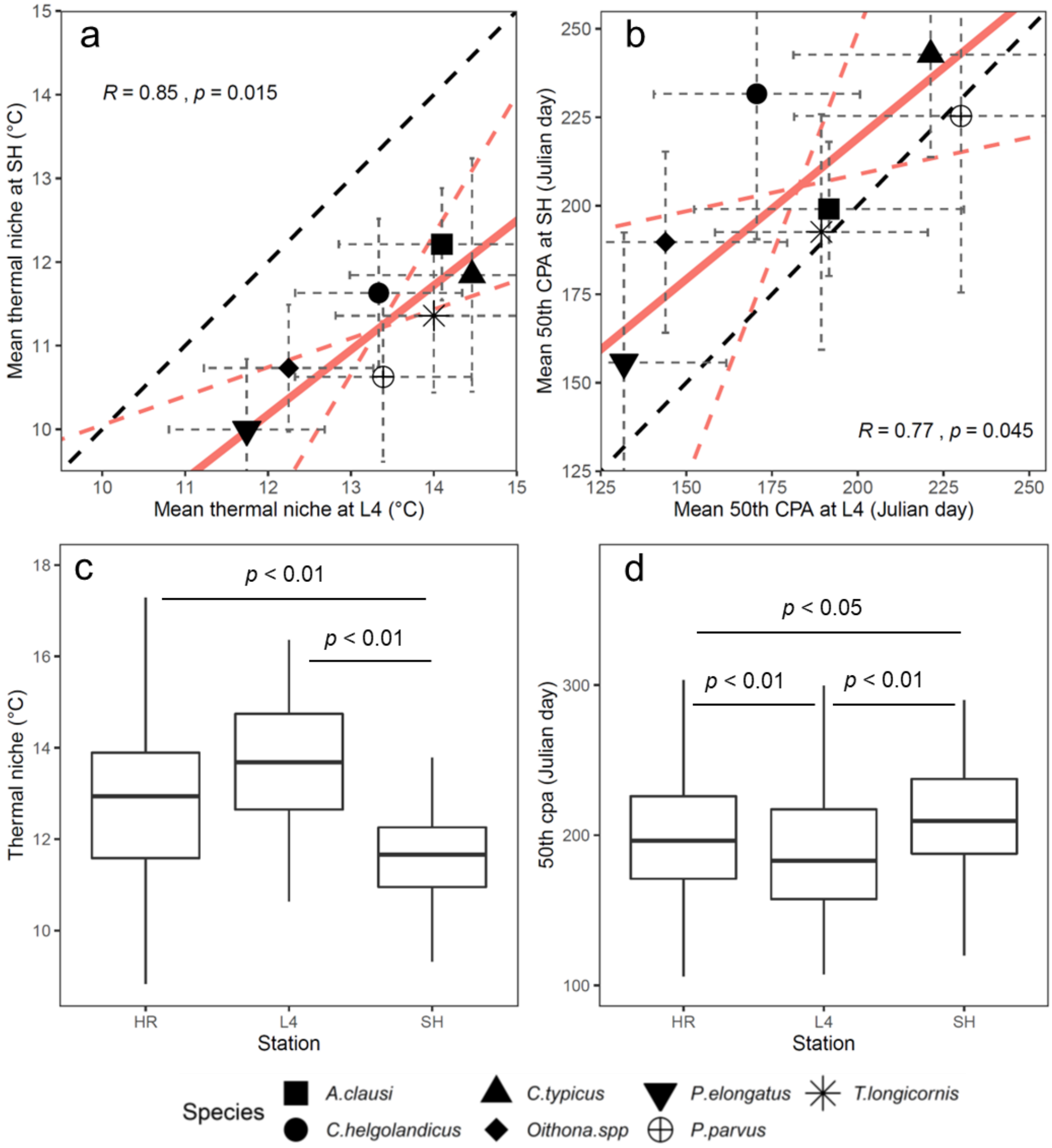


Figure 3.3: Reduced major axis (RMA) regressions between the L4 and SH stations of the specific mean thermal niche values (**a**) and the specific mean phenological indices: the 50th CPA (cumulative percentile of abundance) (**b**). Symbols indicate different species (see legend), horizontal error bars indicate the standard deviation of the specific mean values, red line is the fitted line of regression (95% C.I. is indicated by the red dashed lines), correlation coefficient (R) and p values are reported within both graphs. The black dashed line in both panels is a 1:1 line added for better interpretation. **c** and **d** show the thermal niche (**c**) and phenological indices (**d**) values of all years and species pooled (except *P. elongatus* and *P. parvus*, since these two species are not available for HR station) for each station in forms of boxplots (horizontal thick lines indicate the median value, the lower and upper end of the box correspond to the 25th and 75th percentiles, whiskers indicate approximately the 95% confidence interval of the median). P-values are reported for the pairwise significant differences between stations according to the ANOVA test.

3.4.2 Abundance

Six out of seven species showed a significant decrease in abundance over the years (**Figure 3.4, Figure S 3.** also shows the long-term trend of abundance over years for each month). The only taxon that did not decrease at any station was *Oithona* spp. Looking in more detail, copepod abundances decreased at slightly different times throughout the year at different stations. As shown in **Figure S 3.2**, the decrease of abundance in HR occurred mainly in summer and autumn, whereas in L4 I found the strongest decreases often in the period that straddles between spring and summer, and then between summer and autumn. At SH, significant decreases of abundance were observed in only a few species in a few periods, mainly *P. elongatus* during the cold part of the year and in September.

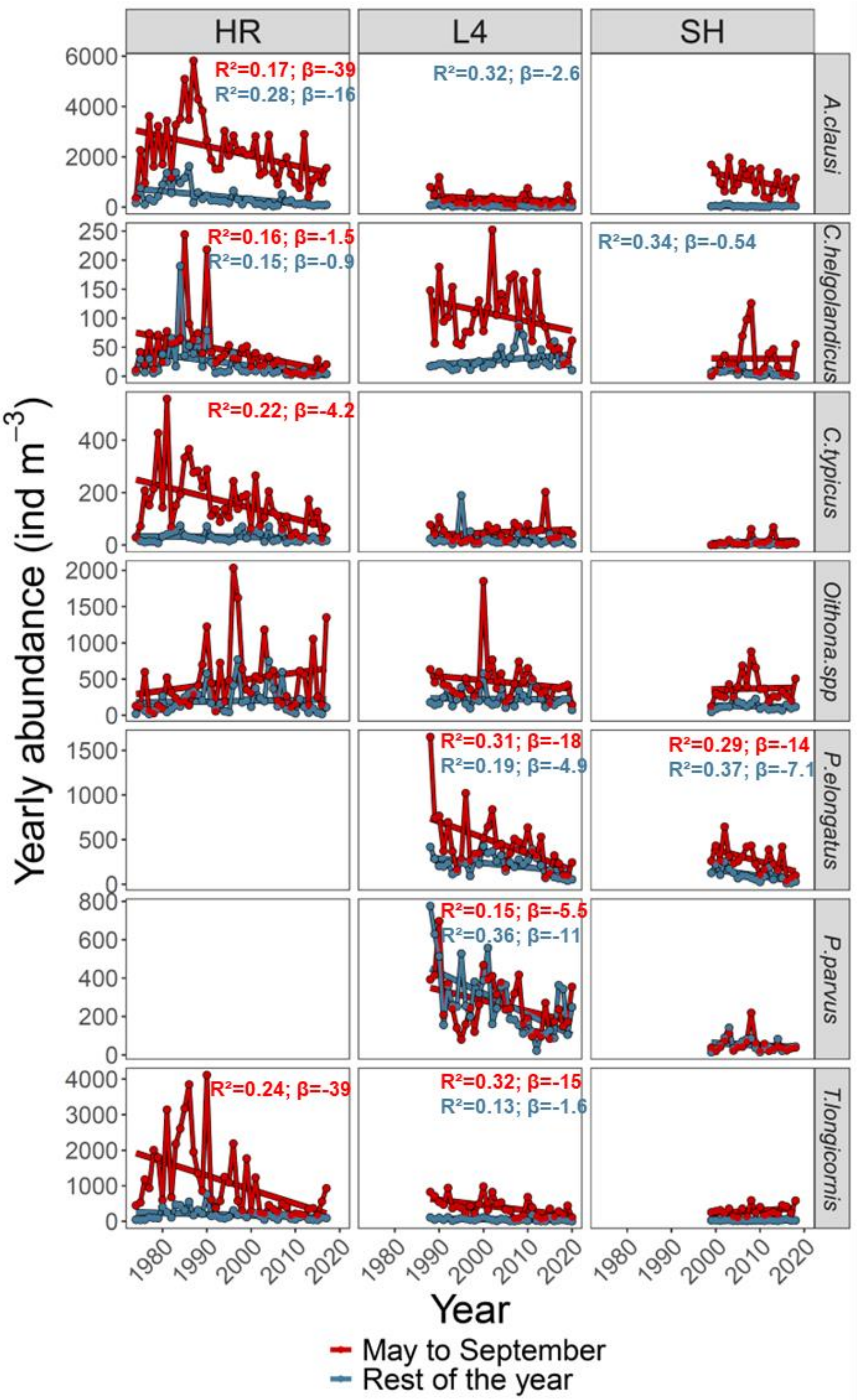


Figure 3.4: Linear regressions between copepod average abundance and years for each species at each station. Points and lines are colour indexed as indicated by the legend at the bottom

where “rest of the year” refers to the months from October to April included. R^2 and slopes (β) are reported only for the significant regressions ($p < 0.05$).

3.4.3 Timing of abundance with warming

At HR, three species (*A. clausi, C. helgolandicus* and *Oithona.* spp.) had at least one timing index (either the 25th or the 50th CPA) negatively correlated with increasing seasonal temperature (*T. longicornis* showed a negative correlation, but this was slightly non-significant ($p = 0.068$)). In L4, *P. parvus* showed a significantly delayed 25th CPA with increasing SST. In SH, no significant correlations were detected except in *C. helgolandicus,* whose 50th CPA got earlier with increasing spring SST. The 50th CPA in *Oithona* spp. was slightly significantly delayed with increasing summer SST. The number of days between the 25th CPA and the 75th CPA was never found to be significantly increasing for any species and station, instead, it significantly decreased for *C. typicus* and *P. parvus* at L4, *A. clausi* at HR and *T. longicornis* at SH. There was no clear evidence that spring abundant species tended to increase earlier in warmer years or that autumn species appeared later in warmer years versus average day of appearance (regressing timing shift in days $°C^{-1}$ vs. average timing index day). *C. helgolandicus* showed a consistent and significant earlier occurrence of its seasonal timing of abundance against years at all stations. *A. clausi, C. typicus* and *T. longicornis* also showed timing advance but only at HR (**Figure S 3.3**). The amplitude decreased over years in two species at HR (*A. clausi, Oithona* spp.) and one in SH (*P. elongatus*), and it increased in one species at L4 (*C. helgolandicus*). Overall, timing indices (25th and 50th CPA) of all species at all stations changed more (became earlier) from the first to the last decade of the respective time series ($F_{1,377} = 23.35$: $p < 0.01$, n = 378 for the 25th CPA and $F_{1,349} = 24.59$; $p < 0.01$; n = 378 for the 50th CPA), than between the 10 coolest and the 10 warmest summers (25th CPA: $F_{1,377} = 0.20$; $p = 0.65$; n = 363 and 50th CPA: $F_{1,349} = 0.18$; $p = 0.66$; n = 363, **Figure 3.5b-d**).

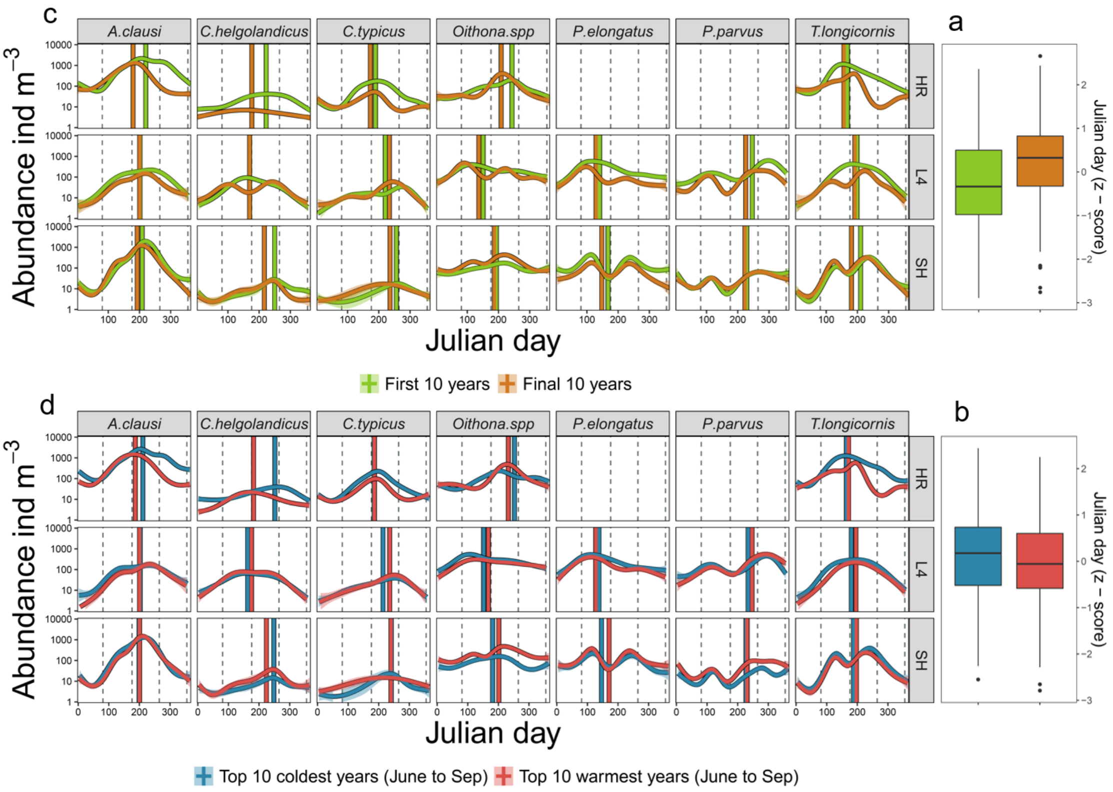


Figure 3.5: **a-b**) Box-plots on the right represent the distribution of the 50th CPA timing index (z-scores scaled by species and stations), showing the direction between different chronological phases (top) and different thermal regimes (bottom): negative values on the y axis mean earlier phenology, positive values mean a later phenology (only the difference between chronological phases is statistically significant for both the 25th CPA and the 50th CPA, unlike the difference between thermal regimes in both timing indices). **b-d**) Seasonal abundance of each species denoted with curved lines which are smoothed averages from the 'loess' function with a span level of 0.75. Vertical lines denote 50th CPA timing index. Colour indexed denotes either the chronological phase (top graph) or the thermal regime (bottom graph). Grey vertical dashed reference lines indicate winter, spring, summer, autumn.

3.4.4 Thermal niches

Realized yearly thermal niches, across all species together, differed significantly both among stations ($F_{2,454}$ =67.40, $p < 0.01$) and among species ($F_{4,454} = 19.55$, $p < 0.01$), but the difference among stations depended on the species (station × species interaction term: $F_{8,454} = 10.62$, $p < 0.01$). Not all 7 species could be used, as HR lacks data on *Pseudocalanus elongatus* and *Paracalanus parvus*. The *post-hoc* pairwise test detected a significant difference in the realized thermal niche means between L4 and SH for all species except *Oithona* spp., **Figure**

S 3.1). Tukey post-hoc pairwise comparison showed that all realized thermal niches differed more between SH and L4 (difference in means = -1.9; 95% C.I.: -2.77, -1.04; $p < 0.01$) than between L4 and HR (difference in means = -0.4; 95% C.I.: -1.12, 0.29; $p = 0.36$) or SH and HR (difference in means = -2.3; 95% C.I.: -3.13, -1.49; $p < 0.01$, **Figure 3.3c**). Species-specific thermal niches in SH correlated significantly with the ones at L4 (**Figure 3.3a**), indicating a consistent difference in thermal niches among species. This correlation was not present when comparing the species-specific thermal niches between L4 or SH with the ones in HR.

3.4.5 Timing of egg production in *Calanus helgolandicus*

To examine potential mechanisms behind phenology shift I examined available time series data on one of the species at one of the sites, namely *C. helgolandicus* at L4. **Figure 3.6** shows long-term seasonal changes for all the four summary variables relating to its population dynamics: *C. helgolandicus* abundance, egg production rate, biomass of potential food and abundance of its potential predators. *C. helgolandicus* abundance decreased over the 30 years in May, June, and July: exactly a month delayed to the long-term decrease of *C. helgolandicus* EPR, which occurred in April, May and June. Food (as total microplankton, mg C m^{-3}) decreased significantly in July and slightly significantly in April, whereas predators abundance (ind m^{-3}) increased significantly between winter and spring and in late autumn.

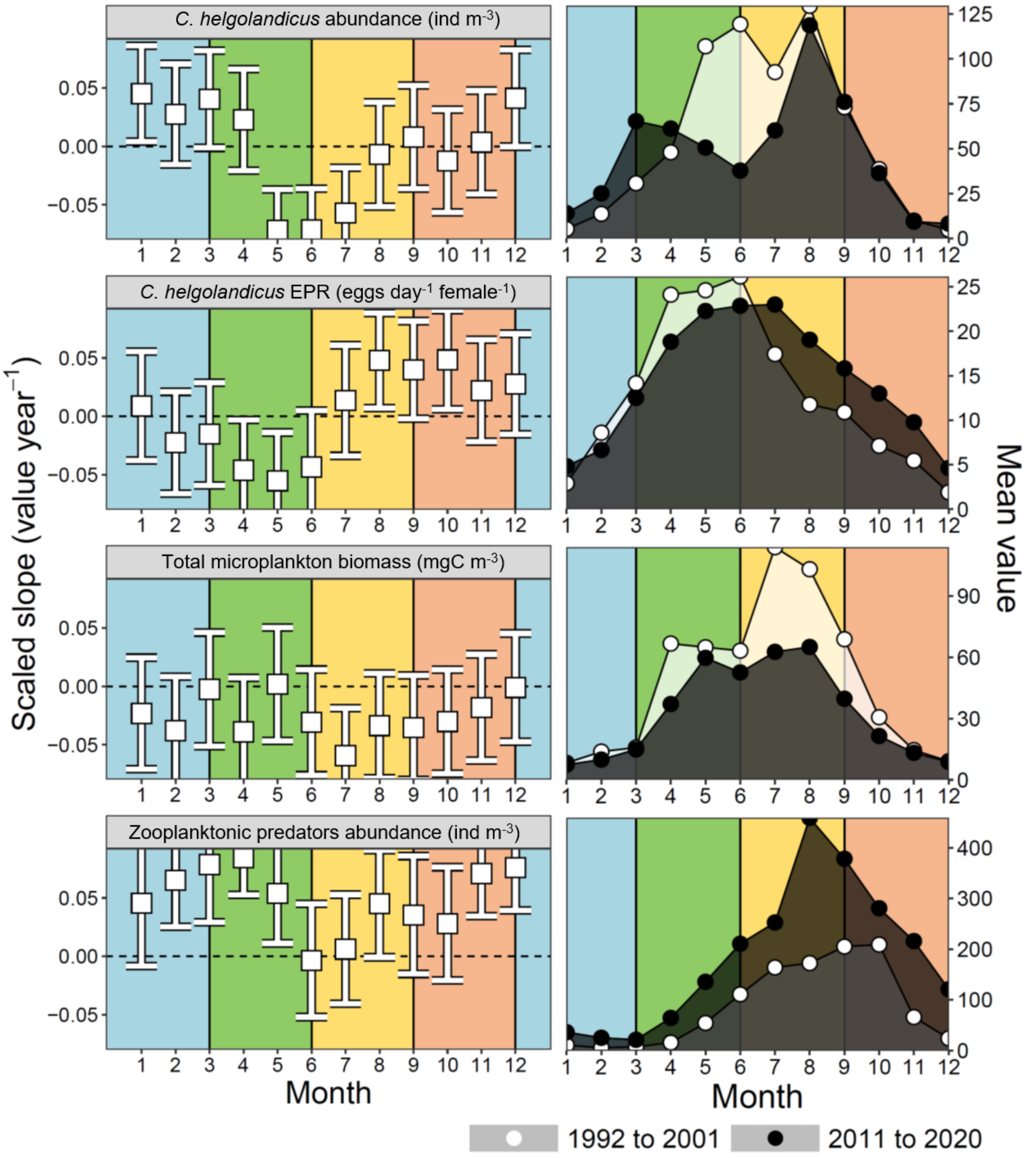


Figure 3.6: Change over years of different variables (indicated above each plot) by each month at location L4. On the left-hand side, points indicate the slope value on the y-axis, calculated from a linear regression of z-scores (meaning that all variables have the same scale limits and center) against years. Each point presents a vertical band that indicates the 95% C.I., thus, bands that do not intersect the 0 (horizontal dashed lines) indicate a significant slope. Background colours refer to seasons, blue: winter, green: spring; yellow: summer; orange: autumn. Predators are calculated as the sum of fish larvae, chaetognaths, and cnidarians. Food concentration (mg C m^{-3}) is calculated as the sum of diatoms, dinoflagellates, flagellates, ciliates, coccolithophorids and *Phaeocystis* (the nanoflagellate functional group, which was not included, is also decreasing overall at L4 as described by Atkinson et al., 2022). The plots on the right-hand side show the seasonal distribution change over time in absolute terms across the first and last decade.

3.5 DISCUSSION

3.5.1 Thermal niche conservation

One basic, important, but often untested assumption of species distribution models which project distribution patterns in space and time, is that the relationship of a species to its environment (and particularly to temperature) is broadly fixed (Cheung et al., 2008; Brun et al., 2016). I tested this assumption using the species thermal distributions at the three different stations, and found that this assumption was not upheld among stations. Comparison between L4 and SH shows, generally for all species, a more summer-centred abundance distribution in SH, whereas in L4, warmer temperature allows them to take advantage of spring too. This difference can be seen both in the simple seasonal abundance distributions and the seasonal relative percentage abundances and in their average timing indices, the 25th and the 50th cumulative percentiles of abundance (CPA), which are later in the year in SH than in L4. From this, I may assume that if the timing of seasonal abundance can change from one latitude to another, as a local plastic response, it can also potentially change over time within the same latitude as sea temperature rises, although this depends on how close the species live to their thermal tolerance limits (Pinsky et al., 2019) and variables other than temperature may explain some of the inter-latitudinal variance (Uriarte et al., 2021). However, this seasonal timing adjustment between different latitudes is not enough to maintain the same realized thermal niche, because SH copepods still experience colder temperature than at L4 (**Figure 3.3c**). One reason for this is that the maximum SST in SH is not as high as that at L4, and the time gap to take advantage of warmer conditions is shorter at higher latitudes. The distribution of the thermal niches within the thermal limits of each station (**Figure 3.7**) clearly shows how copepods at L4 manage to exploit a much larger thermal range (they can thrive at both above and below the local average SST), whereas in SH they can only maximize their abundance in the warmer part of the local thermal range. This narrower thermal range in SH may explain, besides other possible biotic and abiotic factors, why the mean yearly abundance was lower than in L4: all species (except for *A. clausi*) reached significantly higher numbers at L4 than SH (**Figure 3.7**). Interestingly, the way copepods change their seasonal timing between L4 and SH remains consistent inter-specifically. In other words: “cold species” and “warm species” at L4 remain relatively the same “cold species” and “warm species” at SH, and the same applies to “early species” vs. “late species” as well (**Figure 3.3Figure 3.3a-b**). This pattern could mean that, despite having a narrower season length at SH, of the copepod species examined, they cannot greatly overlap their thermal niches with each other, following the principle of competitive exclusion (Gause, 1934). HR has higher and lower temperatures than both L4 and SH, so that copepods tend to have a larger thermal range at their disposal. This could explain why copepods in HR have thermal niches intermediate between those at L4 and SH. Additionally, copepods at HR present a different distribution of seasonal abundances (uni-modal) than at L4 and SH (bi-modal), which could obscure the comparisons (interspecific differences in thermal niches were inconsistent between HR and the other two stations).

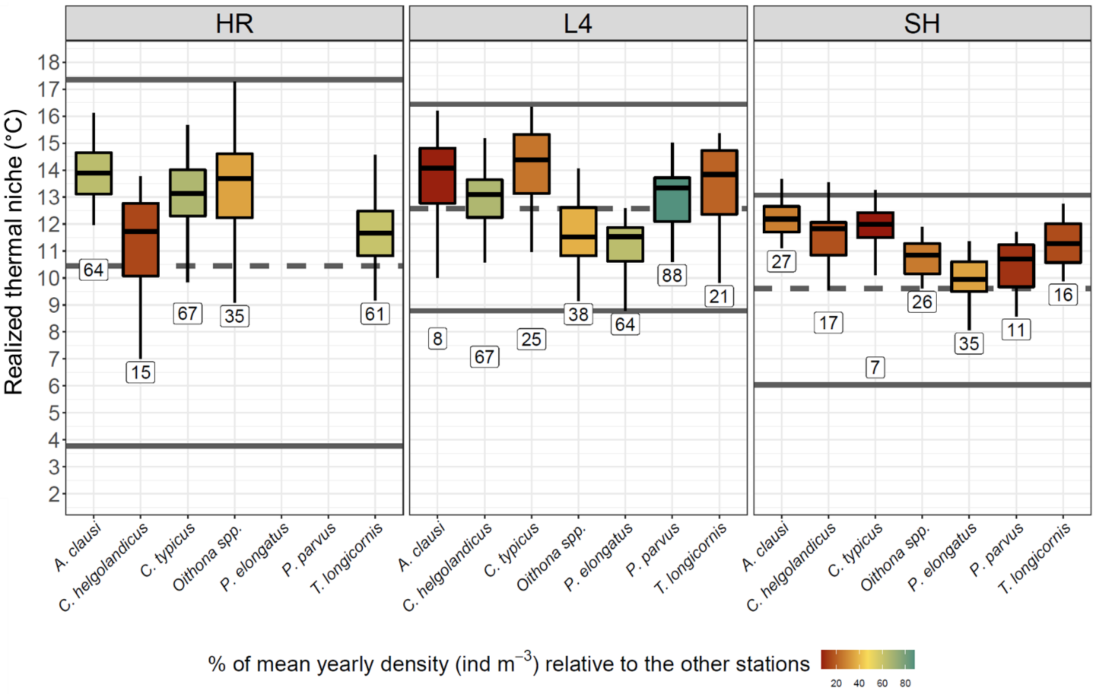


Figure 3.7: Boxplots indicating yearly thermal niche value distribution for each species at each station. The colour of the boxplots refers to the percentage of mean yearly density (ind m^{-3}) relative to the other two stations (as indicated by the legend on the bottom, specific values are reported as well in the labels below each boxplot). Horizontal dashed lines indicate the average yearly mean temperature, horizontal solid lines indicate the upper and lower thermal limits of each station (defined as the average maximum and minimum yearly temperature).

Overall, all species at all stations have conserved the same realized thermal niche over time within each station, with the only exception of *Oithona* spp., whose thermal niche has significantly increased over time (**Figure S 3.4**). In fact, *Oithona* spp. is the only taxon whose abundance significantly increased in summer and never decreased in other parts of the year, whereas the other species have shown the exact opposite (**Figure S 3.2**). An increase in abundance in summer, relative to other periods, indicates an increase in the temperature of the realized thermal niche of that year, and yet, species which instead decreased in abundance in summer did not show an opposite trend (i.e.: a thermal niche which gets colder over time, instead, it remained the same throughout the whole time series). One explanation for this is that temperatures have risen overall and countered the fewer copepods experiencing the warmest season (summer). This could mean that the decrease of abundance in summer has been a response to the warming, which led to these species maintaining the same thermal niche. From this simple concept come two important considerations: first, the seasonal timing change in HR may simply be a consequence of this decrease of abundance in late summer/early autumn, which could also explain why I did not observe significant shifts in timing index at the other two stations, where the decrease of abundance occurred differently (in two distinct parts of the year instead of one). This could also explain why I also did not find the trend of early when warming for spring species and late when warming for late summer species, which is often predicted (Mackas et al., 2012). The second consideration is that this thermal niche conservation was associated with great costs, because although similar temperatures are still experienced by most copepods, their abundance has significantly decreased over the time series.

3.5.2 Varying influence of temperature on phenology across the range of a species

Our second hypothesis, based around the predictions of Beaugrand and Kirby (2018), is that a species should have a different response in their seasonal timing to temperature at the different latitudes or different parts of their thermal range. Specifically, the "width" of the abundance peak of a species should broaden with warming at the cold part of its thermal range, resulting in an increase in the annual abundance, and get narrower with warming at the warm part, with a resulting decrease of annual abundance. By contrast, the same species at the centre of its thermal range is predicted to reduce in abundance during summer under warming, with a tendency to increase its abundance earlier in the year (see Figure 4 in Beaugrand and Kirby, 2018). The species examined here did not show longer duration of high abundance with warming at any station (there were actually cases of decrease) and did not show an increase of annual abundance over time (rather they mostly show a decrease instead). This should indicate that the stations considered do not represent the northern range (the minimum temperature limit) of the species, not even the coldest station (SH). In fact, all the species in my study can be found at higher latitudes than SH (Halvorsen et al., 1994; Bucklin at al., 2000; Potters et al., 2006; Beaugrand et al., 2007; Evjemo et al., 2008)). This may indicate that my stations are somewhat within the central part of the thermal niche of my species, where the calculation of phenological index should reveal an earlier phenology, and the annual abundance should not change substantially, according to the Beaugrand and Kirby model. In my study, instead, I found significant changes in timing indices with temperature almost exclusively in HR, and I found long-term decrease of abundance in all stations.

It is also difficult to determine whether the absence of multiple and strong significant timing shifts in species at SH is due to limited number of years of sampling, or due to intrinsic environmental characteristics of the region. Uriarte et al. (2021), for instance, suggests that phenology in SH is driven more by chlorophyll than by temperature. Stronger timing changes in HR could also be due to the unimodal seasonal distribution of abundance there compared with the bimodal distributions in L4 and SH. In fact, having a single and wide peak of copepod density may make abundance a better proxy for phenology, whereas having a decline in density between the two peaks could imply a greater seasonal mortality, which can potentially obscure the real phenological changes of copepods.

Overall, the timing indices across all species and stations changed more (species abundance was distributed earlier in the year) from the first to the last decade of the respective time series, than between the top 10 coolest to the top 10 warmest summers. Therefore, it is likely that variables other than temperature may be regulating copepod seasonal abundance distribution, or that the effect of temperature increase is indirect and can be detected only on a very long-term scale. SST has a very high inter-annual variability, as illustrated by regressions of annual ‘SST vs. years’ regressions in all the stations, which have shallow slopes and high scatter in HR ($\beta = 0.03$ °C year^{-1}, $R^2 = 0.28$) and L4 ($\beta = 0.02$ °C year^{-1}, $R^2 = 0.21$), albeit significant. Moreover, at SH the SST shows no obvious temporal warming in the 1988-2019 time-series ($\beta = 0.004$ °C year^{-1}, $R^2 = 0.01$, $p = 0.07$), despite warming occurring globally (IPCC, 2021). Another confounding factor is the constant decrease of copepod abundance over years, which does not occur homogeneously throughout the year, but more in some months than others.

3.5.3 Drivers of population dynamics and their influence on phenology

Abundance of copepods across the NW European shelf and NE Atlantic have declined greatly over the last 60 years (Boersma et al., 2015; Capuzzo et al., 2017, Schmidt et al., 2020). Are these changes a cause or a consequence of the lack of clear phenological adjustment with temperature that I showed? The abundance distributions of each species at L4 has changed greatly: from a uni-modal distribution to a bi-modal one (**Figure 3.5c**), as a result of abundance decrease between spring and autumn (**Figure S 3.2).** This change can be seen both between thermal regimes and between the first the final decades, but not in the much shorter and more recent time series at SH, which has a bi-modal distribution throughout its whole time series. In HR however, copepods decreased in abundance mainly in summer, but maintained a uni-modal distribution for each species both over time and over different thermal regimes. Having two peaks of abundance during the year can significantly increase the complexity and difficulty in analysing seasonal timing, especially when the number of peaks also changes over time, as seen in L4. Therefore, this probably could also explain why I see stronger seasonal timing changes in HR, where the peak was uni-modal for the whole time-series, making abundance distribution a more reliable proxy for phenology.

For this reason, I tested the relationship of timing of abundance with the timing of a life cycle event which is less influenced by mortality: egg production. For *C. helgolandicus,* the seasonal timing indices of abundance did not correlate with those of EPR, as also found by Maud et al., (2015) and Cornwell et al., (2018). This lack of correlation may indicate that the drivers of EPR and adult abundance are different, probably because of the stronger effect of mortality on abundance (than on EPR). However, **Figure 3.6** shows that the *C. helgolandicus* abundance decrease over time occurs in a period of the year which is a month delayed from the decrease of the EPR. This delay (of ~ 1 month) roughly coincides with the average development time of this copepod species, indicating a relationship between the decline of abundance and egg production on a long-term scale. This decrease in EPR (and consequent decrease in abundance) may be due to a decrease in the environment quality for *C. helgolandicus* in almost all months of the year, with particularly abundant predators and scarce food in the warmer summer months. This is manifested in reduced spring/summer egg production rates and lower abundance, but the shift to later egg production may have sustained the population density in autumn and winter. Similarly, Schmidt et al. (2020) showed from the Continuous Plankton Recorder (CPR) survey dataset, that microplankton significantly decreased over a period of 60 years during the months from May to September. However, at L4 I noticed that the decrease of EPR over the study period started in April, which coincides with a decrease of microplankton and a significant increase of predators (**Figure 3.6**). Indeed, the month of April appears to be a crucial time at L4, as the beginning of the temperature-dependent phase, when copepods tend to show strong negative correlations between adult body size and temperature, most likely because of high ratios of predators to available food, and potentially high and varying degrees of competition for food (Chapter 2). Overall, the compound effects of warmer summer temperatures, changes in food and predators are likely to have negative effects on copepods across the NW European shelf, but their relative roles will likely vary regionally.

3.6 CONCLUSION

Both temperature-related phenological shifts and to some extent a degree of thermal niche conservation were evident. However, the effects were far from clear, and obscured by major seasonally-specific changes in abundance that are likely related to station-specific balances of food and predation controls. These non-temperature related drivers of population dynamics severely challenge the ability to understand and predict shifts in plankton phenology in relation to temperature. Moreover, the earlier seasonal timing was demonstrated more clearly across years than with temperature. Therefore, it is difficult to determine if the actual effect of global warming on plankton phenology is obscured, and possibly underestimated, or simply not directly correlated with seasonal distribution of species abundance. However, time series are lengthening worldwide and are becoming ever-better networked across the NW European shelf. Linking these into coherent analyses will unravel the drivers of the profound, climate-related changes that are occuring in the pelagic food web.

CHAPTER 4:

Thermal niche conservation and latitudinal shift: contrasting trends of adjacent copepod populations in the North Sea and NE Atlantic

4.1 ABSTRACT

Shifts in the latitudinal distribution of ectothermic species are described as one of the three universal responses of biota to climate warming. However, in marine environments there is still limited understanding of what controls the extent of such range shifts. I used a 60-year time-series dataset of 29,880 samples across 8 copepod taxa taken from the ocean basins around the British Isles, in order to assess the distributional responds of species to climatic warming over multiple decades. Most taxa shifted northward with warming, but the greater shifts were in the NE Atlantic. Instances of thermal niche conservatism (where a species adjust its geographical/seasonal distribution in order to experience the same temperature) were detected in the NE Atlantic, whereas such conservation was not found for the species in the North Sea, where the temperature has risen more rapidly, but species shifted north less intensely, showing thermal plasticity. These sharply contrasting patterns between adjacent populations may be due to different biotic interactions (e.g.: lack of food, predators, competition). Whatever the cause, responses to warming that differ between taxa and marine areas may help to restructure food web connections and complicate our ability to project future species distributions based on assumptions of a fixed thermal niche.

4.2 INTRODUCTION

In response to climate change, it has been predicted that some marine species will exhibit shifts in their biogeographical range to maintain within their preferred environmental conditions, especially so in planktonic species (Beaugrand et al., 2002; Parmesan and Yohe, 2003; Cheung et al., 2009). Latitudinal range shifts are considered one of the three universal responses to climate change, alongside phenological shifts (Edwards and Richardson, 2004; Atkinson et al., 2015) and the temperature-size response (Atkinson, 1994; Daufresne et al., 2009; Horne et al., 2017). Studying the latitudinal range shift is essential to better understanding the mechanisms behind such redistribution in space, as the potential implications of such outcomes. Of course, we ultimately will wish to be better able to predict and model future biological outcomes. Species distribution models assume that the relationship of a species distribution to its environment is broadly fixed and predictable (Cheung et al., 2008, Brun et al., 2016, Beaugrand and Kirby, 2018). It is to be acknowledge, however, that temperature may not be the only driver of shift of abundance latitudinal distribution and other cofounding factors may exist (Freer et al., 2021). Furthermore, resilience to climate change, defined as "niche plasticity" and achieved through genetic adaptation, could obscure predictions of distributional responses to climate change using such models (Sandford and Morgan, 2011; Dam, 2013).

Species-specific quantification of the biogeographical shift in response to climate change is still lacking in pivotal marine organisms such as copepods. These animals are excellent candidates for climate change studies since they are ectothermic (hence very sensitive to thermal changes), multivoltine (multiple generations in a year) and planktonic (so their response can be detected temporally and geographically, often rapidly). Furthermore, planktonic organisms are not commercially exploited, so any long-term change potentially relatable to global warming is not obscured by direct human activities such as fishing (Richardson, 2008). Some evidence suggests that plankton are even more sensitive to climate change than are physical environmental variables, because the non-linear responses of biological communities can amplify subtle environmental perturbations (Taylor et al., 2002).

Copepods comprise up to 80% of the zooplankton assemblage, thus they significantly contribute to the marine trophic web. They represent an important link of energy between the unicellular primary producers and higher trophic levels. It is therefore important to understand the processes and mechanisms of range shifts and thermal niche conservatism in these animals. While range shifts among marine plankton have been demonstrated in recent years (Beaugrand et al., 2002; Hays et al., 2005, Poloczanska et al., 2013), most of these did not consider whether a species/group of animals is simply tracking their thermal optimum, exhibiting thermal niche conservatism, or is also performing thermal niche plasticity (some degree of *in-situ* resilience to global warming). I aim to look for objective evidence for or against a fixed environmental niche by calculating the change in the percentage of copepod abundance north of a certain isotherm. I analysed one of the most intensively-sampled sea areas in the world, with ~60 years of spatial-temporal data, comprising two contrasting sub-areas at similar latitudes, namely: a) the slowly warming (~0.008°C year^{-1}) North-East Atlantic and b) the very rapidly warming (~0.019°C year^{-1}) North Sea (outcomes from current study). Having two adjacent zones with such a different warming rate makes for an excellent test of the reactiveness of the copepod biogeographic shift response. By studying range shifts of a suite of 8 ecologically important taxa in these adjacent areas over 58 years of warming I aim to understand the mechanisms of range shift and to test whether the principle of thermal niche conservation holds for these copepod species.

4.3 METHODS

4.3.1 Data collection

Copepod abundance data were obtained by the Continuous Plankton Recorder (CPR) activity, created by the David Johns Marine Biological Association of the UK (MBA) and published by The Archive for Marine Species and Habitats Data (DASSH) in 2021. The CPR devices have been in use since 1931, making it the longest-running plankton sampling survey in the world (Richardson et al. 2006). CPR devices sample plankton while being towed behind ships of opportunity at a depth of ~ 6 to 7 m. Plankton is filtered by a scrolling mesh of 270 µm and sandwiched by a second mesh, before being fixed in 4% formaldehyde. Data are never collected less than 10 km from the coastline to avoid interference from local land conditions. For my study, I used the abundance values (measured as individuals m^{-3}) of eight copepod taxa. Four of these were identified to the specific level: *Calanus finmarchicus, Calanus helgolandicus, Centropages typicus, Temora longicornis*, three taxa were identified to genus level: *Acartia* spp., *Oithona* spp., *Pseudocalanus* spp, and another was a mix between *Paracalanus* spp. and *Pseudocalanus* spp. (generally referred to as “*Para-Pseudocalanus* spp.”). The geographical area considered for the analysis was between 20° W and 8° E longitude and between 45° N and 64° N latitude (**Figure 4.1**), over a 60-year time span from 1958 to 2018. Sea surface temperature (SST) values were obtained by the Hadley Centre of the UK Meteorological Office (http://www.metoffice.gov.uk/hadobs/hadisst/). These monthly temperature values are taken during daylight, for a 1° latitude-longitude grid and are the product of a mix of observation and interpolation. For a more detailed description of the SST dataset and its production process, see Rayner et al. (2003).

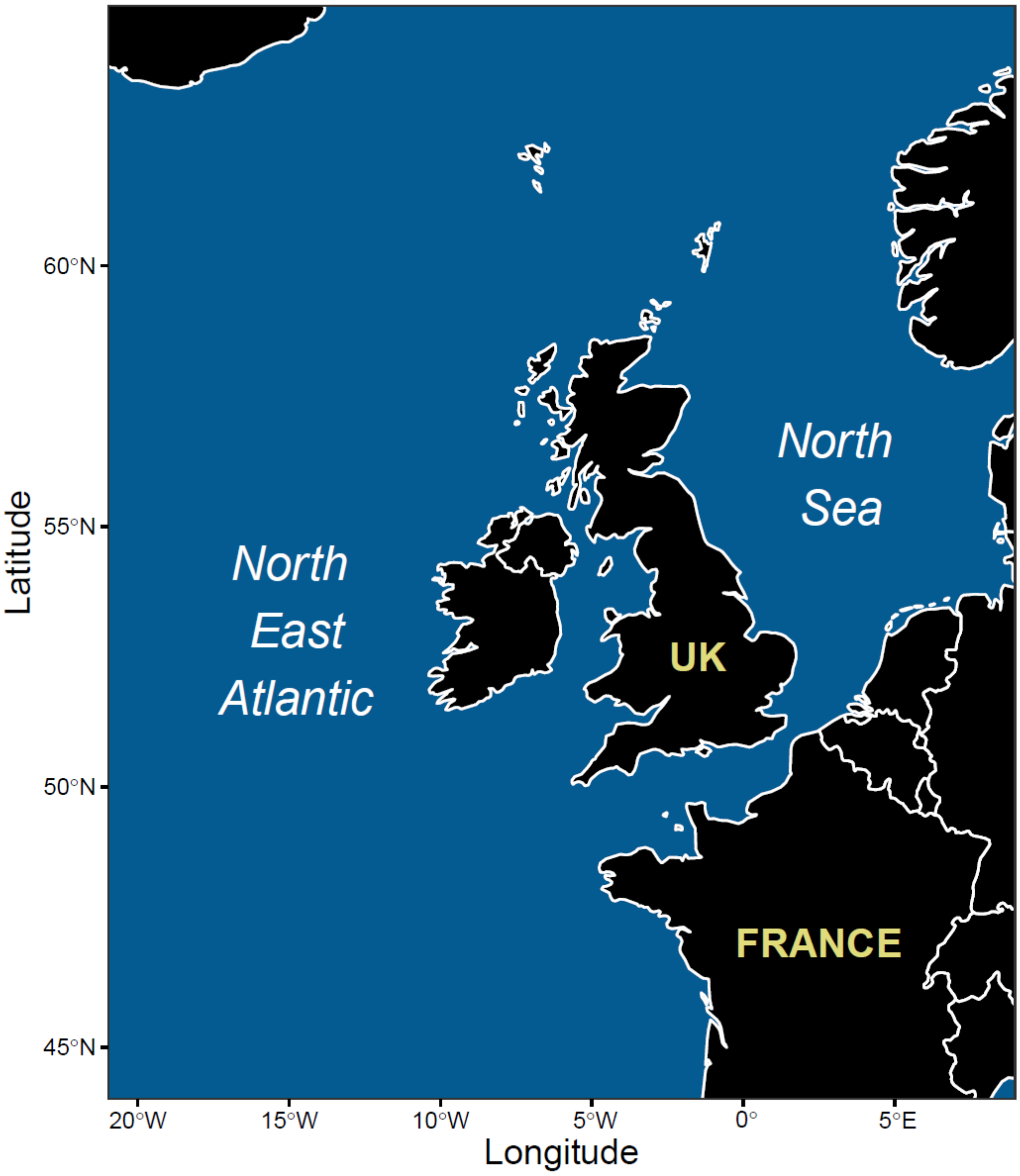


Figure 4.1: Map of the area considered.

4.3.2 Analysis

Data analysis followed the approach of Chivers et al. (2017): Copepod abundance and SST data were averaged by year and by each 1° latitude ×1° longitude section from the area between 45°N and 64°N latitude, and between 20°W and 8°E longitude. The number of samples, equal for each taxon, was very similar for each month of the year (mean count: ~18892; SD: 1203) and each hour of the day (mean count: ~9491; SD: 292), hence showing no major biases toward a certain part of the year, or time of the day (see **Figure S 4.1**). I interpolated copepod abundance by kriging the five-year averages of $\log_{10}$(Abundance + 1) values for each taxon.

To avoid potential biases, I only considered grid cells that were sampled more than 25% of times during the whole time series (for geographical data coverage, see **Figure S 4.2**). Kriging was performed using packages “sp” (Pebesma and Bivand, 2005), “gstat” (Pebesma, 2004) and “automap” (Hiemstra et al., 2009) in R. The former package was used to fit the best fitting variogram by using the autovariogram function for each species and each five-year period. The sea surface temperature (SST) values were used to calculate the latitude of the isotherms at 10, 11, 12 and 13 °C at each 1° longitude in each five-year period (these isotherms were chosen to straddle the centre of the geographic area and taxon ranges sampled, see **Figure 4.2**). To do this, I performed polynomial regressions between the averaged SST against latitude at each longitude degree. I assessed the latitudinal change over time of these isotherms by regressing the average latitude of the isotherms against year. I calculated the 25th, 50th and 75th quantile of latitude for copepod abundance across all longitudes through the use of the function wtd.quantile in R (package: Hmisc (Frank and Harrel, 2021)). I then calculated the average percentage copepod abundance north of each isotherm, relative to the abundance south of the same isotherm, for each five-year period and for each taxon (following Chivers et al., 2017). These percentage values were linearly regressed against time (the five-year periods) and against temperature, represented by the mean latitude of each corresponding isotherm, in order to see whether copepods managed to change their latitudinal distribution at the same rate as the isotherms change (i.e.: with warming). These regressions were made both for the whole area (45°N-63°N; 20°W-8°E) and for two distinct marine areas: the North-East Atlantic (CPR areas ID: B4, B5, C3, C4, C5, D4, D5, E4, E5) and the North Sea (CPR areas ID: B1, B2, C1, C2, D1, D2) (see **Figure S 4.3**). In the end, I applied kriging interpolation for each taxon at each five-year period for spring (April, May, June) and summer (July, August, September). This was done to calculate the percentage of spring abundance relative to the sum of spring and summer, as a proxy for shift in timing of seasonal abundance (phenological shift). I regressed spring proportions of abundance against average SST, the slopes obtained were then regressed against the slopes of proportion of abundance north of the ‘isotherm vs. isotherm latitude’, to see the relationship between geographical and seasonal shift across all taxa and between the two marine areas.

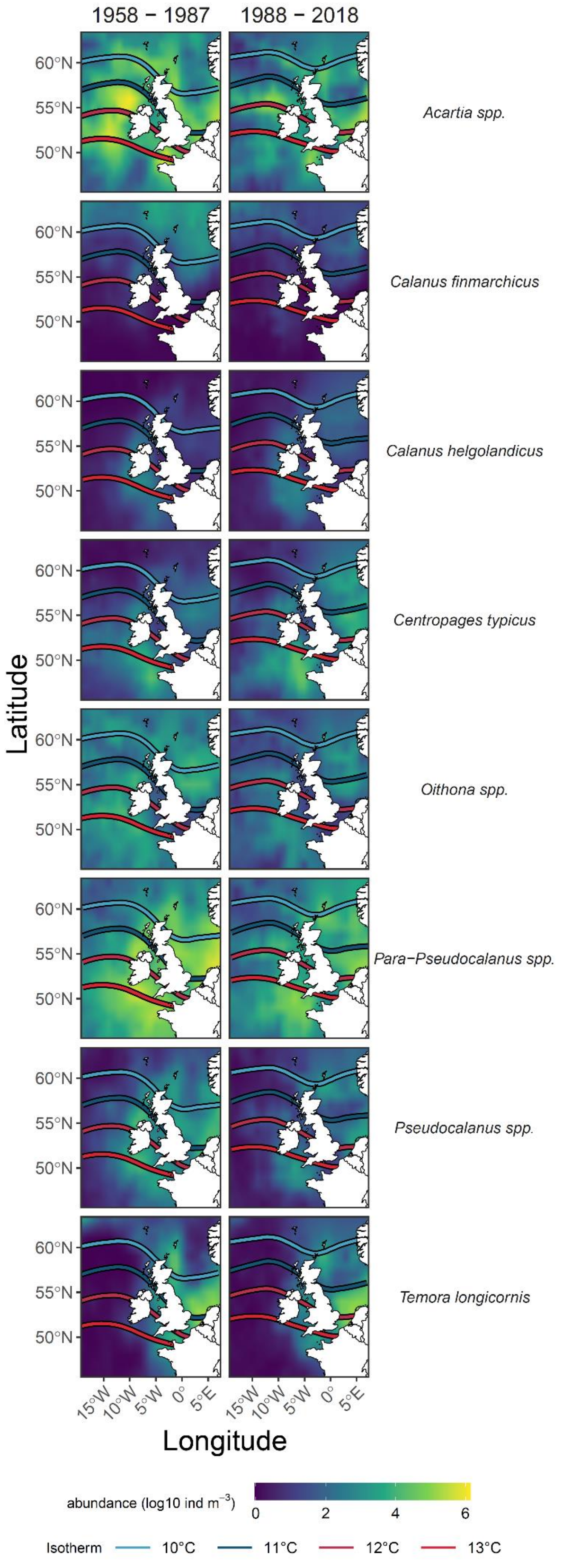


Figure 4.2: Geographic distribution of each copepod taxon in the whole area during the first (1958 – 1987) and last (1988 – 2018) 30 years of the time series. Bright areas indicate high copepod abundance (log(x+1)) as indicated by the legend on top-right. Coloured lines indicate

the four isotherms at different temperatures (10, 11, 12, 13 °C) as indicated by the legend on bottom-right.

4.4 RESULTS

The mean SST of each five-year period has significantly increased over time both in the North-East Atlantic (NEA) and the North Sea (NS) (**Figure 4.3a**) in the period 1958 to 2018. The increase in SST over time in the NEA has been at a slower pace, at around half the warming rate, than in the NS (~0.008°C year^{-1} in the NEA, and 0.019°C year^{-1} in the NS). The latitude of all isotherms (10, 11, 12, 13 °C) significantly increased in both marine areas (**Figure 4.3b**) and is significantly correlated with the mean SST of the whole area (**Figure S 4.4**). In the NEA, the warmer isotherms, 12 and 13°C, significantly increased in latitude over time, whereas the latitude of colder isotherms, 10 and 11°C, showed an initial reduction, representing cooling, between 1958 and 1987, and then a latitudinal increase, illustrating a warming period until 2018 (**Figure 4.3b**). In the NS, the colder isotherms shifted northward by a great latitude than did the warmer isotherms: the coldest isotherm (10 °C) shifted northward on average by ~0.09°N latitude degree per year (**Figure 4.3b**).

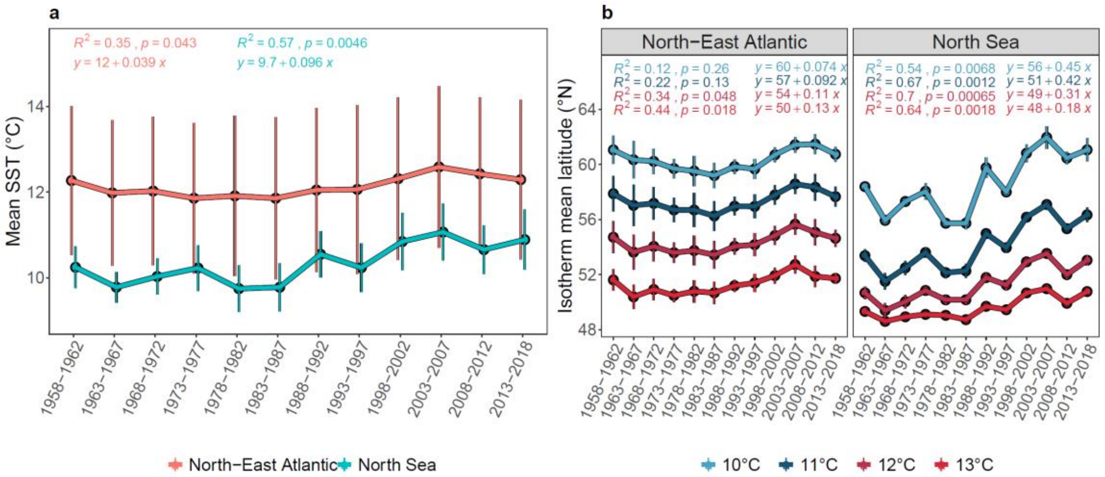


Figure 4.3: **a**) Mean of the estimated SST values over the twelve five-year periods. Colours refer to the different marine areas considered as indicated by the legend at the bottom. On top are reported the R², p-value and the equation from the linear regressions between the y and x axes. **b**) Mean latitudes of the 10, 11, 12 and 13 °C isotherms (colour indexed as indicated by the legend at the bottom) for each five-year period.

The slope of 'mean abundance vs. SST' when calculated for each latitudinal degree, is usually negative at the south and positive at the north (**Figure 4.4**). These trends can also be visualized in **Figure 4.2**, which compares the estimated abundance of each taxon between the first and the last half of the whole time-series (maps showing the estimated distribution of each taxon at each ten-year period can be seen in the Supplementary Material: **Figure S 4.5-12**). Quantiles of latitude for copepod abundance showed different biogeographic shift directions throughout the whole time-series in the Northeast Atlantic: an initial southward shift in the first half of the times-series, followed by a northward shift during the last decades (see *Acartia* spp. and *Centropages typicus* in **Figure 4.5**). This trend seems to be analogous to the change of isotherm latitudinal shift direction (a period of cooling temperatures followed by period of warming in the NE Atlantic), so I calculated the change of quantile of latitude for copepod abundance against time during the cooling period, from 1958-1962 to 1978-1982, and during the warming period, from 1983-1987 to 2003-2007, see **Table 4.1**. Slopes of quantiles of latitude for copepod abundance against mean isotherm latitude were only positive when significant, which means a northern expansion/shift with increasing temperature, and greater in the NEA than in the NS (**Figure 4.6**, the linear regression model fit lines can be seen in **Figure S 4.3**). The percentage of copepod abundance north of each isotherm (11, 12° C) in the NS showed significant negative slopes with warming (with mean isotherm latitude) for all taxa, whereas in the NEA slopes were significantly negative only for *Oithona* spp. (and only with the 13°C isotherm), the only significant positive slope was detected for *Pseudocalanus* spp. for the 11°C isotherm (**Figure 4.7**, the linear regression model fit lines can be seen in **Figure S 4.4**).

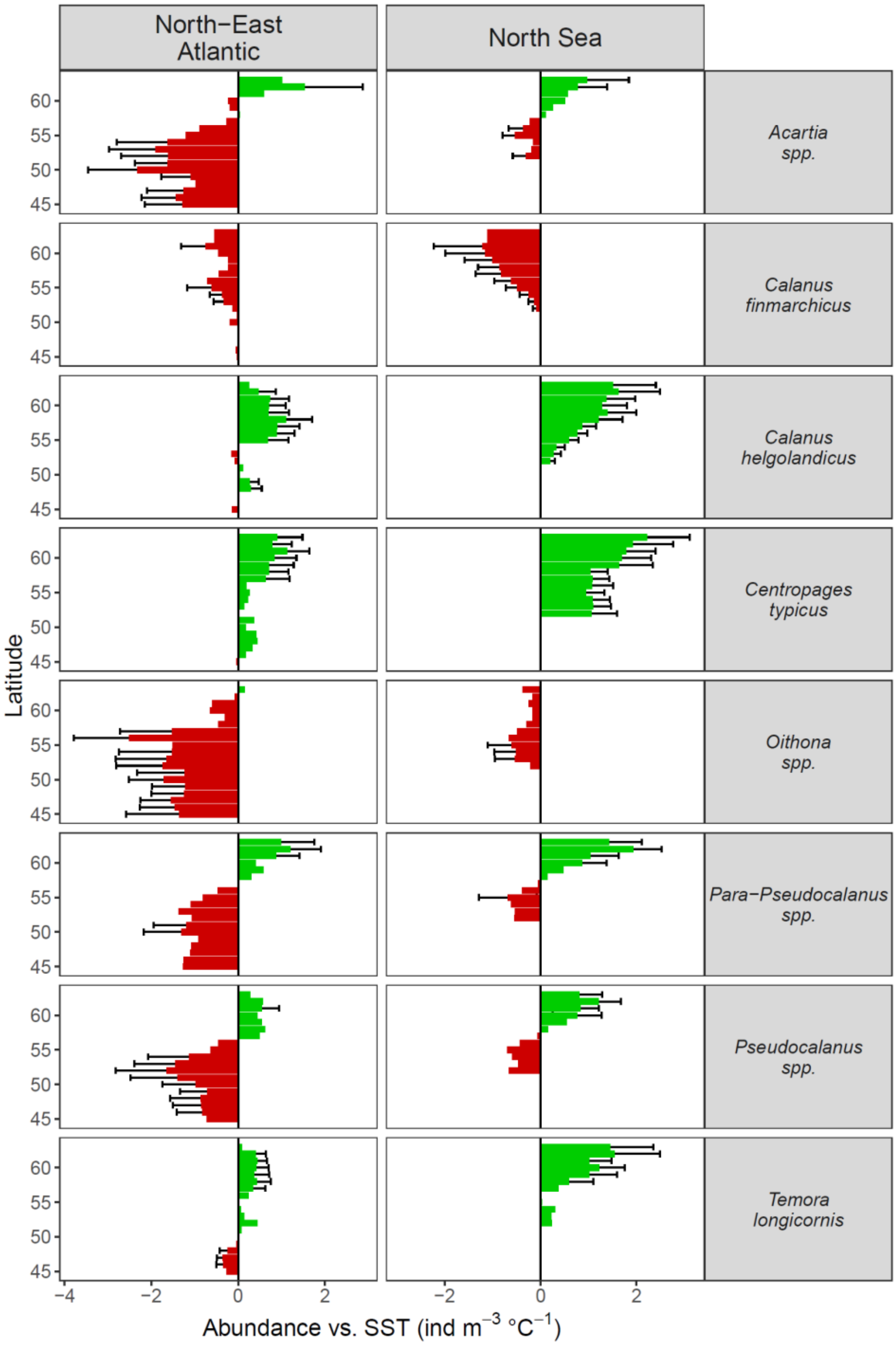


Figure 4.4: Bars indicate the slope values (x-axis) from the linear regressions of mean copepod abundance vs. mean sea surface temperature (SST), for each latitudinal degree (y-axis). Green bars on the right of the vertical black line indicate an increase, while red bars on the left indicate a decrease of copepod abundance with warming within that latitude. Horizontal error bars indicate the 95% confidence interval and are reported only for significant slopes.

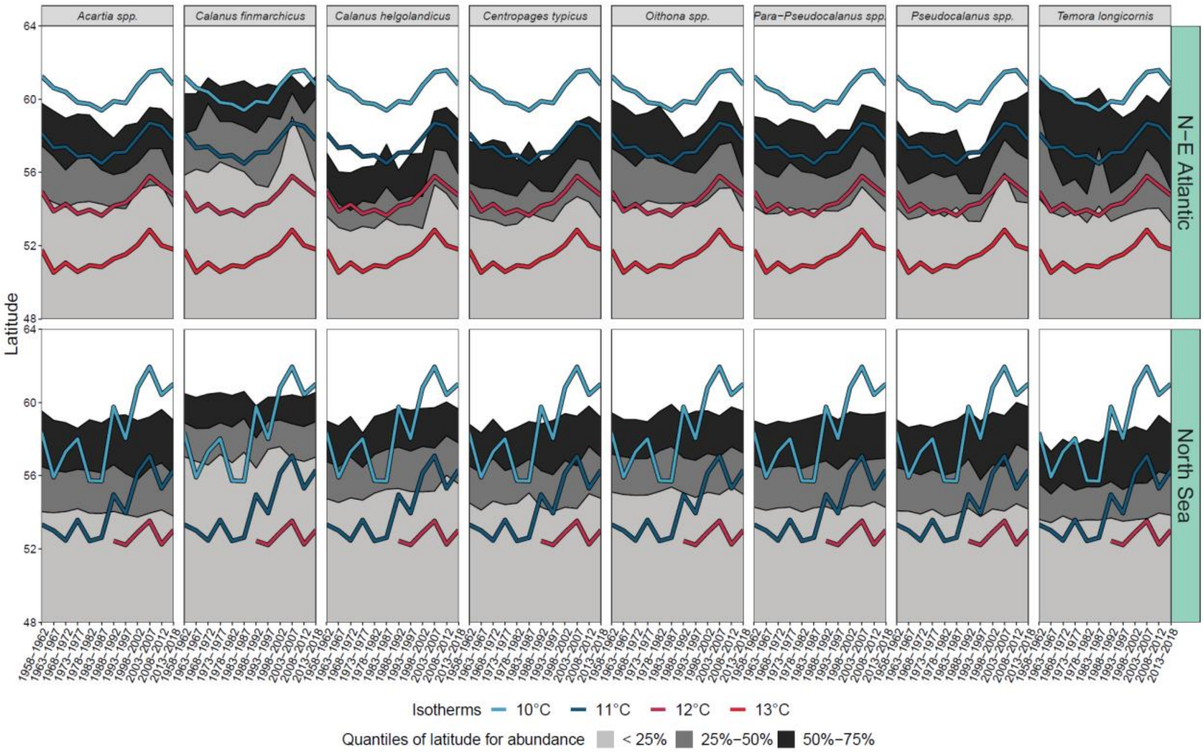


Figure 4.5: Long-term trends of the 25%, 50% and 75% quantiles of latitude for copepod abundance indicated by the three coloured bands. Superimposed lines indicate the long-term trend in the latitude of isotherms (colour indexed for four different temperatures as indicated by the legend on the bottom).

Table 4.1: Total movement in km during the cooling period (from 1958-1962 to 1978-1982) and warming period (from 1983-1987 to 2003-2007) for each taxon in each of the two areas. Total movement is calculated as the mean of all three quantiles (25th, 50th, 75th) of latitude for copepod abundance vs. time (five-year periods) and then converted from latitudinal degree (°N) into km. Positive value mean northward and negative mean southward movements.

Taxon	Total movement (km) during the cooling period	Total movement (km) during the warming period	Area
Acartia spp.	-136.49	168.91	Northeast Atlantic
Calanus finmarchicus	111.39	240.48	Northeast Atlantic
Calanus helgolandicus	-157.99	97.90	Northeast Atlantic
Centropages typicus	-21.32	-93.58	Northeast Atlantic
Oithona spp.	-147.46	-122.57	Northeast Atlantic
Para-Pseudocalanus spp.	-64.59	102.35	Northeast Atlantic
Pseudocalanus spp.	-115.22	265.24	Northeast Atlantic
Temora longicornis	-445.02	77.03	Northeast Atlantic
Acartia spp.	-75.85	23.37	North Sea
Calanus finmarchicus	24.87	43.61	North Sea
Calanus helgolandicus	17.34	52.50	North Sea
Centropages typicus	-23.56	25.45	North Sea
Oithona spp.	5.16	-54.85	North Sea
Para-Pseudocalanus spp.	1.57	30.39	North Sea
Pseudocalanus spp.	-36.47	50.46	North Sea
Temora longicornis	-13.62	74.30	North Sea

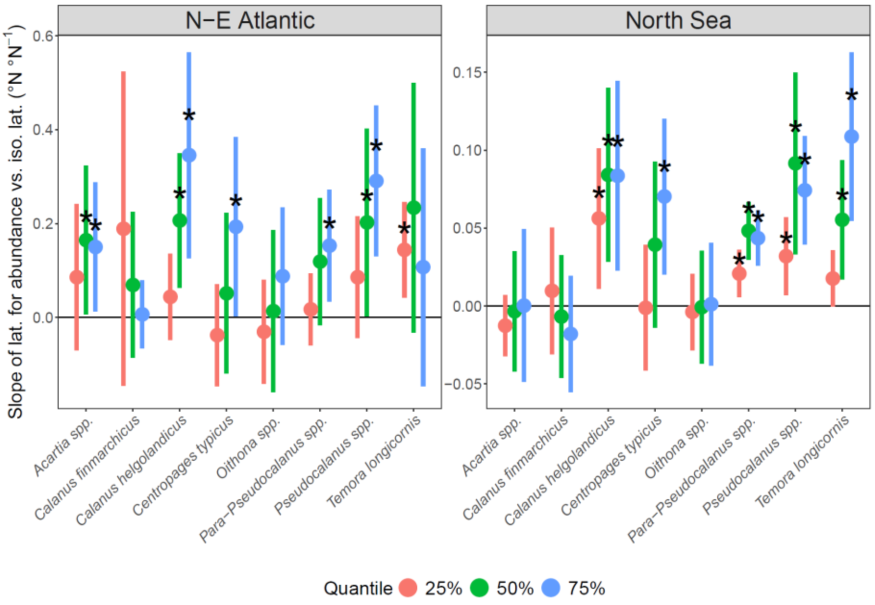


Figure 4.6: Taxon-specific linear regression slopes of quantiles of latitude for copepod abundance vs. mean isotherm latitude (°N °N^{-1}). Points above the horizontal black line indicate a northward expansion of copepod abundance with warming, indexed by the latitude of the isotherm at 11°C. Colours refer to the three different quantiles of latitude for copepod

abundance (25%, 50%, 75%). Vertical error bars indicate the 95% confidence interval, asterisks indicate significant slopes (* = $p \leq 0.05$). Note that the scales of the y axes in the two panels are different (for better visualization), so slopes values for the North Sea are much lower than the ones for the N-E Atlantic.

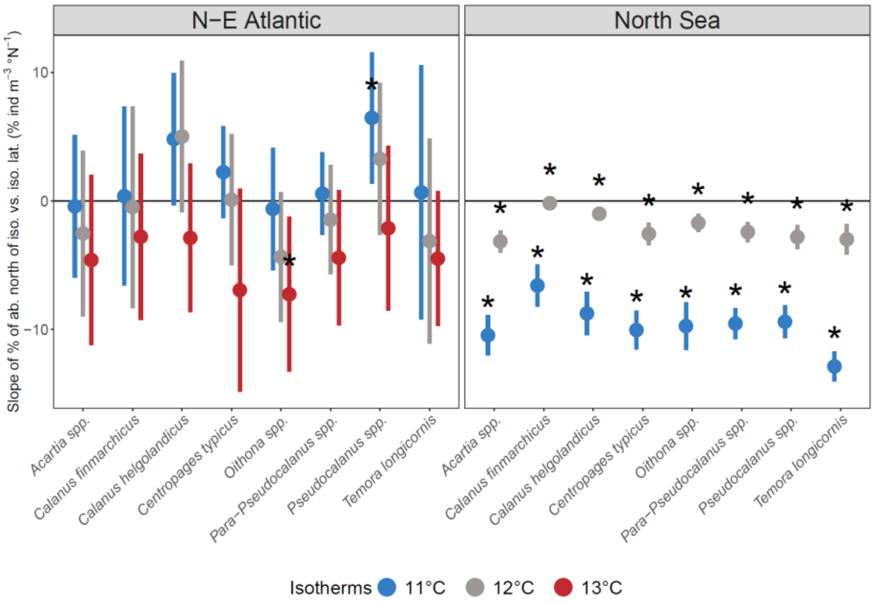


Figure 4.7 Taxon-specific linear regression slopes of percentage abundance north of isotherms vs. mean isotherm latitude (% ind m^{-3} $°N^{-1}$). Colours refer to the three different isotherms indicated at the bottom (I did not include the 13°C isotherm because it never intersects the North Sea). Vertical error bars indicate the 95% confidence interval, an asterisk next to the symbol denotes a significant slopes (* = $p \leq 0.05$).

In the NS marine area, all taxa, except *Temora longicornis*, showed significant increase of spring proportion of abundance with warming; in the NEA marine area only three taxa showed the same pattern. However, no taxon showed a decrease in spring proportion of abundance in either of the two marine areas. In the NS, across all taxa, I found a positive and significant relationship between the change of relative spring abundance with warming, and the change of proportion of abundance north of the isotherm with warming (**Figure 4.8**).

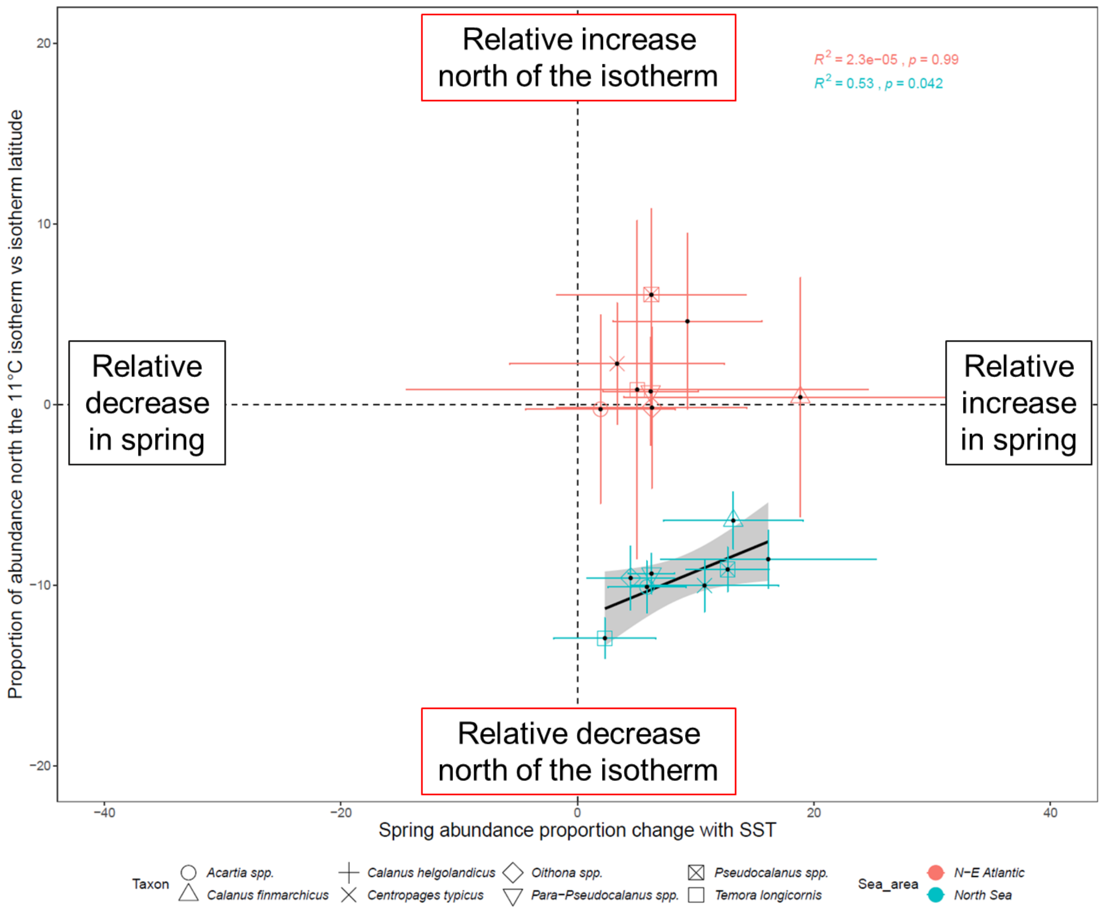


Figure 4.8: Relationship between phenological shift and range shift responses, respectively indexed by spring abundance proportion with warming and abundance proportion north of the isotherm with warming (with isotherm latitude). Labels explaining the trend of the two axis have been added onto the plot to better guide the viewer in interpreting the correlation. Points indicate the corresponding slopes, shapes and colours refer to the different taxa as indicated by the legend at the bottom. Error bars indicate the 95% C.I. Vertical and horizontal dashed lines indicate 0 (no change). Black straight line indicates the linear regression fit when significant; grey area indicate the 95% C.I. of the linear regression fit. The significant regressions from the North Sea indicates a positive relationship between phenological shift and range shift.

4.5 DISCUSSION

Both the North-East Atlantic (NEA) and the North Sea (NS) have experienced a rising temperature, on average, between 1958 and 2018. The Atlantic waters showed an initial period of cooling, especially in the northern part of the range, up until around 1987, whereas the NS has warmed up steadily and more rapidly. The difference in pace of warming between the two areas was an important reason for them to be consider separately, whilst there are other advantages for such a distinction of analysis into the two marine areas. Despite having a less steep increase in temperature over time, the NEA had more intense changes in copepod latitudinal distribution (generally steeper slopes in **Figure 4.6** and **S 4.13**) with warming, where warming is indexed by latitude (°N) of the 11°C isotherm. In this marine area, all of the taxa considered have expanded their geographical distribution northward, with the only exception of *Calanus finmarchicus* and *Oithona* spp.. *C. finmarchicus* also did not show a decreased proportion of abundance north of the north-moving isotherm, which seems contrary to expectation (i.e.: taxa not shifting latitudinally would be expected to change their proportion of abundance north of the north-moving isotherm). It is likely that the very northward skewed latitudinal distribution of *C. finmarchicus* is confounding my analysis because I am analysing only the very southern limit of its geographical distribution (Sundby, 2000). By contrast, *Oithona* spp. does show a decreased proportion of abundance north of the north-moving isotherm, while not shifting northward, as expected. This relatively stationery position could be possible if there is sufficient *in-situ* thermal plasticity: the copepod can maintain the same biogeographical range by adapting other measures of response (such as phenological shift, as found in Chapter 2). In the NS marine area, *C. finmarchicus* and *Oithona* spp, also did not show signs of northward shift. These three taxa are drastically decreasing in abundance along the whole latitudinal range of the NS (**Figure 4.4**). All the other taxa showed a northward shift but with much smaller latitudinal change than in the NEA: ~0.12 °N northward shift with warming in the NEA vs ~0.015 °N northward shift with warming.

Did my findings show evidence of niche conservatism or thermal plasticity of taxa? When comparing the change of abundance percentage north of a specific isotherm with warming, I found strong differences between the two marine areas. In the NEA all taxa except *Oithona* spp. managed to shift their latitudinal distribution at the same pace as that of the isotherms, implying niche conservatism, and one taxon (*Pseudocalanus* spp.) expanded northward even faster than the isotherm (**Figure 4.7**). Specifically, *Acartia* spp., *Calanus finmarchicus, Calanus helgolandicus, Centropages typicus, Para-Pseudocalanus* spp. and *Temora longicornis*, all showed signs of niche conservatism, because their percentage abundance north of the isotherms did not significantly change with warming. In the NS marine area, by contrast, no taxon managed to maintain the same latitudinal shift as the isotherms. All taxa decreased significantly and more intensely in their relative abundance north of the isotherms. These clear failures of abundances to track the north-moving isotherms in the NS may be exacerbated by the shortest latitudinal range, compared to the NEA area, coupled with a very fast latitudinal shift of the isotherm. However, the difference in latitudinal distance moved between the two marine areas is real, even when using normalized quantiles of latitude for copepod abundance that are normalized to account for the difference in sample area size between the NEA and the NS (**Figure S 4.15**).

What is preventing copepods in the NS from shifting their range and conserving their thermal niche as they do in the NEA? In theory, biogeographical shifts should be greater where temperature rise more quickly (Beagurand et al., 2018), yet I found the opposite. Our results suggest that other factors other than temperature may be at play. It is possible that when copepods are advected to an area with suitable abiotic conditions they may encounter other constrictions such as low food quality/abundance, changing pressures from predators, prey, parasites, and of course competition (Gilman et al., 2010). These factors will also be themselves sensitive to the new biotic and abiotic changes, leading to potentially chaotic trophic interactions that are technically impossible to predict (Hastings and Powell, 1991). For instance, it is possible that the uneven latitudinal distribution of chlorophyll concentration may play a crucial role in copepod biogeographic redistribution: the NS, indeed, has a greater chlorophyll concentration in the southern part of the range (close to continental European coastline) and lower concentration in the north (Quante et al., 2016), due to the different hydrographic zones (Krause et al., 1987). The NEA on the other hand presents a more homogeneous hydrography and distribution of chlorophyll, which can sometimes even increase at northern latitudes (Campbell and Aarup, 1992). This is also in agreement with Hays et al. (2005) and Pitois and Fox (2006), who found a greater copepod production in the southern NS than in the northern part. Further exploration is required to see if this difference can explain why copepods in the NS shifted northward less than in the NEA.

Ultimately, another difference detected between the two marine areas is that copepods in the NS increased their relative abundance in spring more than the studied copepods in the NEA (**Figure 4.8**). Moreover, in the NS, the taxa that decreased more north of the isotherms, were also the ones that increased in spring the least; thus, taxa that shifted their seasonal timing more, may not have needed to shift their geographical distribution so much (and *vice versa* – strong range shifters, had less need for large shifts in seasonal timing of abundance). This could be seen as a trade-off between two different strategies of plastic adaptation to warming, which requires further investigation. In conclusion, my findings show that different responses to climate warming (geographic shift and niche conservatism, or *in-situ* niche plasticity, or failed response and abundance decline) can occur not only among different taxa, but also among different hydrographic areas. This biogeographical patchiness may add complexity to models that predict responses to global warming, but it needs to be considered to better estimate the past, current and future development of marine ecosystems in a changing environment.

CHAPTER 5:

Interrelationships among the three universal responses to climate change

5.1 INTRODUCTION

The previous chapters have investigated each of the three proposed universal responses to warming individually, namely shifts in body size (Chapter 2), phenology (Chapter 3) and geographic range, for seven species or genera of copepod (Chapter 4). In brief, the second chapter benefitted from a large dataset with many body length measurements that allowed a rigorous comparison of the temperature-size response (TSR) of major copepods. An important finding was that body mass trends across seasons within a year were greatly influenced by abundance in April, which is the start of the temperature-dependent phase in the annual cycle. Furthermore, I also showed a long-term body size reduction with warming across 27 years. The third chapter was the most challenging, both in terms of execution and interpretation. Traditional phenology indices such as times of bud burst, flowering or egg laying cannot easily be applied to marine copepods, rather we followed the more typical approach for planktonic taxa, using indices of timing of seasonal increases in abundance. However, this approach of using abundance does mean the outcomes are also influenced by other factors besides for example temperature and physiology, such as food and mortality. Moreover, I suggest that the long-term decline in abundance during specific periods of the year, has changed the seasonal distribution of abundances so much so, that the “true” warming-related phenological adjustment may have been obscured. All of this “noise” could possibly explain why I did not find a consistent phenological response, or trend, across different stations and species. Nevertheless, copepods, in some cases, adjusted their timing of seasonal abundance with a changing thermal regime both in the long term, within the same location, and from one latitude to another. The fourth chapter showed that copepods are shifting their biogeographical distribution poleward concomitant with the shifting isotherms. However, it was only in the NE Atlantic that copepods tracked their thermal niche by shifting north. In the North Sea, despite rapid warming and northward movement of the isotherms, the copepod ranges were more static, such that much of their population remained in increasingly warm waters (Chapter 4). To explain this surprising finding, I hypothesize that the greater abundance of food in the southern part of the North Sea may have prevented the shift northward.

Most studies to date on the responses of biota to warming have only examined one or two of these universally-observed responses. Only one study, to my knowledge, has explored possible relationships between these responses, and it was only between the phenological and biogeographical redistribution (Chivers et al. 2019). This final chapter aims to explore the interrelationships among all three of these responses that were explored individually in the previous chapters. Is there any sort of relationship between these responses? For instance, do species that shift more intensely in phenology also shift strongly in range shift and/or adult body size? Or perhaps there is a sort of trade-off, by which species that predominantly undergo a certain phenotypic adjustment do not display other types of responses? I will use different types of metrics for each taxon and each kind of response (for example, a metric that measures how much a species has shifted in range, phenology and adult body size), then I will regressed these metrics of one response against the metrics of another. However, since I have only 7 taxa available (8 in Chapter 4), I will integrate new species data, when possible, in order to increase my sample size. To conclude, besides discussing possible explanations and ecological implications of these relationships, I will report limits and unanswered questions that arose from this whole thesis, with suggestions for future studies. Furthermore, theorizations and speculations on current and previous findings, such as “escape vs. expansion”, “plastic vs. niche tracker”, and adaptation will be a central point of this last chapter. For the sake of simplicity, I will divide this chapter into two sections, one for the relationship between adult body size change and phenological shift (**5.2**) and one for the relationship between phenological shift and range shift (**5.3**), each one subdivided in methods, results and discussion.

5.2 RELATIONSHIP BETWEEN BODY SIZE CHANGE AND PHENOLOGICAL SHIFT

5.2.1 Methods

I tested the relationship between species-specific temperature-size response (TSR, %µgC °C^{-1}) values (measured in Chapter 2) with the shift in seasonal timing of abundance across years (from Chapter 3, I did not use shift across temperature because time was a better explanatory variable). In order to increase my original sample size, which was only seven taxa, I integrated field TSR values from the meta-analysis by Horne et al. (2016), which added *Euterpina acutifrons* and *Oncaea* spp. to consider a total of nine taxa (n = 9). These two additional taxa were chosen because they were also present consistently in the whole L4 time-series (1989 to 2019), enabling me to also calculate their seasonal timing of abundance shift. Other matching taxa could not be found for the TSR-phenological shift relationship test. Next, I regressed the individual body size in April of each year and taxon against the timing of abundance (average between the 25th and the 50th cumulative percentile day of abundance) of each year and taxon (n = 91), both axes values were normalized across years for each species. I also regressed laboratory-measured TSR values from the literature (Horne et al., 2016) with the shift in seasonal timing of abundance from my study (Chapter 2), although only five taxa (n = 5) could be used. I thereby assessed whether there is an relationship between the TSR measured under benign laboratory conditions and phenological shift.

5.2.2 Results

A significant negative correlation between TSR across seasons and seasonal shift of timing of abundance (mean between the 25th and 50th cumulative percentile of abundance day year^{-1} at L4) was found. Thus, species that lost more body mass from one generation to another with warming, tended to show the least degree in shift of their timing of abundance over years less **Figure 5.1**. I did not find any significant relationship between laboratory-measured TSR values and annual shift in seasonal timing of abundance at L4.

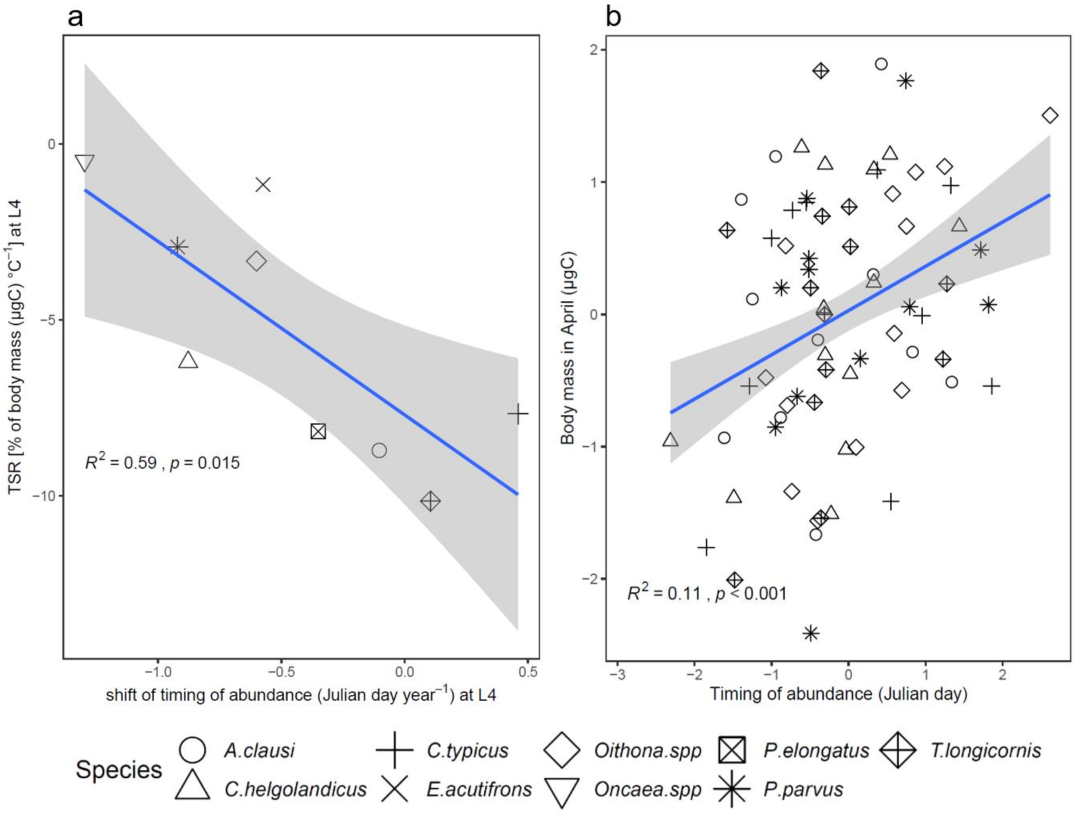


Figure 5.1: **a**) Linear regression between temperature-size response (TSR), measured as % of body carbon mass change °C-1 at L4 (temperature measured over seasons, see Chapter 2) and timing of abundance shift (measured as the day of the year derived from mean between the 25th and the 50th cumulative percentile of abundance of each year vs time (year^{-1}) at L4, see Chapter 3). **b**) Linear regression between body mass in April of each year (µg C, values normalized across years for each species, see Chapter 2) and the timing of abundance of each year (day of the year derived from mean between the 25th and the 50th cumulative percentile of abundance of each year). For both plots, each point shape indicates one taxon as indicated by the legend at the bottom. The blue straight line indicates the linear regression fit line, the grey band indicates the 95% interval of confidence. R² and p values are reported at the bottom-left of each plot.

5.2.3 Discussion

It is still not simple to determine whether the relationship between the change of seasonal body size with temperature (TSR) *in situ* and seasonal timing of abundance (phenological shift) that I found is due to methodological limitations rather than an actual physio-ecological relationship. In fact, in years with early phenology, copepods tended to show a smaller body size in April (**Figure 5.1b**), which is in agreement with the fact that, in April, body size is correlated with copepod abundance. This is because abundance in April and seasonal timing are strictly correlated, as I found in Chapter 2. Therefore, why are the overall TSR values of all species correlated with the shift in timing of abundance? I suggest that the explanation lies in how the TSR is calculated: TSR is determined from the regression between body size and temperature throughout the temperature-dependant phase (TDP, the period from April to October), and I have shown that the starting body mass of the TDP (in April) tends to influence the steepness of the 'size vs. temperature' regression slope. Consequently, in years with early phenology, abundance at the start of the TDP (April) will be greater than average, implying a decreased individual adult copepod body mass in April too (as found in Chapter 2). Therefore, species whose timing of abundance substantially shifts to early in the season are also the ones that have a lower TSR overall (see scheme in **Figure 5.2** for a better understanding of this concept).

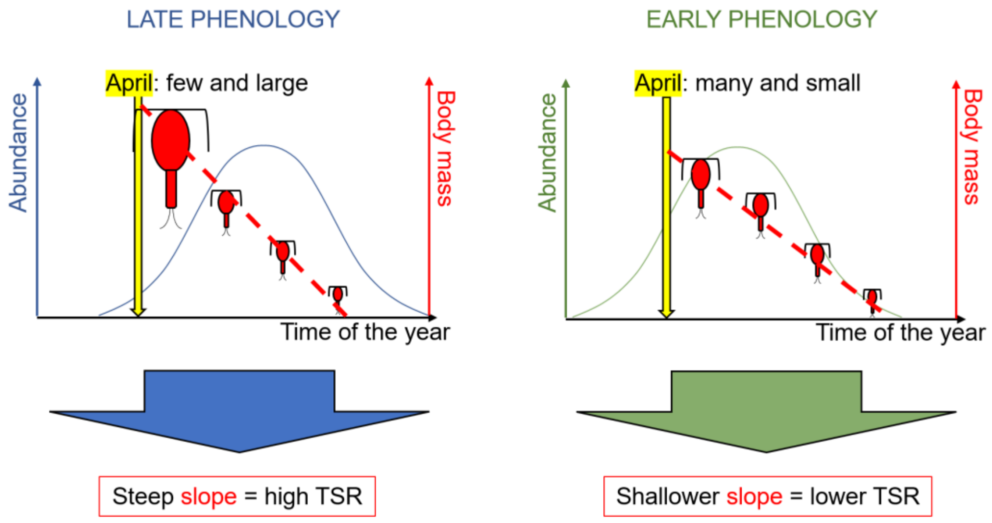


Figure 5.2: Schematic explanation for how the temperature-size response may change with a shift in seasonal timing of abundance. Left: a late-season abundance peak where the abundance in April is lower, which is associated with a greater body size in April (as found in Chapter 2). Right: an early-season abundance peak where the abundance in April is greater, hence individual body size in April is smaller. A greater body mass at the beginning of the temperature-dependent phase (April) steepens the slope of body size vs. temperature, from which the temperature-size response (TSR) is calculated.

With only five taxa available for the test, I did not find a relationship between the lab-measured TSR values and the *in situ* shifts in seasonal timing of abundance. Therefore, more species-specific data, either more lab TSR values or more phenological shift ones, are required before concluding how a species TSR (measured under controlled conditions) relates to shifts in timing of its seasonal abundance.

5.3 RELATIONSHIP BETWEEN PHENOLOGICAL SHIFT AND RANGE SHIFT

5.3.1 Methods

The observed positive relationship between the warming-related shift in proportion of abundance north of the isotherms and the warming-related shift in proportion of abundance in spring versus summer (**Figure 4.8**), observed only in the North Sea, could indicate that species whose distributions shift more intensely in latitude, also shift more intensely in seasonal timing of abundance. To investigate this idea further, I first explored the trend of spring relative abundance with warming for each taxon at each latitude. Then, I estimated the spatio-temporal density distribution of copepods across the same areal and time span I considered in Chapter 4, but for each month of the year. I thereby calculated more accurate indices of timing of abundance from the monthly Continuous Plankton Recorder (CPR) data. I used the Centre of Gravity (COG) method (Edwards and Richardson, 2004), which works better with monthly data, instead of the cumulative percentile of abundance that I used in Chapter 3 with daily/weekly/biweekly data:

$$COG = \sum(N_m * M)/\sum N_m$$

COG is expressed in month of the year; N_m is the estimated abundance of each taxon at each month M.

COG was calculated on the yearly mean abundance of each five-year group in each of the two areas (North-East Atlantic vs. North Sea). I then regressed COG values against the average sea surface temperature (SST) from April to September inclusive, for each taxon. To compare warming-related seasonal shifts in abundance with warming-related latitudinal shifts, I then regressed average taxon-specific slopes from 'COG vs. SST' regressions against the corresponding average taxon-specific slopes from regressions of 'proportion of abundance north of the isotherm vs. isotherm latitude'.

5.3.2 Results

Each taxon exhibited a significant shift in timing of abundance toward the early part of the year, with increasing temperature in both the NE Atlantic and North Sea, with a range of days advanced °C^{-1} between ~8 and ~36 (**Figure 5.3**).

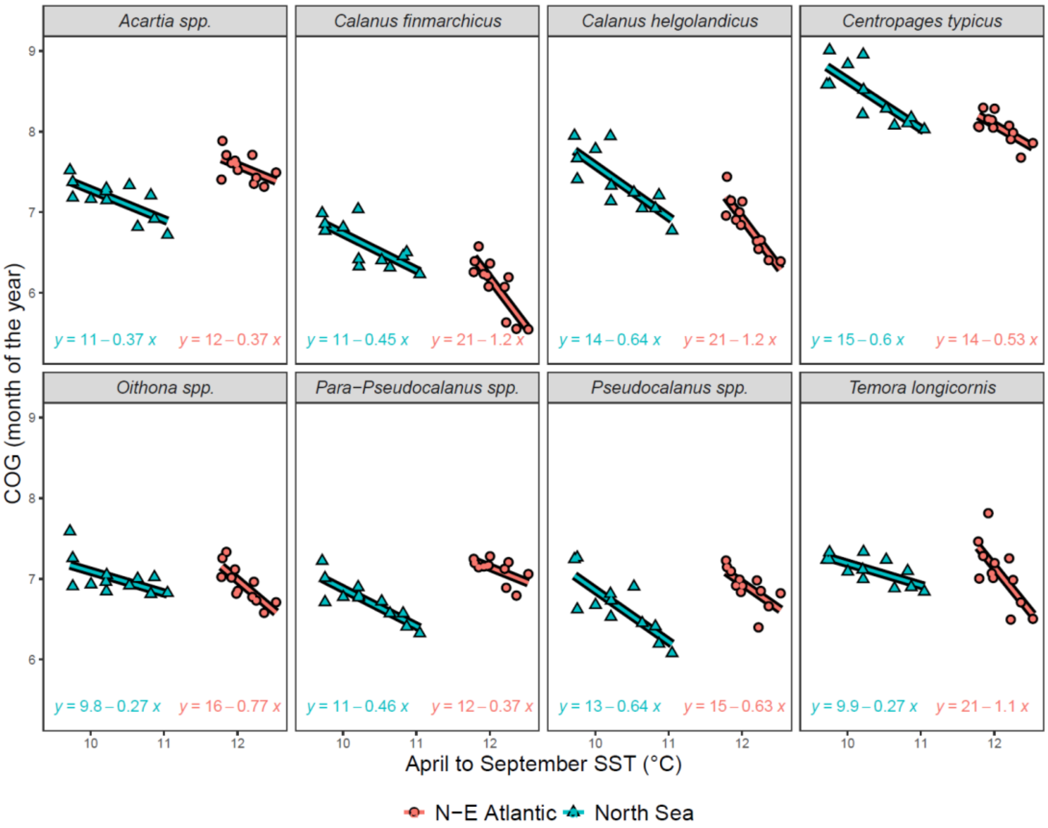


Figure 5.3: Linear regressions between the Centre of Gravity (COG, measured as month of the year) of seasonal abundance and the April-to-September sea surface temperature (SST, measured as °C). Shapes and colours refer to the two different marine areas as indicated by the legend at the bottom. All regressions are statistically significant ($p < 0.05$).

When regressing ‘COG vs. SST’ slopes against slopes of ‘proportion of abundance north of the isotherms vs. isotherm latitude’, no significant relationships were found in either of the areas. Sensitivity of the seasonal timing of copepod abundance to average April-September temperature (COG vs. SST) was greater at higher latitudes than southern ones across all taxa in the North Sea and in one taxon (*T. longicornis*) in the N-E Atlantic; only two taxa (*Acartia* spp. in the N-E Atlantic and *C. finmarchicus* in the NS) showed an opposing trend: abundance shifting to later-season with warming in the southern compared to northern latitudes (**Figure 5.4**).

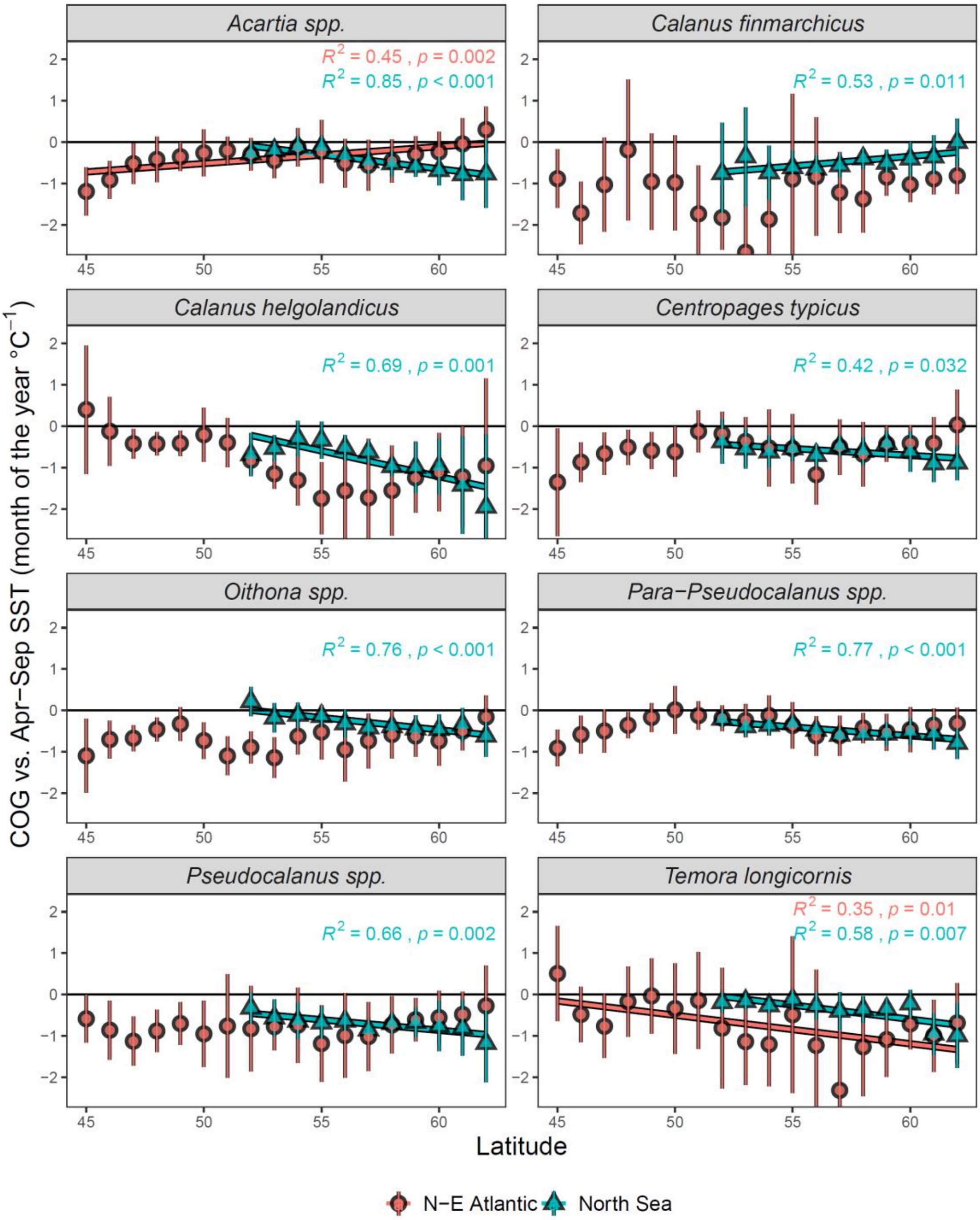


Figure 5.4: Plots show phenological sensitivity to temperature (y axis) vs. latitude (x axis). Units on the y axis are slopes expressed as Centre of Gravity (COG) month with warming (month of the year °C^{-1}). Different points and shapes refer to the two areas as indicated by the legend at the bottom. Vertical error bars for each point indicate the 95% confidence of interval for each slope on the y-axis. Horizontal black line indicates zero (no phenological shift). R² and p values are reported for the significant regressions between y and x axis for each taxon and both marine areas.

5.3.3 Discussion

In this chapter, I have revealed new insights into shifts in seasonal timing of abundance with warming, by using data from the CPR. Compared to the shifts in timing I measured at the three sampling stations (in Chapter 3), these new seasonal shifts in timing of abundance are much more consistent: abundance of all taxa shifted toward earlier in the year with warming, showing much stronger regression slopes (up to -36 days °C^{-1} with CPR data vs. maximum -13 days °C^{-1} with data from the three stations). This difference in thermal sensitivity of seasonal timing between fixed stations and the CPR data may arise because of the greater (and two-dimensional) area covered by the CPR, hence reduced "noise" compared with the punctiform nature of fixed sampling stations, where both plankton abundance and environmental variables present high variability from one sample point to another. It is not possible to exclude other methodological differences, like sampling the whole water column in stations vs. sampling just the surface water in CPR and the different taxonomic levels of identification (at species level in stations and often at genus level in CPR), that may partly explain this different outcome.

In Chapter 4, I found a correlation between seasonal and biogeographical shift (**Figure 4.8**), by using relative spring abundance as an index of phenology. In the current chapter, I aimed to replicate the same regression by using more accurate timing indexes (COG, instead of spring relative abundance). However, although showing a similar trend to that in Chapter 4, it was not statistically significant ($p = 0.09$). Therefore, is the correlation between seasonal and biogeographical shift found in Chapter 4 just a false positive (the fit line seems to hinge only on 2 or 3 taxa at the extremes: *T. longicornis* and *Calanus* spp., **Figure 4.8**)? Or is the second regression in this chapter a false negative due to not having enough observations for confirming the correlation (only 8 taxa)? Theoretically, it would be reasonable to expect species that tend to shift more in seasons to also shift more in latitude, because species more sensitive to temperature should be more likely to occupy new seasons and new latitudes with warming. However, it is important to acknowledge that these two responses are measured through abundance shifts, which are not direct phenotypic reactions to warming at the individual level. Instead, the measured responses represent a combination of phenotypic responses and changes in abundance (reflecting births, deaths and movement in the population). An optimal temperature in a new season or latitude is not enough alone to allow the "colonization" by new individuals, because survival is also determined by biotic interactions within the new season or latitude, such as prey availability, competition, predation, mortality, etc. So, a new niche (seasonal or geographical), may be optimal in terms of temperature, but not necessarily in terms of biotic interactions and vice versa. Another important aspect to consider is what "shifting" actually means: is it an "escape" (i.e. individuals disappear from one niche which is not optimal anymore and appear in a new niche that is now optimal) or is it an "expansion" (i.e. individuals appear in a new niche that has now optimal conditions, but still remain in the original niche)? According to this simple categorization, based on the biogeographical shift trends of chapter 4, I could label, by visualizing the change of abundance with warming at different latitudes (**Figure 4.4**), *Acartia* spp., *Para-Pseudocalanus* spp. and *Pseudocalanus* spp. as "escapers", *Calanus helgolandicus, Centropages typicus* as "expanders". *Temora longicornis* seems to be in an intermediate situation between escaper and expander, whereas *Calanus finmarchicus* and *Oithona* spp. are both decreasing across the whole latitudinal range, with the only difference being that the first taxon is decreasing more in the north, whereas the second is decreasing more in the south. Regarding the phenological shift, it is less intuitive to define which taxa are "escaping" and which are "expanding". From the analysis performed in chapter 3, species seemed to generally expand toward spring, although I detected, in the English Channel, a decrease of abundance over time in the period between spring and summer, which may appear as a sign of "escaping". However, that part of the year, from which they should be "escaping", is not the warmest one across all seasons, so other factors than temperature may be involved.

Here, my findings on seasonal timing (performed on CPR data) showed that copepods tended to advance their timing of abundance with warming more at higher latitudes than at lower ones, but only in the North Sea (**Figure 5.5**). In fact, this would indicate that copepods are indeed occupying northern latitudes with warming, but they are occupying also the early season (spring) more in the north than in the south. In fact, spring abundance in the North Sea is increasing with warming relatively more than summer abundance, and this increase is greater in the northern than the southern latitudes (**Figure 5.5**).

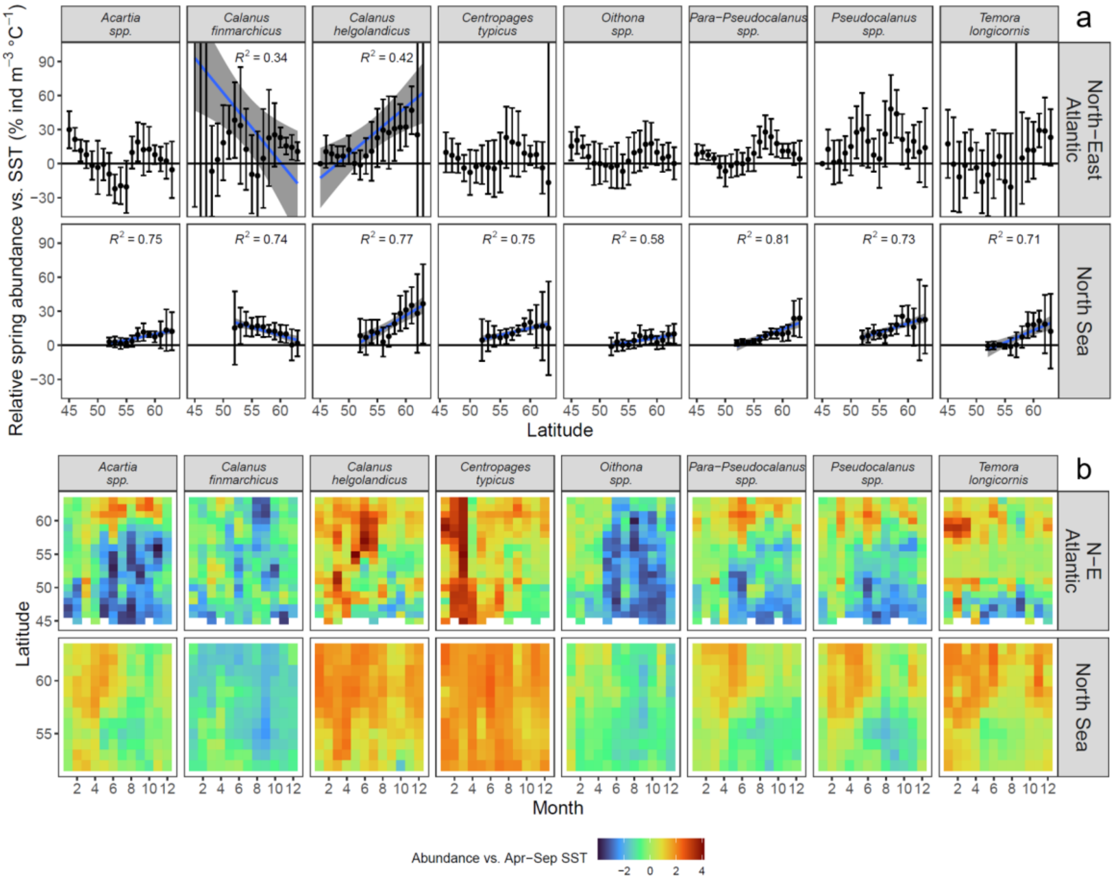


Figure 5.5: **a**) Linear regressions between relative (to other seasons) mean spring abundance (% individuals m^{-3}) vs. sea surface spring temperature (SST, measured as °C). Fit lines (in blue) are reported only for statistically significant regressions ($p < 0.01$), grey bands indicate the 95% confidence of interval. R² values are also reported for significant regressions. **b**) shows the abundance sensitivity to temperature across a seasonal-latitudinal scale. Abundance (ind m^{-3}) vs. April-to-September SST (°C) are reported for each month and latitude, colour indexed as indicated by the legend at the bottom.

5.4 CONCLUSION

In this chapter, I provided new insights for how these three universal responses are related to each other. The relationship between body size change and phenological shift may be due to how the temperature-size response is calculated. Nevertheless, the relationship between body size at the start of spring and phenology is real and is likely to have ecological consequences. Considering the high phenological shifts detected in this Chapter from the CPR data, combined with the continuous increase of temperature globally, body size in April could be predicted to continue to decrease in the next decades, possibly leading to cascade effects on the rest of the marine trophic web. However, my hypothesis is relying on the body size data from only one sampling station (L4) and seven taxa, so it is unsure whether this relationship occurs predominantly locally, or if it can be referenced to a broader area. I therefore encourage more studies on this subject, by measuring the *in situ* TSR values in different stations, at different latitudes, possibly on more zooplanktonic taxa, and compare these values with the corresponding phenological shifts (from the same stations or possibly from the CPR dataset). I suspect that meta-analyses, although being a quicker and more feasible method, may lead to unconclusive outcomes since all of the *in situ* TSR studies are based on very few temporal repetitions (except for Evans, 1981, who sampled over 10 years) and generally across few species. Furthermore, mechanisms behind the size-density relationship are still to be explained, thus, laboratory experiments (e.g. microcosm) are suggested in order to fully investigate this phenomenon.

It must be clarified that the three plastic responses to climate change that are investigated in this thesis are not all the same conceptually. Body size change is a phenotypic physiological reaction, whereas phenology and range shifts could be considered as “niche compensatory” mechanisms: spatio-temporal rearrangements that reflect the thermal niche of a species in a changing environment. Both responses are then quite similar in concept, thus it would make sense to see them correlated: species that shift intensely in phenology also shift intensely in range and could be labelled as “niche trackers”, due to their temperature sensitiveness, whereas species that do not shift, or shift little, in phenology also do not or shift little in range and could be labelled as “plastic”, due to their local resilience to warming (as suggested by Chivers et al., 2017). However, here I propose a revision of this categorization, for several reasons: first, shifting toward a new latitude or a new season may not just be an index of thermal sensitivity, but also (or only) a function of local survival in the new niche, determined by different biotic interactions, as previously discussed. So being able to thrive as a population in a new temporal or geographical niche is also a sign of phenotypic plasticity, that could precede the local genetic adaptation, as many authors suggest (West-Eberhard, 2003; Fierst, 2011; Dam, 2012; Wund, 2012). Two kinds of plasticity could be then labelled: thermal plasticity (local survival in the original spatio-temporal niche) and ecological plasticity (local survival in the new spatio-temporal niche, although ecological plasticity in the old niche may occur as well if local food and/or predators undergo ecological adjustments due to long-term warming). Following this reasoning, a species that does not shift in phenology and or in range does not necessarily mean that is more thermally resilient, but it could simply be less “ecologically plastic”: unable to colonize new temporal or geographical niches (see schematic in **Figure 5.6**). At this stage, my work is not able to confirm with high confidence the positive correlation between range shift and phenological shift, thus new studies on both kinds of responses on more and different zooplanktonic taxa (from the CPR dataset possibly) are required and encouraged. However, if these two plastic responses are indeed related, and if plasticity is indeed the precursor to local adaptation, would this mean that species that are intrinsically able to expand (possess ecological plasticity, **Figure 5.6a**) or escape (possess ecological plasticity but no thermal plasticity, **Figure 5.6b**) into a new spatio-temporal niche are more favourite to adapt to climate change than species that are less ecologically plastic (**Figure5.6c-d**)? It is possible that species with no (or less) ecological plasticity could be late in the “race” for adaptation, meaning that it could be more difficult for them to shift and adapt to new niches that are already occupied by the ecologically plastic ones. Of course, this is all very theoretical, but I suggest that it could be useful as a point of reference for future empiric studies and models. Another question that has arisen from this thesis that still requires an answer is why is there such a difference in terms of range shift between two adjacent seas (North-East Atlantic and North Sea) and also why is the phenological and range shift relationship (if confirmed) only found in the North Sea? I suspect that food availability and distribution could play a role in this, in fact, chlorophyll not only is overall found at much higher concentration in the North Sea, but it seems to disproportionally prevail at greater levels in the southern part of the Nort Sea, whereas in the Atlantic phytoplankton appears more evenly distributed, but overall less abundant. I therefore encourage a broader study on phenological and range shifts across multiple marine geographic sectors and taxa, to investigate which bio-environmental parameters (besides temperature) are behind the geographical shift in zooplankton. The latest Overview of the Coupled Model Intercomparison Project Phase 6 (CMIP6, Eyring et al., 2016) has definitively highlighted the copepod food availability decline at mid latitudes combined with increasing temperature (Ratnarajah et al. 2023). Therefore, given the inestimable ecological importance of zooplankton, it is imperative to exhaustively investigate the responses to warming within an integrated and broader framework, that integrates multiple disciplines, such as oceanography, hydrography and evolutionary biology), in order to better predict future ecological dynamics and act accordingly through tempestive environmental policies.

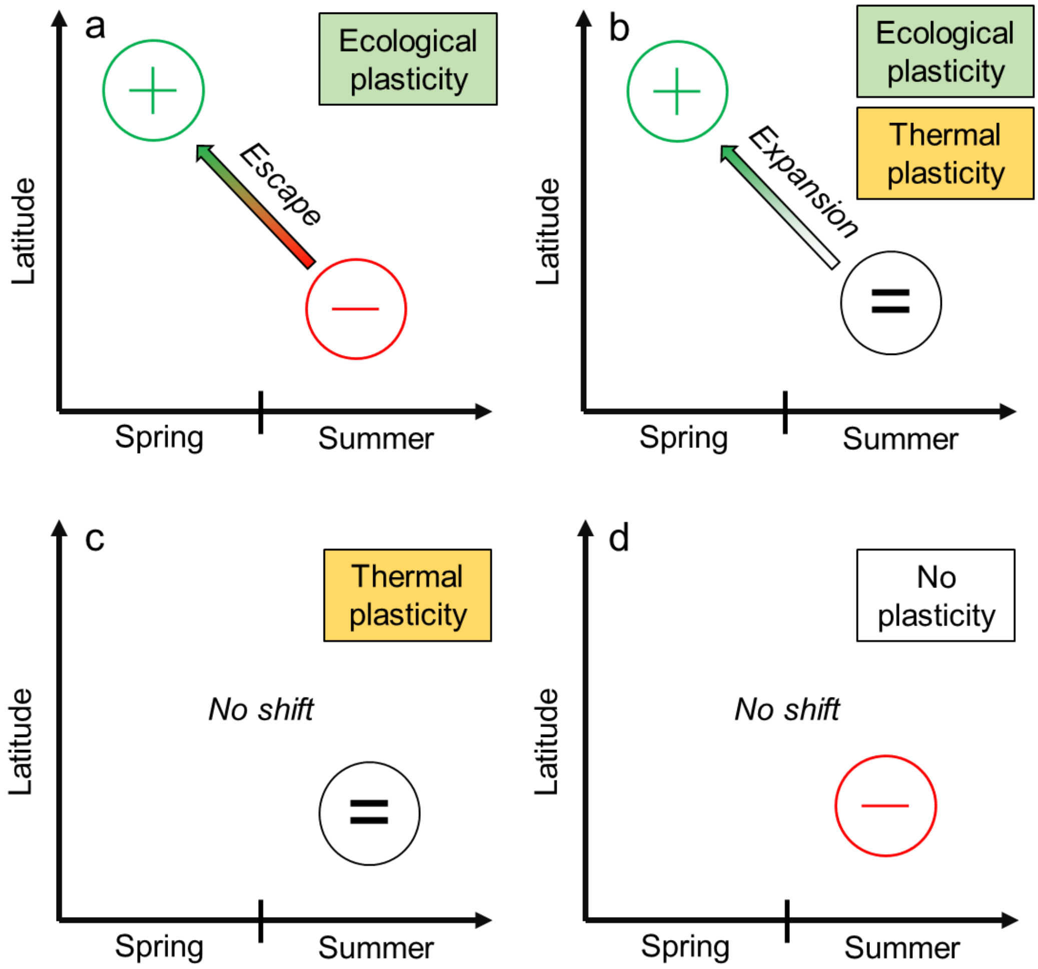


Figure 5.6: Schematic representation of escape vs. expansion situations and how these relate to the two types of plasticity: ecological and thermal. "+", "-", "=" symbols indicates, respectively, increase, decrease, and no change of abundance with warming. **a**) case in which abundance shifts by decreasing with warming in the south/summer niche, due to lack of thermal plasticity, and increasing with warming in the north/spring niche, due to ecological plasticity; **b**) case in which abundance shifts by remaining stable in the south/summer niche, due to thermal plasticity, and increasing in the north/spring niche, due to ecological plasticity; **c**) case in which abundance does not change in the summer/south niche, due to thermal plasticity, resulting in no shift; **d**) case in which abundance decreases in the south/summer niche, due to lack of thermal plasticity.

6 SUPPLEMENTARY MATERIAL

Table S 2.1: Prosome length (PL) - body mass (DW = dry weight; CW = carbon weight) conversion equations used for each species, and corresponding reference. Correlation coefficient *r* is reported as well where available. Carbon weight was obtained assuming this to be 0.4 of dry weight (Kiørboe et al., 1985). Note that for *O. similis* we measured from the top of the prosome to the connection between the first and the second segment of the urosome (as done in Sabatini and Kiørboe 1994).

Species	**Equation**	**Reference**	***r***
A. clausi	DW (µg) = 15.2PL(mm)$^{2.97}$	Klein Breteler et al., 1982	0.97
P. parvus	DW (µg) = 19.1PL(mm)$^{2.74}$	Hay et al., 1991	
O. similis	CW (µg) = 2.85PL(mm)$^{2.16}$	Sabatini & Kiørboe 1994	0.94
C. typicus	DW (µg) = 17.8PL(mm)$^{2.45}$	Klein Breteler et al., 1982	
T. longicornis	DW (µg) = 31.3PL(mm)$^{3.06}$	Klein Breteler et al., 1982	0.99
P. elongatus	DW (µg) = 19PL(mm)$^{2.73}$	Klein Breteler et al., 1982	0.97
C. helgolandicus	DW (µg) = 15.4PL(mm)$^{2.71}$	Hay et al., 1991	

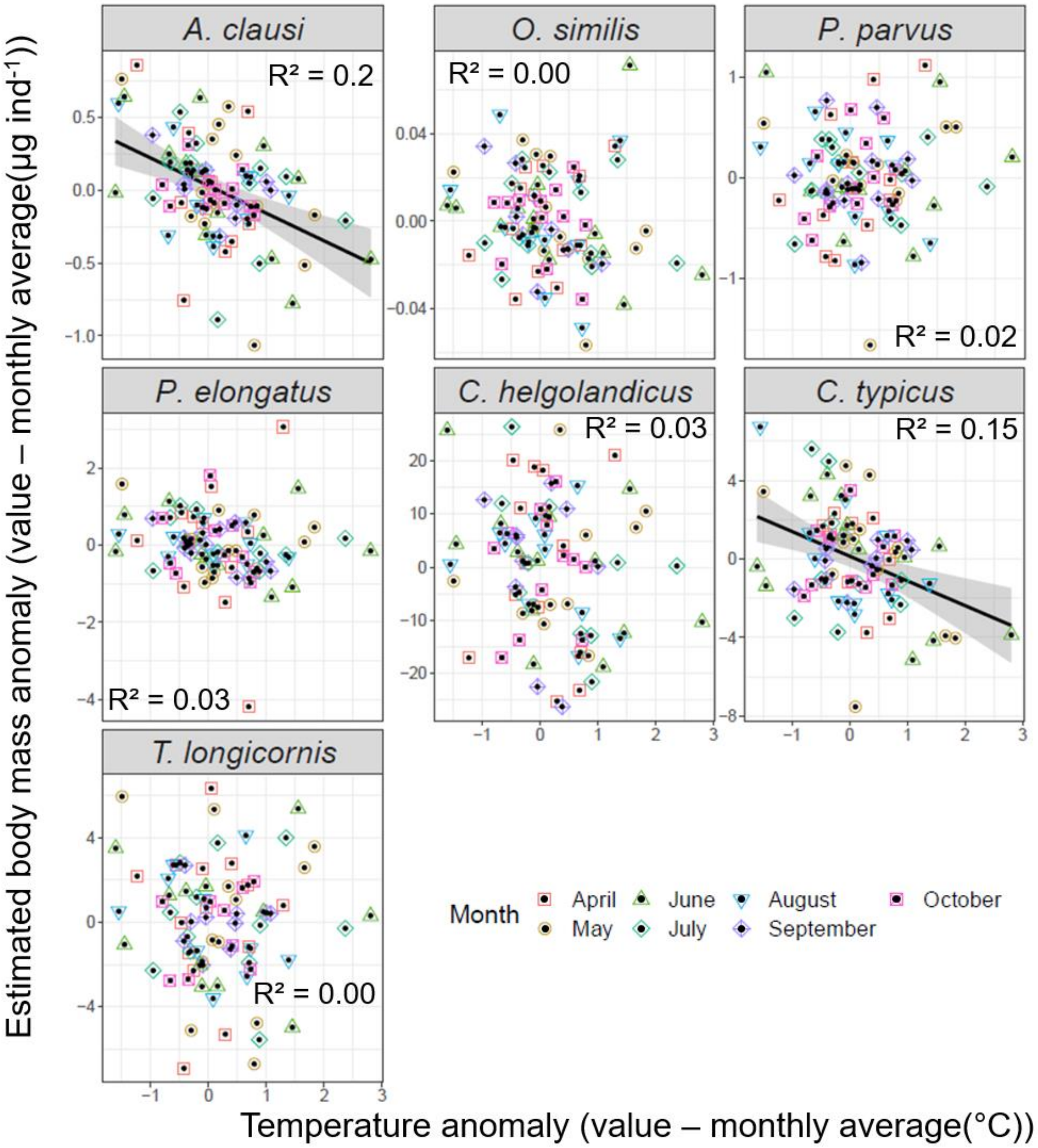


Figure S 2.1: Carbon mass anomaly versus temperature anomaly for each species; colours and shapes refer to different months as indicated in the legend on bottom-right. *R²* values are reported for each species-specific regression, straight black lines indicate significant regressions.

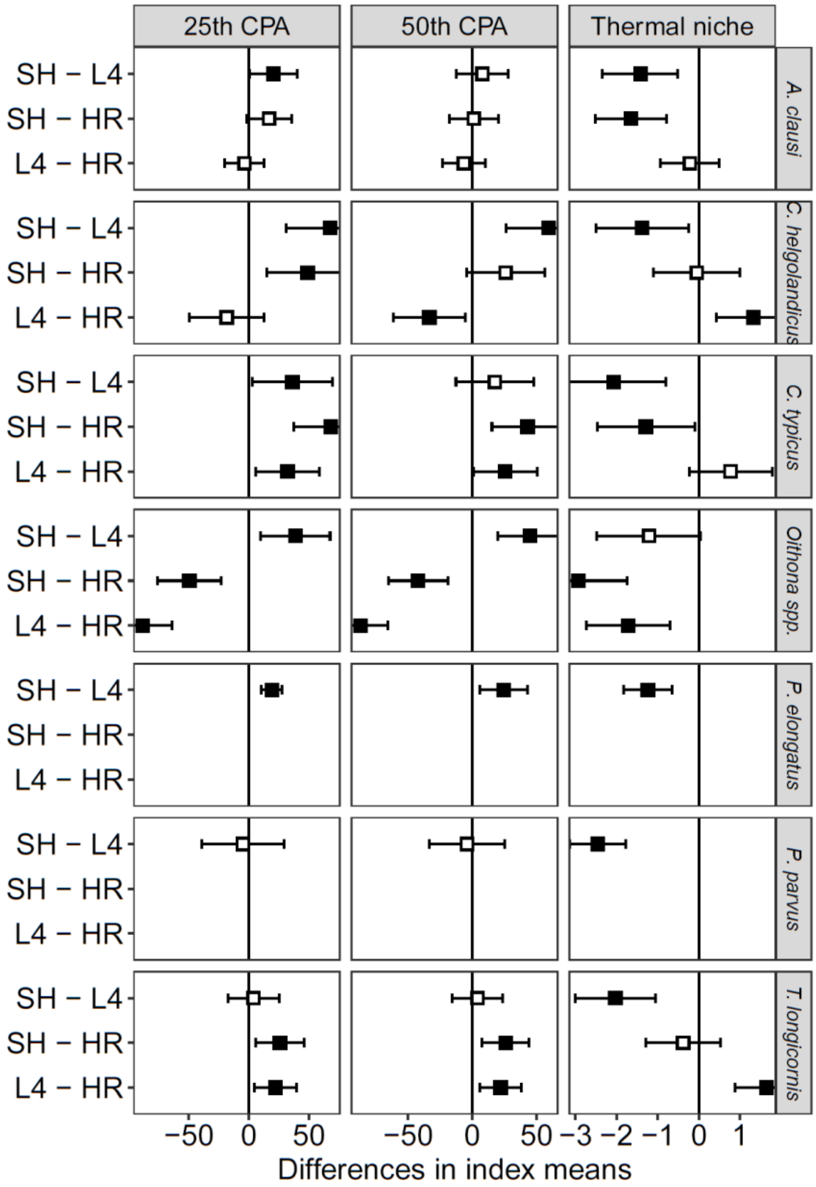


Figure S 3.1: Results of the Post-hoc pairwise comparison test on phenological indices (25th and 50th CPA) and thermal niches of each species between stations. Squares represent the difference value in means, horizontal error bars indicate the 95% CI. Black squares indicate significant differences ($p < 0.05$). In the first two columns, the position of each square relative to the vertical line indicates an earlier (on the left) or later (on the right) phenology of the first station compared to the second one at each comparison (for example: the top left square indicates that *A. clausi* has a significantly later phenology in SH compared to L4). On the third column, the position of the square indicates a colder (on the left) or warmer (on the right) thermal niche between the first and the second station.

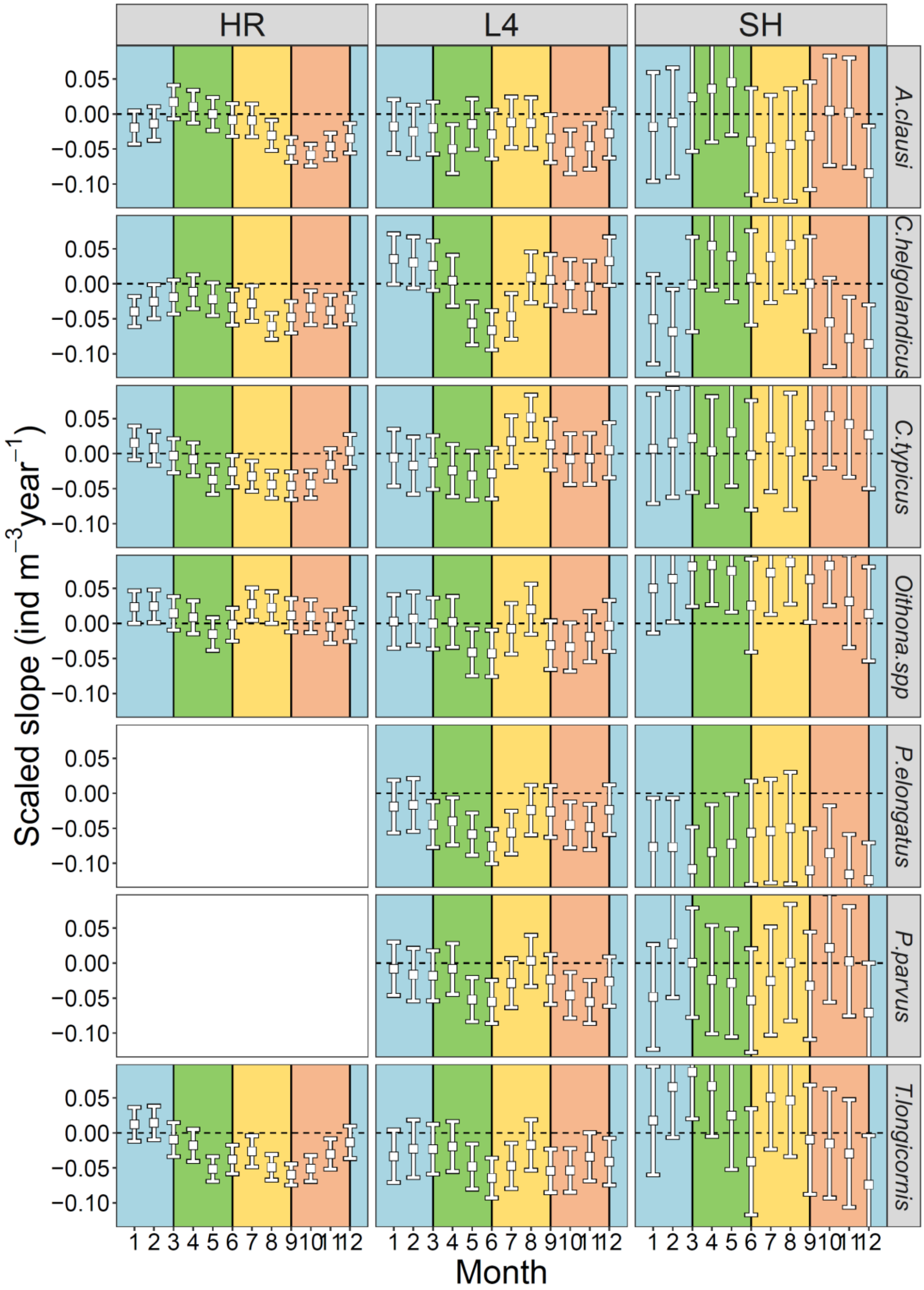


Figure S 3.2: Points indicate the slope of mean log(abundance+1) vs. years for each month, each species at each station. Vertical bars indicate the 95% confident interval, horizontal dashed lines refer to the value 0 (bars that do not intersect this line indicate a significant slope). Background colours refer to the four seasons (cyan: winter, green: spring, yellow: summer, orange: autumn).

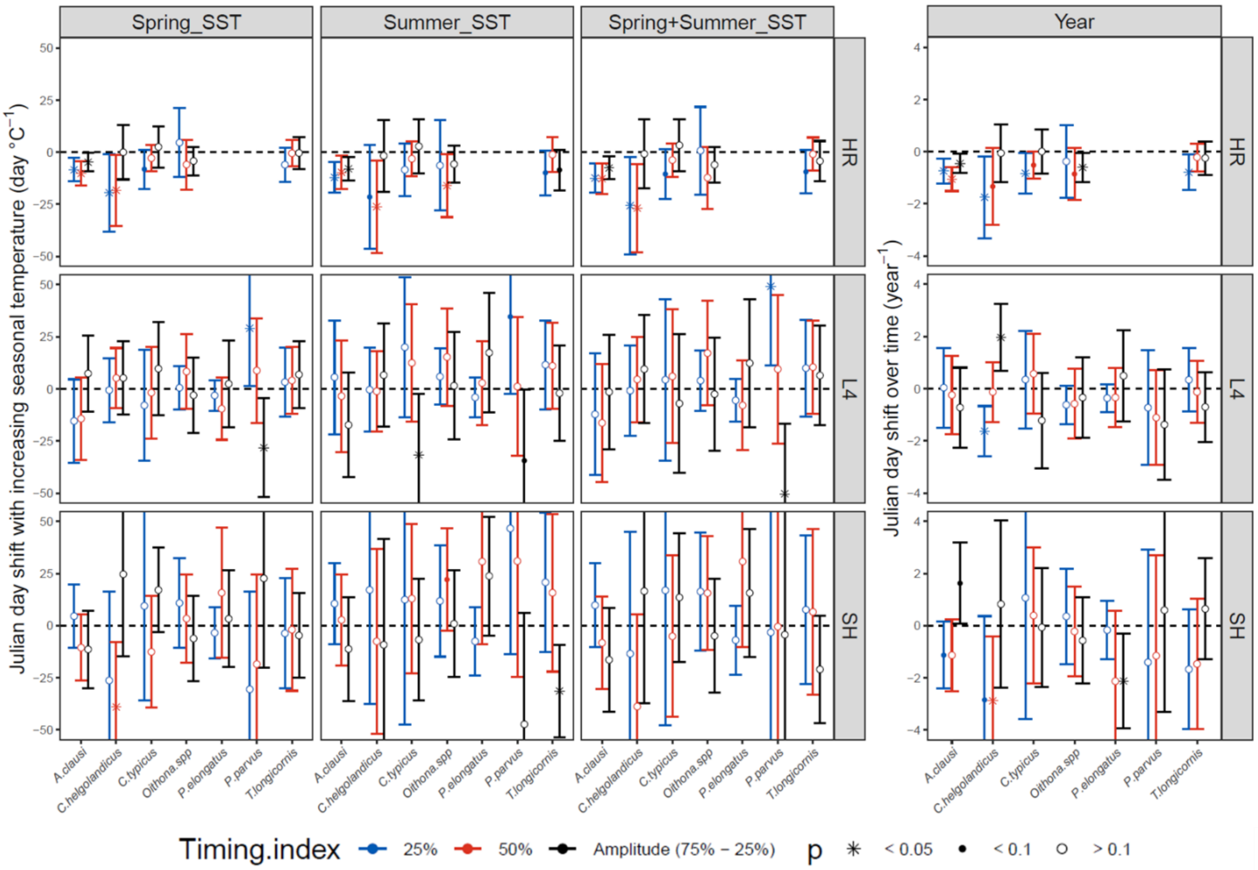


Figure S 3.1: Slope values from the 'phenological indices vs. SST' linear regressions, for each station and different sets of SST, last column shows regressions of 'phenological indices vs. years'. Symbols shape indicates the p values levels and symbol colour refers to the timing index (as according to the legend below).

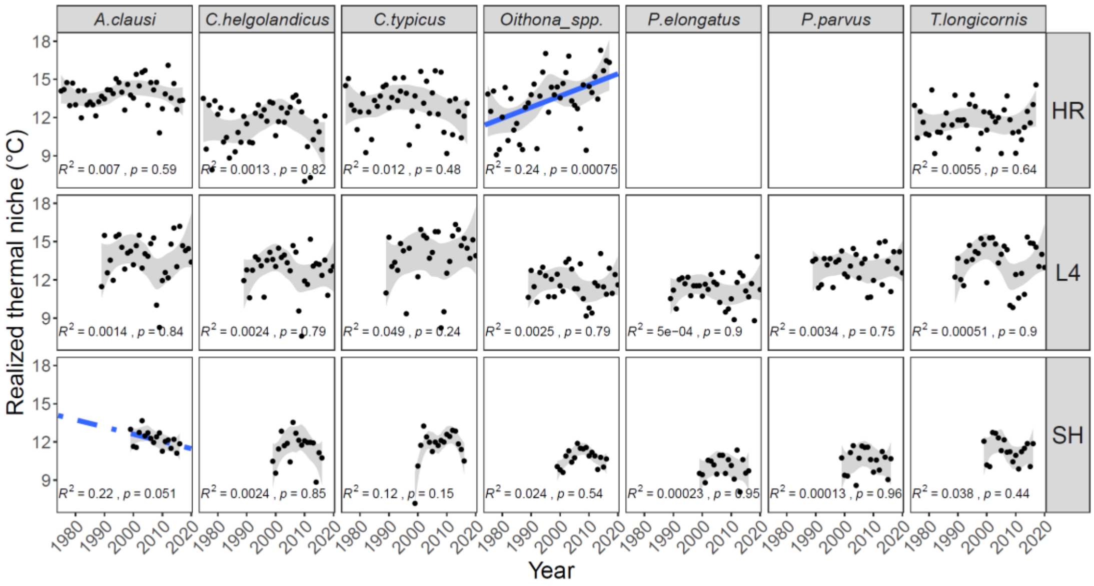


Figure S 3.4: Scatterplots show the annual realized thermal niches over years of each species (columns) at each station (rows). At the bottom of each panel are reported the R² and p values of all linear regressions, whose fit lines are reported only when significant (straight line) or barely significant (dashed lines). The grey bands indicate the 95% confidence interval of the loess smooth regression with a α (or span value) of 0.75.

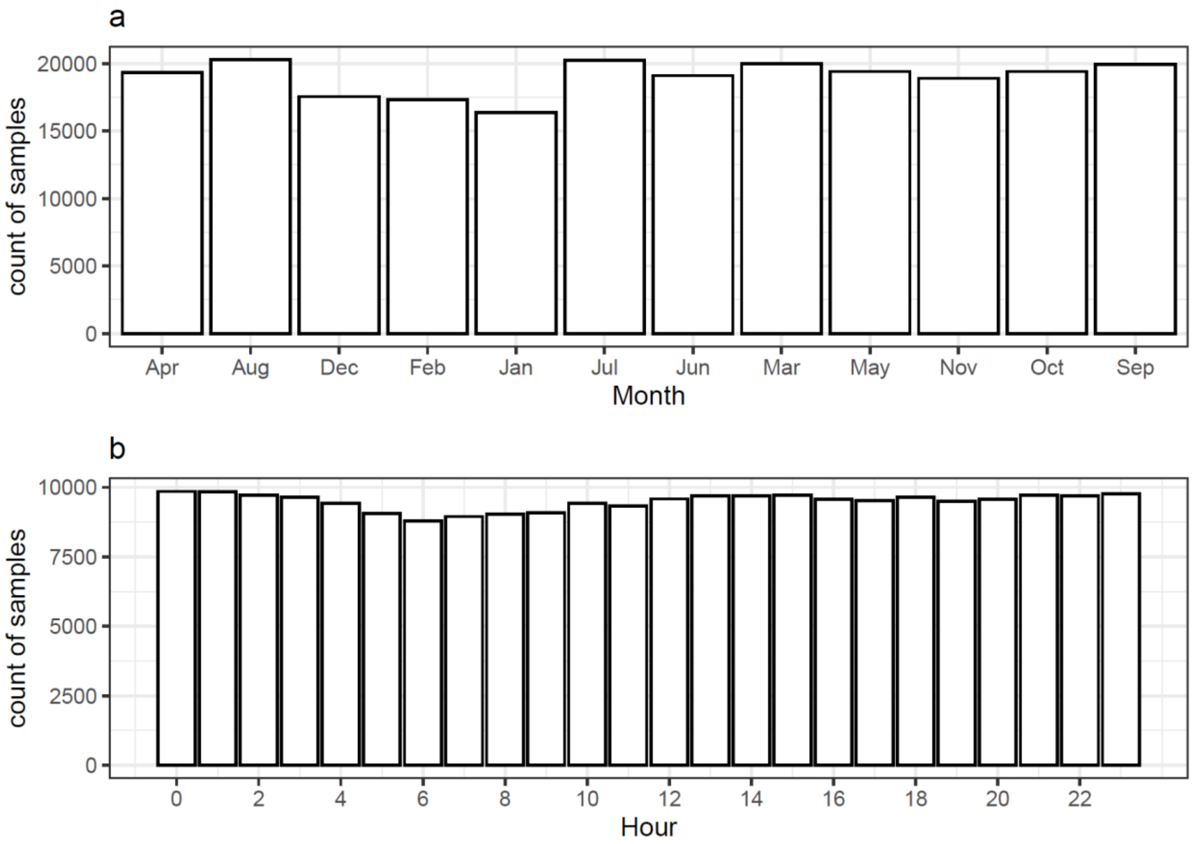


Figure S 4.1: Histograms showing the distribution of number of samples across months of the year (**a**) and across hours of the day (**b**)

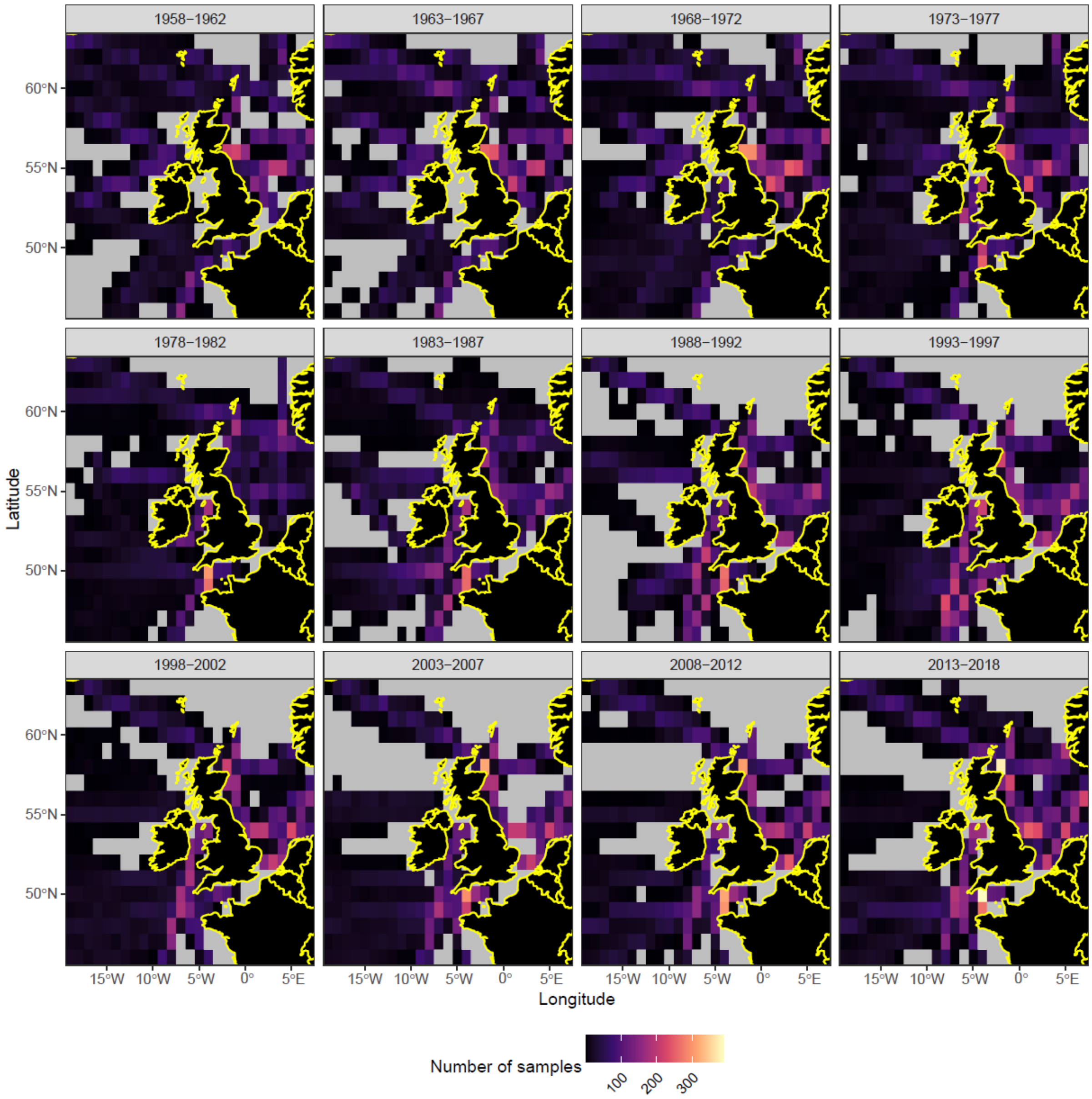


Figure S 4.2: Number of samples at each five-year period. Gray cells indicate no samples.

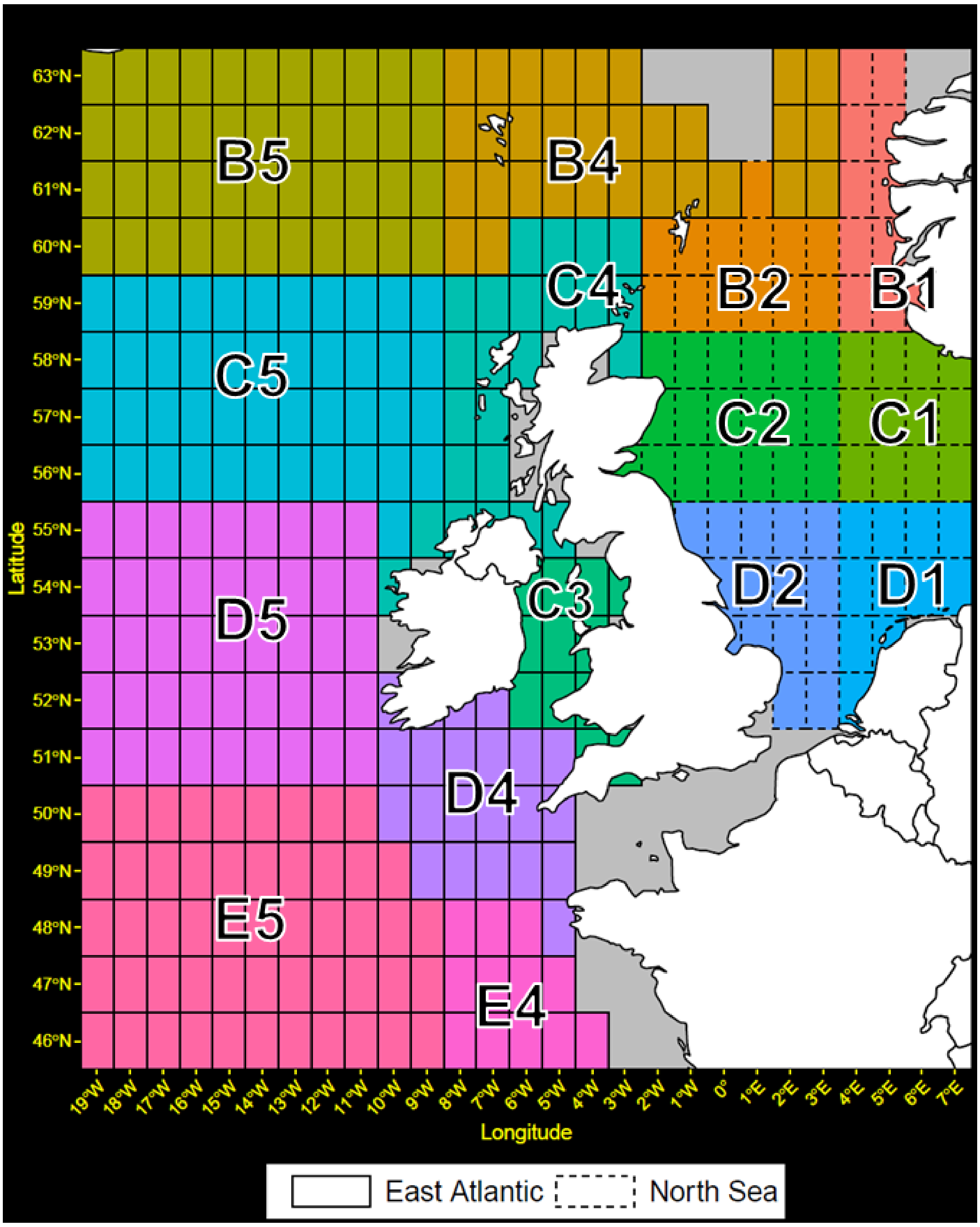


Figure S 4.3: Continuous plankton recorder standard areas. Straight and dashed lines indicate how I divided the North East Atlantic and the North Sea.

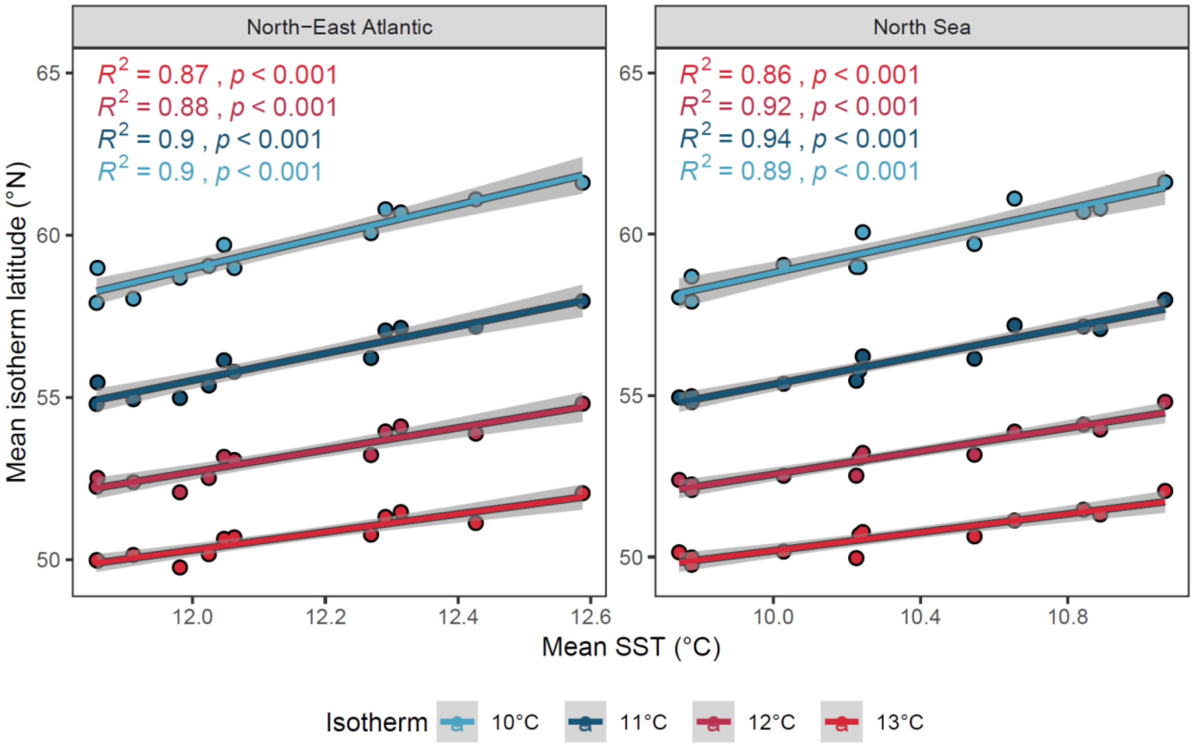


Figure S 4.4: Mean isotherm latitude against mean SST for both areas (points indicate each five-year period, lines indicate the linear regression fit model, R^2 and *p* values are reported on top-left of each panel for all regressions; colours refer to the four different isotherms considered as indicated by the legend at the bottom).

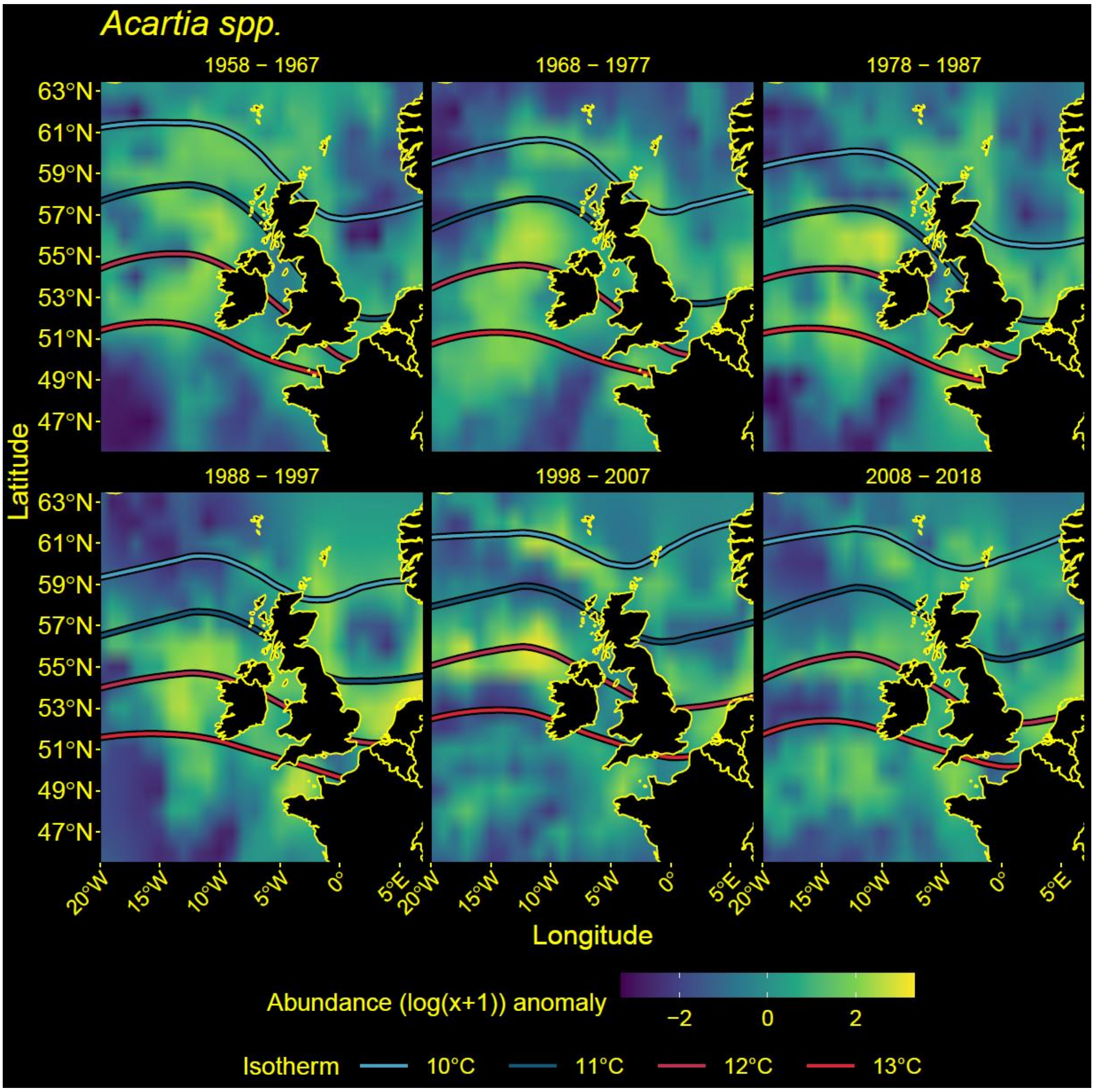


Figure S 4.5: Mean copepod abundance (log(x + 1)) of combined pairs of five-year periods. Values are reported as anomaly values (estimated value – mean value) calculated for each ten-year group, in order to show the relative distribution of each ten-year period and the comparison with the other ten-year periods. Lines indicate the four different isotherms, colour indexed as according to the legend at the bottom.

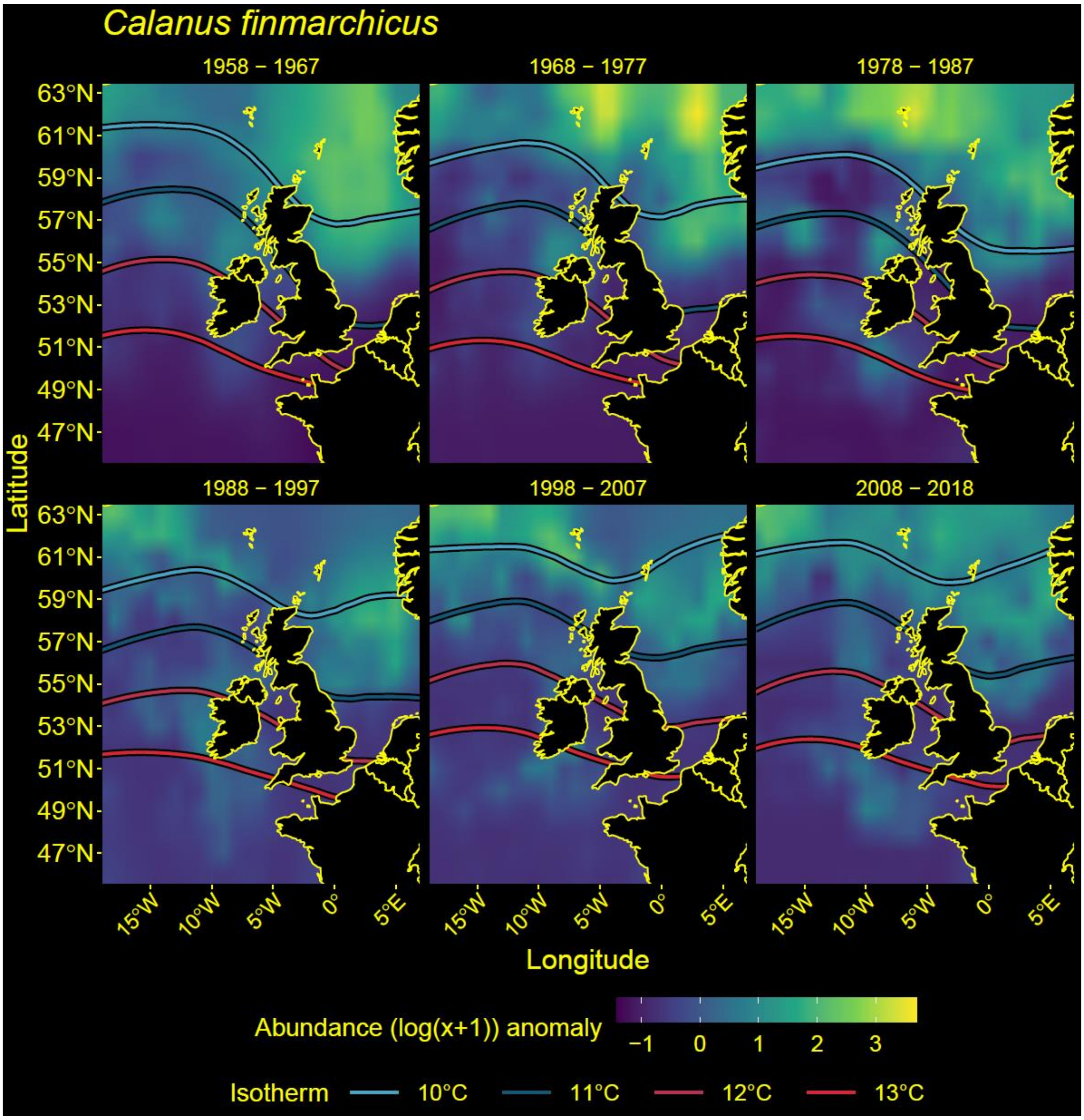


Figure S 4.6: Mean copepod abundance (log(x + 1)) of combined pairs of five-year periods. Values are reported as anomaly values (estimated value – mean value) calculated for each ten-year group, in order to show the relative distribution of each ten-year period and the comparison with the other ten-year periods. Lines indicate the four different isotherms, colour indexed as according to the legend at the bottom.

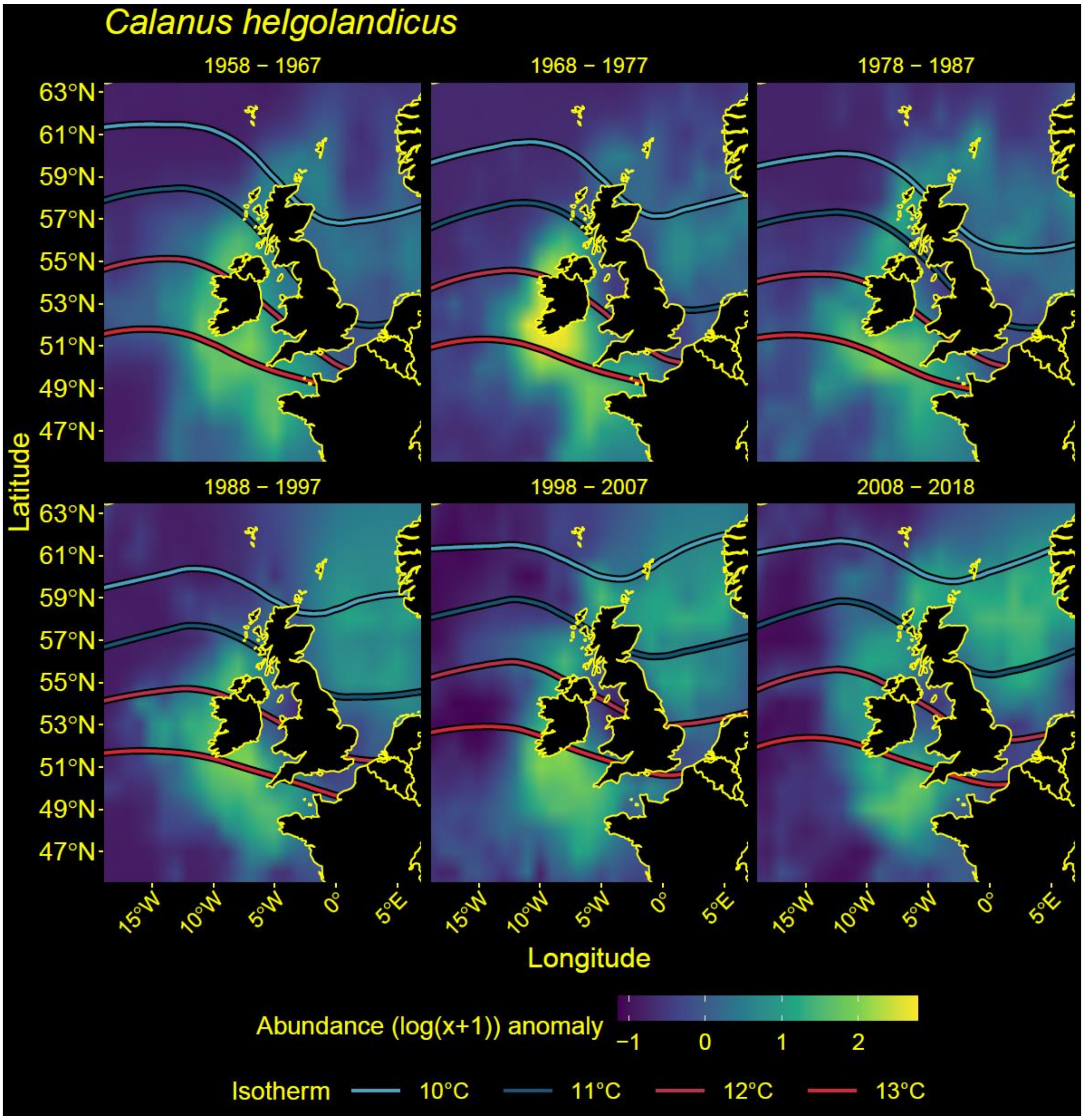


Figure S 4.7: Mean copepod abundance (log(x + 1)) of combined pairs of five-year periods. Values are reported as anomaly values (estimated value – mean value) calculated for each ten-year group, in order to show the relative distribution of each ten-year period and the comparison with the other ten-year periods. Lines indicate the four different isotherms, colour indexed as according to the legend at the bottom.

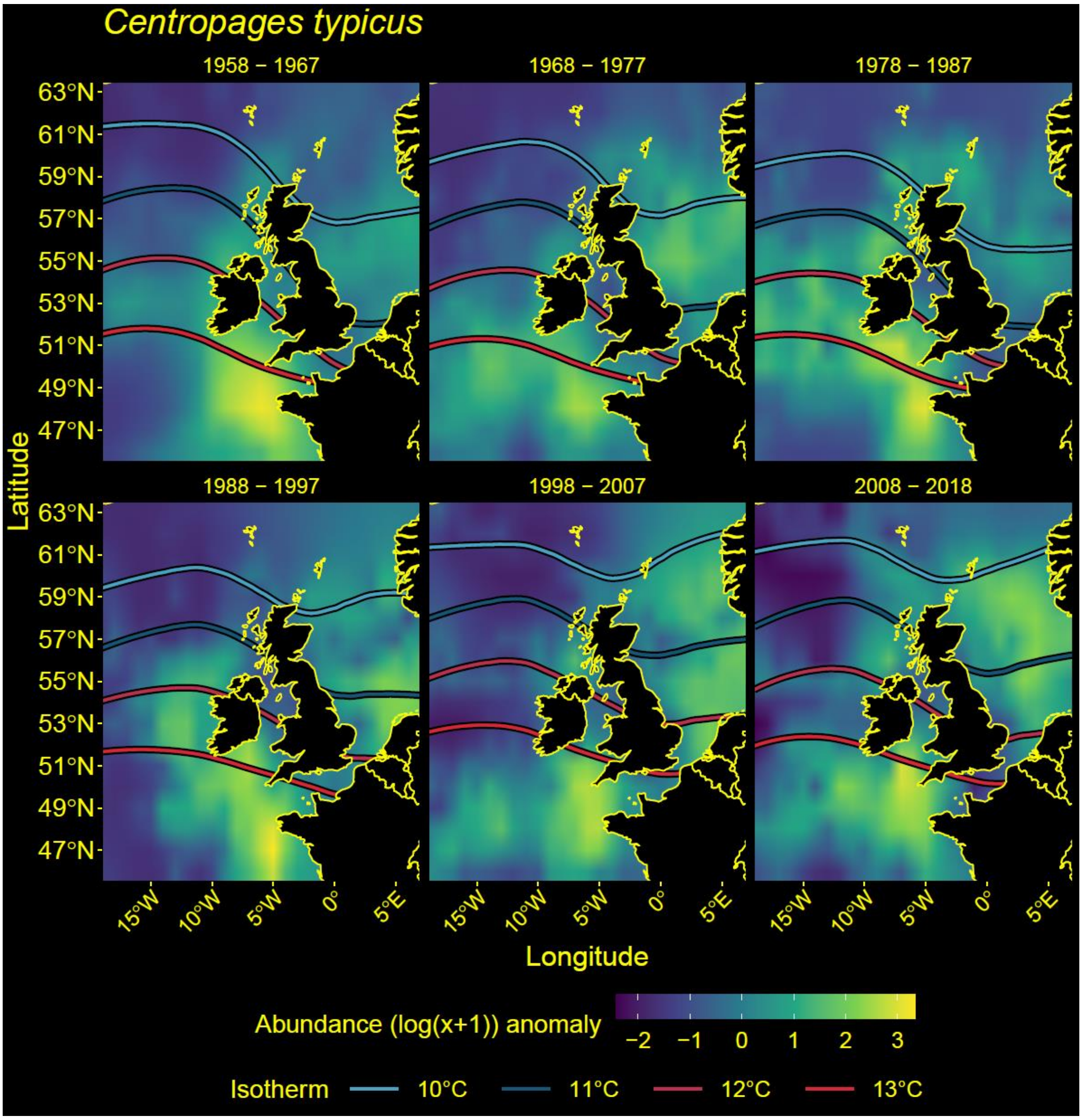


Figure S 4.2: Mean copepod abundance (log(x + 1)) of combined pairs of five-year periods. Values are reported as anomaly values (estimated value – mean value) calculated for each ten-year group, in order to show the relative distribution of each ten-year period and the comparison with the other ten-year periods. Lines indicate the four different isotherms, colour indexed as according to the legend at the bottom.

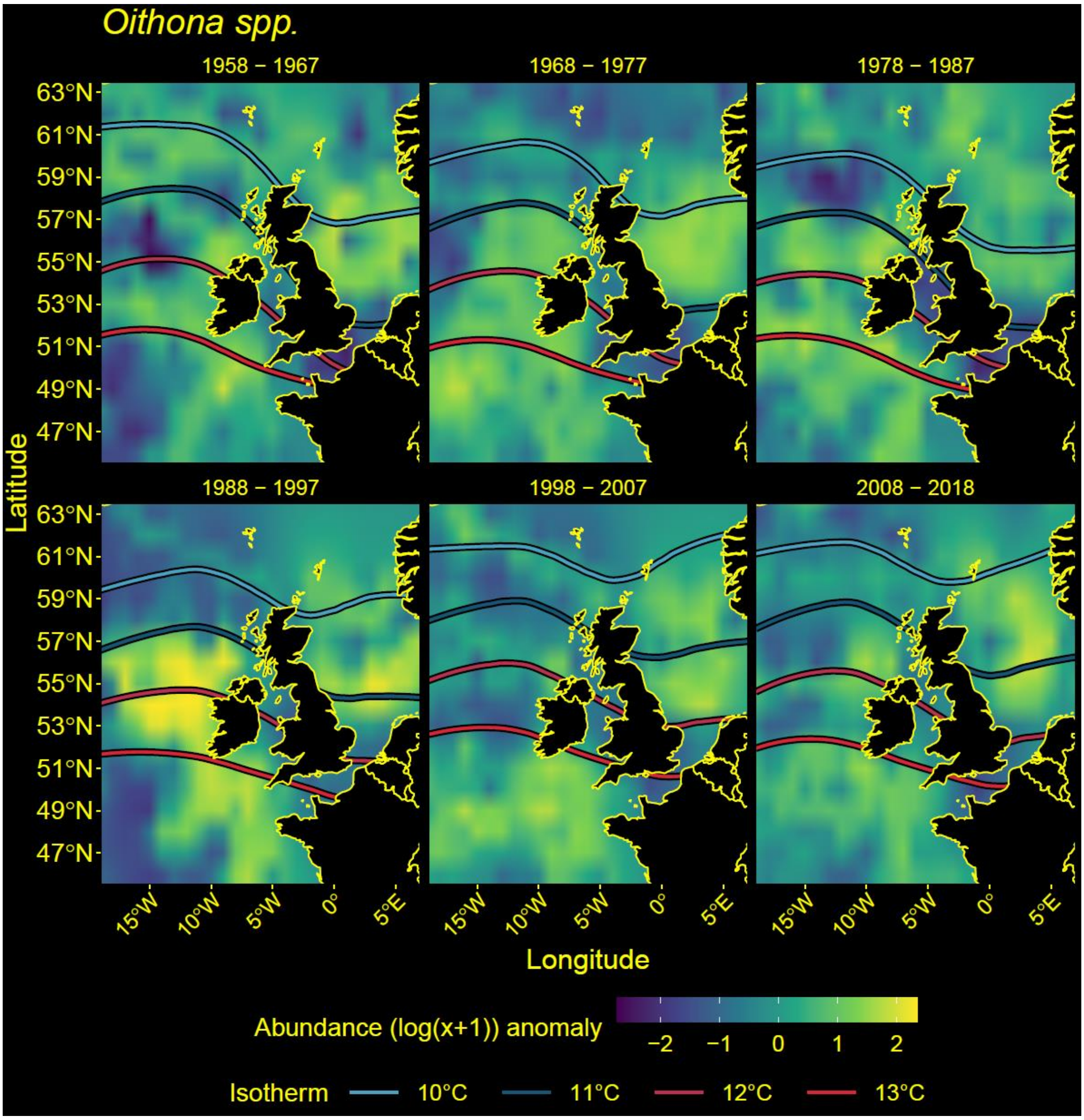


Figure S 4.9: Mean copepod abundance (log(x + 1)) of combined pairs of five-year periods. Values are reported as anomaly values (estimated value – mean value) calculated for each ten-year group, in order to show the relative distribution of each ten-year period and the comparison with the other ten-year periods. Lines indicate the four different isotherms, colour indexed as according to the legend at the bottom.

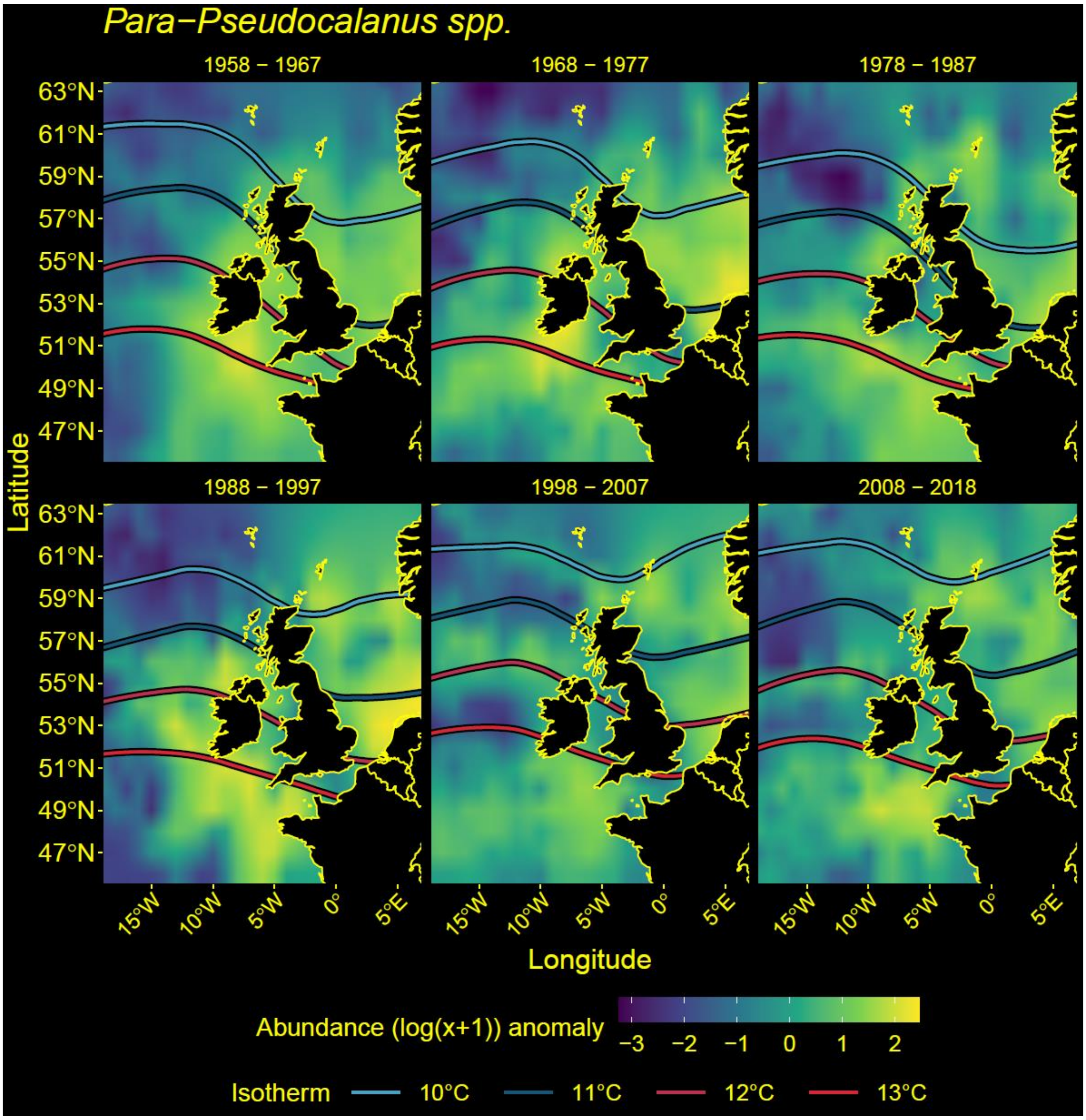


Figure S 4.10: Mean copepod abundance (log(x + 1)) of combined pairs of five-year periods. Values are reported as anomaly values (estimated value – mean value) calculated for each ten-year group, in order to show the relative distribution of each ten-year period and the comparison with the other ten-year periods. Lines indicate the four different isotherms, colour indexed as according to the legend at the bottom.

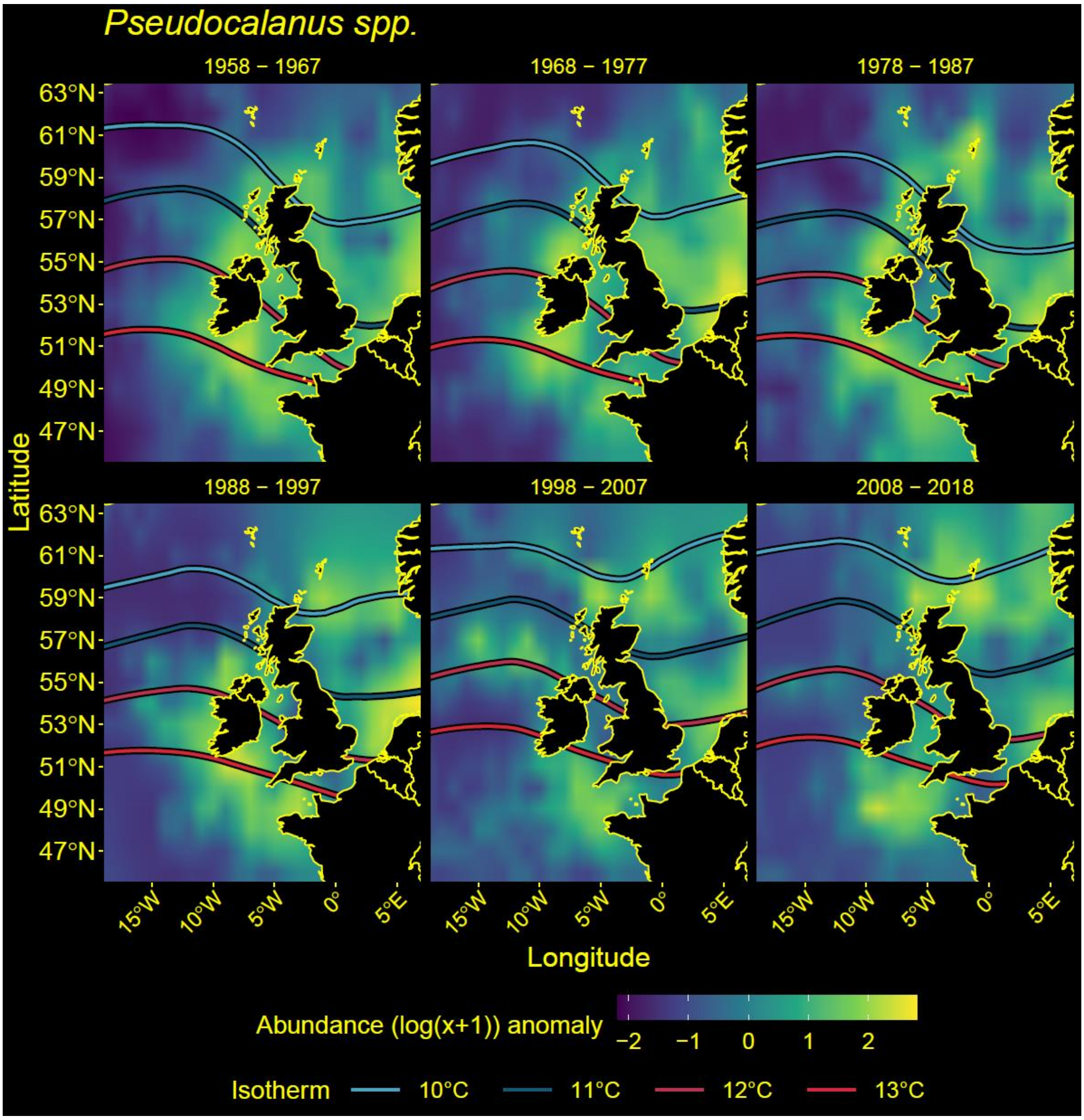


Figure S 4.11: Mean copepod abundance (log(x + 1)) of combined pairs of five-year periods. Values are reported as anomaly values (estimated value – mean value) calculated for each ten-year group, in order to show the relative distribution of each ten-year period and the comparison with the other ten-year periods. Lines indicate the four different isotherms, colour indexed as according to the legend at the bottom.

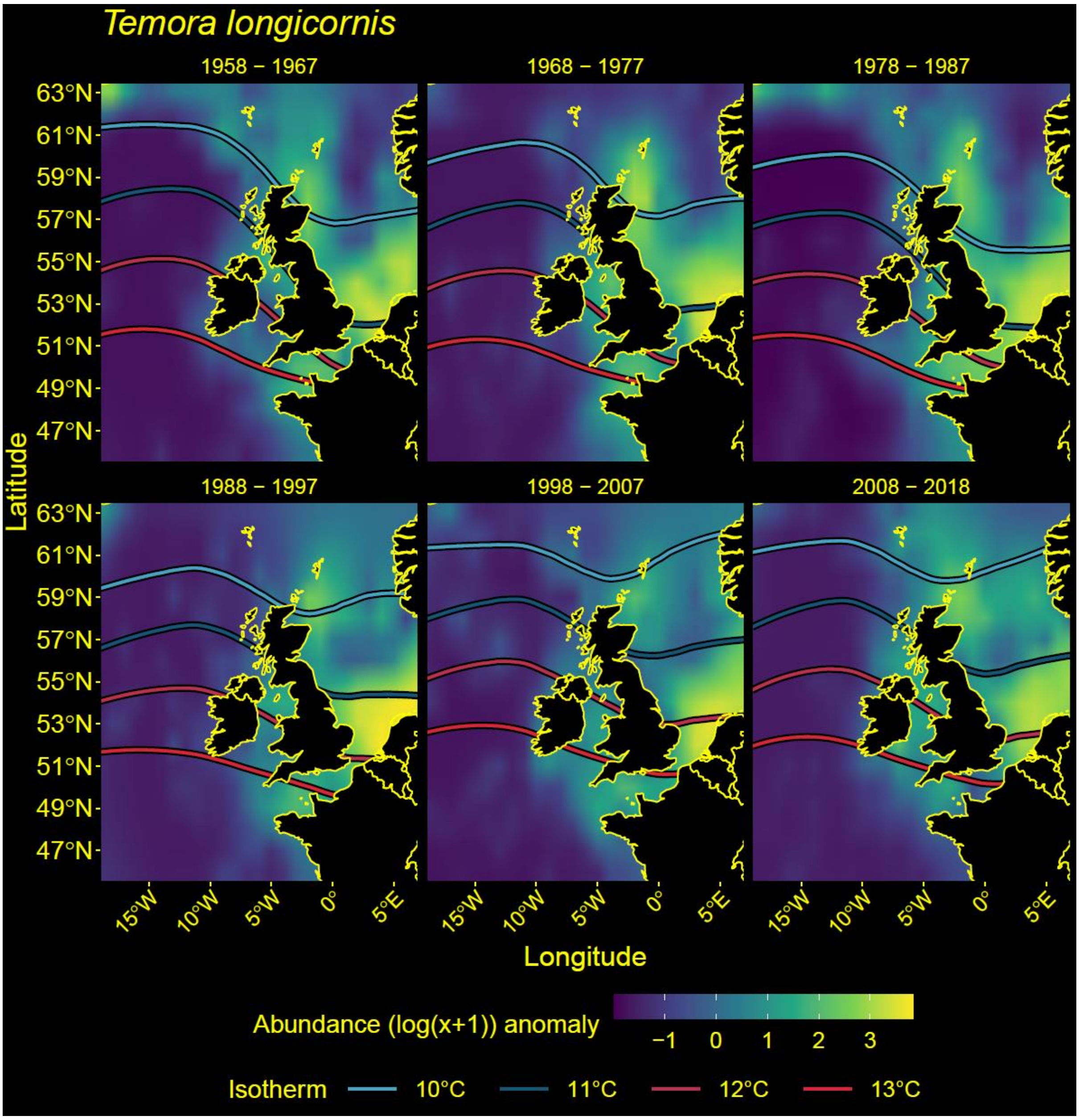


Figure S 4.12: Mean copepod abundance (log(x + 1)) of combined pairs of five-year periods. Values are reported as anomaly values (estimated value – mean value) calculated for each ten-year group, in order to show the relative distribution of each ten-year period and the comparison with the other ten-year periods. Lines indicate the four different isotherms, colour indexed as according to the legend at the bottom.

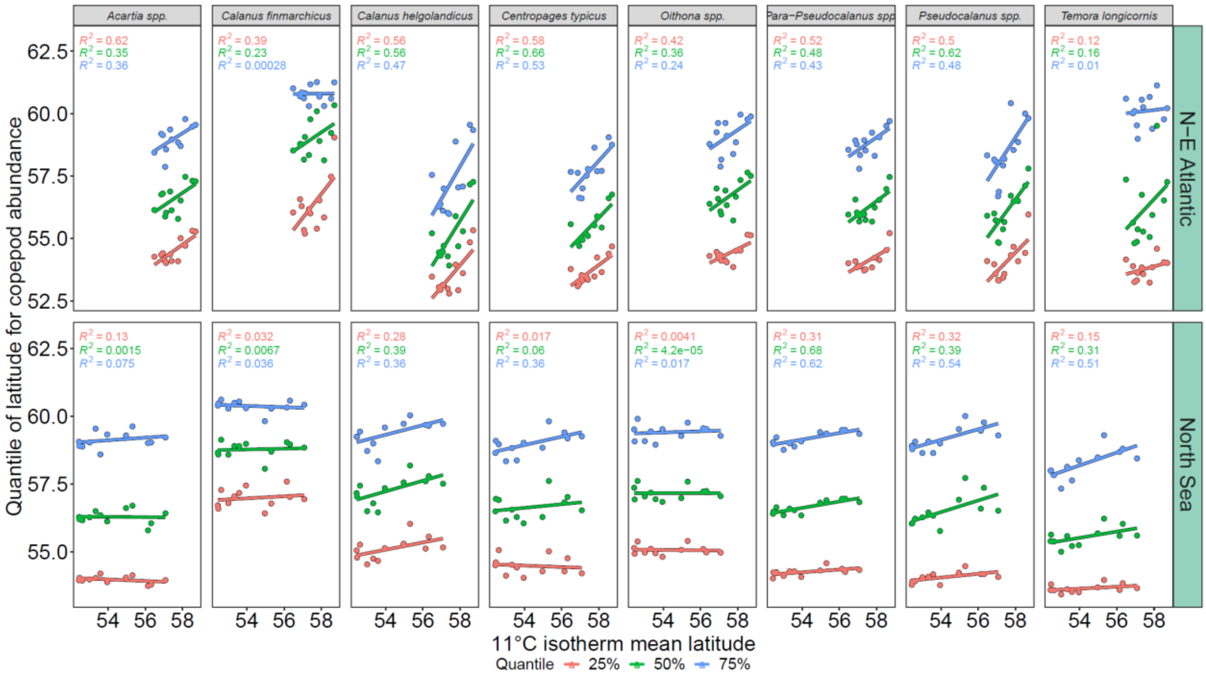


Figure S 4.3: Linear regressions between quantiles (25%, 50%, 75%) of latitude for copepod abundance and the mean latitude of the isotherm at 11°C. Positive regressions mean a northward expansion/shift with increasing isotherm latitude (i.e.: increasing temperature within that marine area). R² and *p* values are reported for each regression. Different colours refer to the three quantiles (25%, 50%, 75%) as indicated by the legend at the bottom.

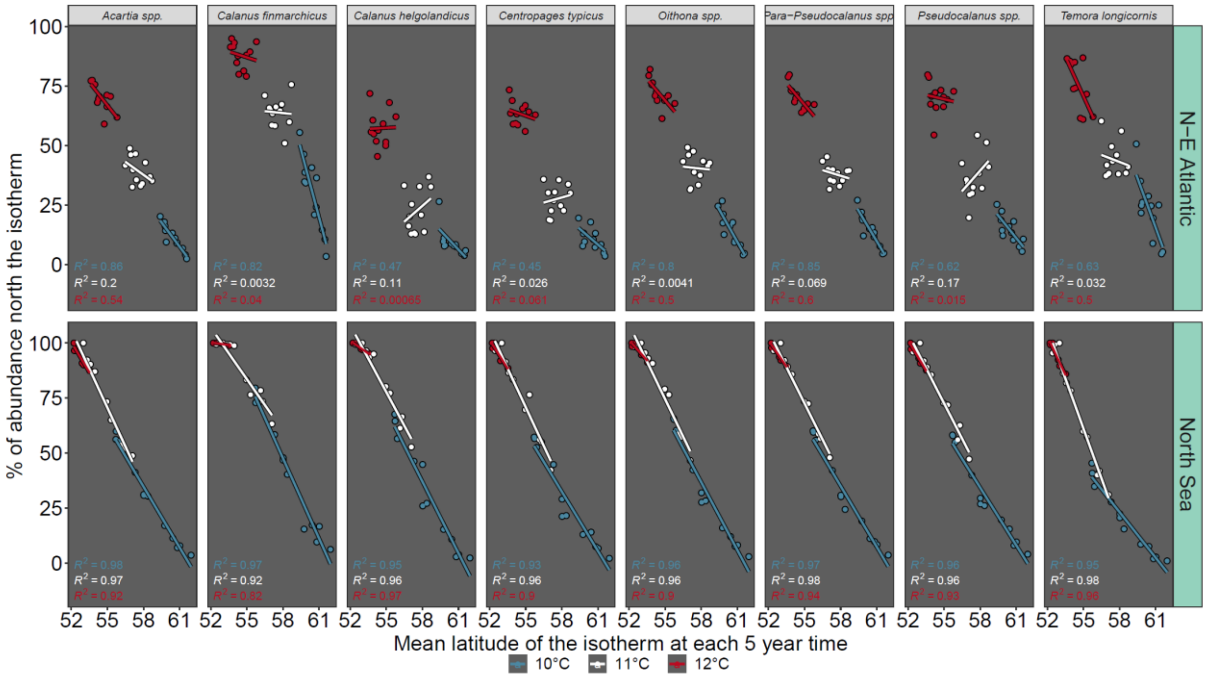


Figure S 4.4: Linear regressions between percentage of abundance north of each isotherm (10, 11, and 12 °C) and the mean latitude of each isotherm. R² values are reported for each regression. Different colours refer to the three isotherms (10, 11, 12 °C) as indicated by the legend at the bottom.

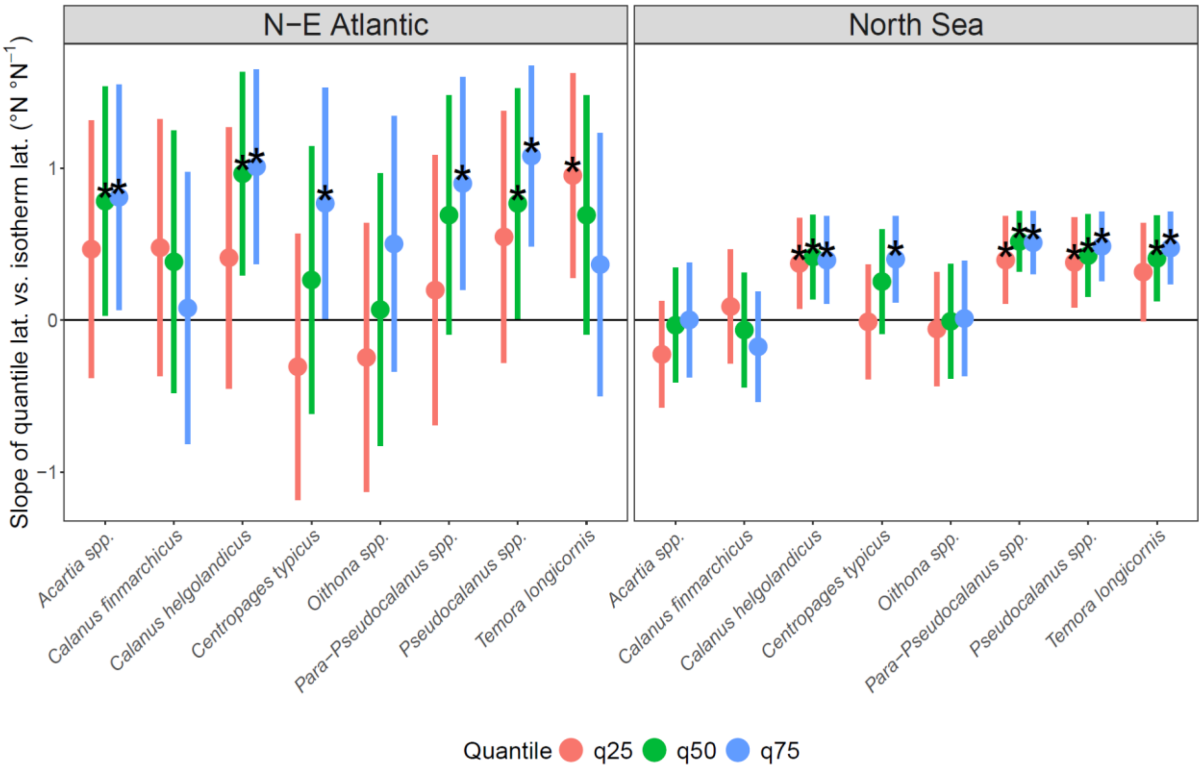
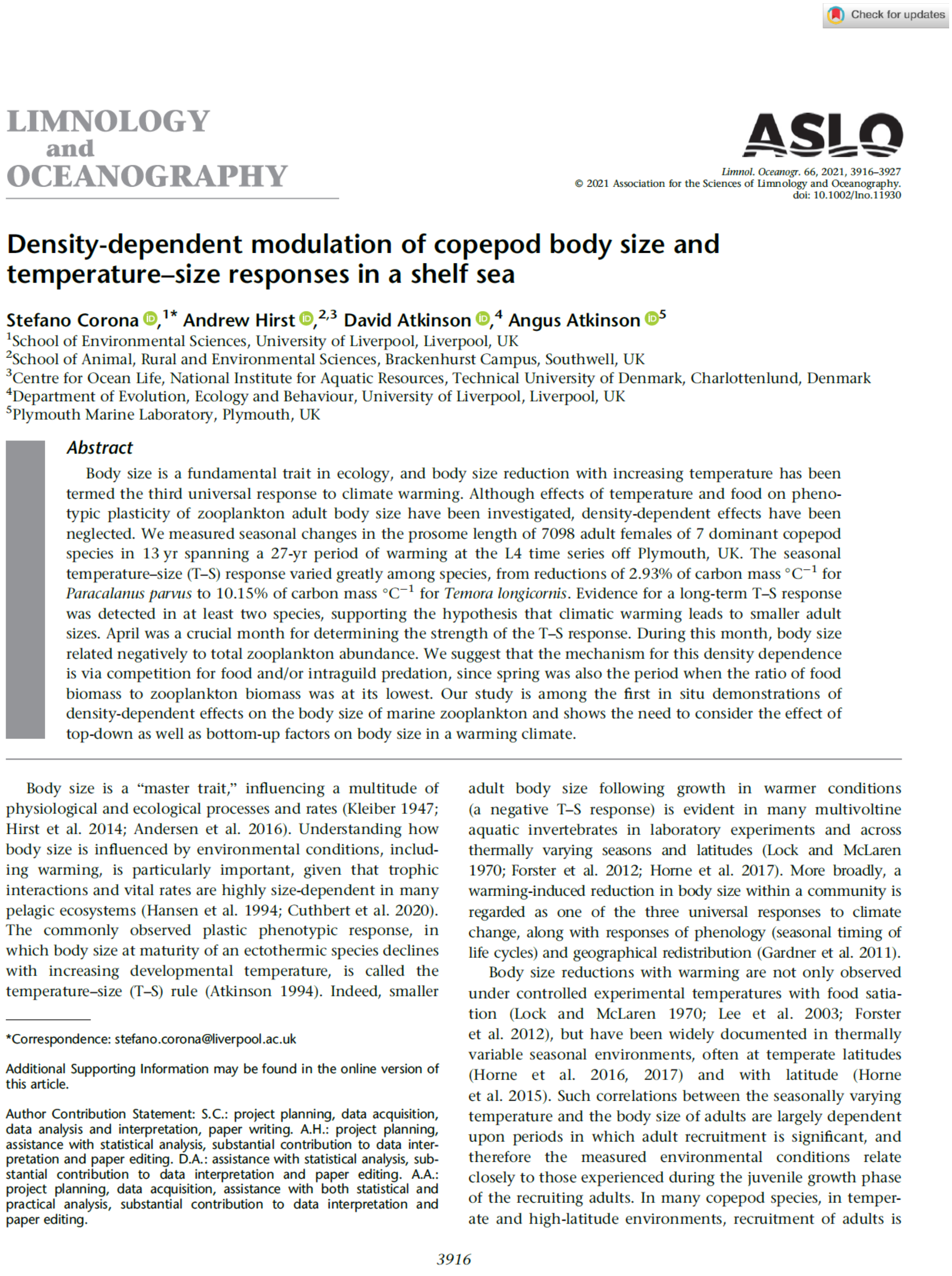


Figure S 4.5: Same plot as the one in Figure 4, with the only difference that here latitudes values have been normalized (z-scores) for each sea area, in order to account for the difference in sample area size between the NEA and the NS.

7 SUPPLEMENTARY APPENDIX

Front page of published paper: Corona, S., Hirst, A., Atkinson, D., Atkinson, A., 2021. Density-dependent modulation of copepod body size and temperature–size responses in a shelf sea. Limnology and Oceanography, **66**: 3916-3927. https://doi.org/10.1002/lno.11930



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Density-dependent modulation of copepod body size and temperature–size responses in a shelf sea

Stefano Corona,[1*] **Andrew Hirst**,[2,3] **David Atkinson**,[4] **Angus Atkinson**[5]
[1]School of Environmental Sciences, University of Liverpool, Liverpool, UK
[2]School of Animal, Rural and Environmental Sciences, Brackenhurst Campus, Southwell, UK
[3]Centre for Ocean Life, National Institute for Aquatic Resources, Technical University of Denmark, Charlottenlund, Denmark
[4]Department of Evolution, Ecology and Behaviour, University of Liverpool, Liverpool, UK
[5]Plymouth Marine Laboratory, Plymouth, UK

Abstract

Body size is a fundamental trait in ecology, and body size reduction with increasing temperature has been termed the third universal response to climate warming. Although effects of temperature and food on phenotypic plasticity of zooplankton adult body size have been investigated, density-dependent effects have been neglected. We measured seasonal changes in the prosome length of 7098 adult females of 7 dominant copepod species in 13 yr spanning a 27-yr period of warming at the L4 time series off Plymouth, UK. The seasonal temperature–size (T–S) response varied greatly among species, from reductions of 2.93% of carbon mass $°C^{-1}$ for *Paracalanus parvus* to 10.15% of carbon mass $°C^{-1}$ for *Temora longicornis*. Evidence for a long-term T–S response was detected in at least two species, supporting the hypothesis that climatic warming leads to smaller adult sizes. April was a crucial month for determining the strength of the T–S response. During this month, body size related negatively to total zooplankton abundance. We suggest that the mechanism for this density dependence is via competition for food and/or intraguild predation, since spring was also the period when the ratio of food biomass to zooplankton biomass was at its lowest. Our study is among the first in situ demonstrations of density-dependent effects on the body size of marine zooplankton and shows the need to consider the effect of top-down as well as bottom-up factors on body size in a warming climate.

Body size is a “master trait,” influencing a multitude of physiological and ecological processes and rates (Kleiber 1947; Hirst et al. 2014; Andersen et al. 2016). Understanding how body size is influenced by environmental conditions, including warming, is particularly important, given that trophic interactions and vital rates are highly size-dependent in many pelagic ecosystems (Hansen et al. 1994; Cuthbert et al. 2020). The commonly observed plastic phenotypic response, in which body size at maturity of an ectothermic species declines with increasing developmental temperature, is called the temperature–size (T–S) rule (Atkinson 1994). Indeed, smaller adult body size following growth in warmer conditions (a negative T–S response) is evident in many multivoltine aquatic invertebrates in laboratory experiments and across thermally varying seasons and latitudes (Lock and McLaren 1970; Forster et al. 2012; Horne et al. 2017). More broadly, a warming-induced reduction in body size within a community is regarded as one of the three universal responses to climate change, along with responses of phenology (seasonal timing of life cycles) and geographical redistribution (Gardner et al. 2011).

Body size reductions with warming are not only observed under controlled experimental temperatures with food satiation (Lock and McLaren 1970; Lee et al. 2003; Forster et al. 2012), but have been widely documented in thermally variable seasonal environments, often at temperate latitudes (Horne et al. 2016, 2017) and with latitude (Horne et al. 2015). Such correlations between the seasonally varying temperature and the body size of adults are largely dependent upon periods in which adult recruitment is significant, and therefore the measured environmental conditions relate closely to those experienced during the juvenile growth phase of the recruiting adults. In many copepod species, in temperate and high-latitude environments, recruitment of adults is

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*Correspondence: stefano.corona@liverpool.ac.uk

Additional Supporting Information may be found in the online version of this article.

Author Contribution Statement: S.C.: project planning, data acquisition, data analysis and interpretation, paper writing. A.H.: project planning, assistance with statistical analysis, substantial contribution to data interpretation and paper editing. D.A.: assistance with statistical analysis, substantial contribution to data interpretation and paper editing. A.A.: project planning, data acquisition, assistance with both statistical and practical analysis, substantial contribution to data interpretation and paper editing.

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