Calanus helgolandicus in the western English Channel: population dynamics and the role of mortality

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by

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Statement of Originality

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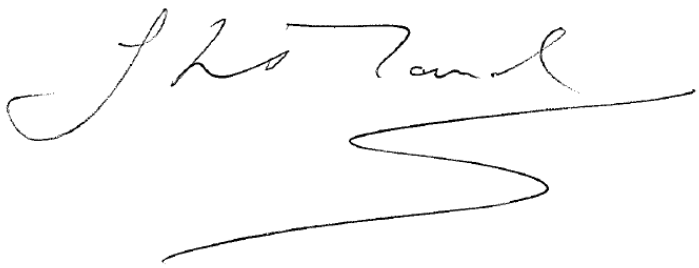

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Collaborations:

All chapters: L4 mesozooplankton and microplankton weekly time series sampling and identification were undertaken by Plymouth Marine Laboratory (PML) technicians and plankton analysts. Weekly egg production experiments were collected and analysed by Andrea McEvoy, who also made the ongoing time series data since 1992 available. Routine CTD and water sampling data (temperature, salinity, O_2, turbidity, chlorophyll-*a*) were produced by PML scientists.

Chapter Two: Egg hatch success and abnormal nauplii data were collected throughout 2013 by J. L. Maud (JLM). All data analysis was performed by JLM.

Chapter Three: *C. helgolandicus* egg hatch success and naupliar abnormalities data were collected by J. L. Maud during 2013 and 2016; 2015 data collected by N. Djeghri, PML (ND). *C. helgolandicus* eggs were collected for fatty acid analysis by JLM; chemical fatty acid analysis undertaken by D. White, PML (DW). Mesozooplankton biomass conversions were performed by A. Atkinson, PML (AA) and M. Lilley, QMUL (ML). Mortality rate estimations and all data analyses were performed by JLM.

Chapter Four: *C. helgolandicus* stage composition 2002-2004 was undertaken by D. Bonnet and A. G. Hirst, QMUL (AGH); 2012-2013 stage composition was undertaken by JLM. The collection and neutral red staining of mesozooplankton, sample processing and identification was undertaken by JLM. Mesozooplankton biomass conversions were performed by AA and ML. Young fish trawl abundance data from L4 were provided by N. Halliday, MBA (NH). Mortality rate estimations and all data analyses were performed by JLM.

Chapter Five: Gelatinous zooplankton collection, DNA extraction, PCR amplification and visualisation using gel electrophoresis gel and cleaning of PCR products was undertaken by JLM. Sequencing data processing was undertaken by MR DNA. Bioinformatic processing and species analysis was performed by JLM.

Publications:

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

Maud, J. L., A. G. Hirst, A. Atkinson, P. K. Lindeque and A. J. McEvoy. (In review). Mortality of *Calanus helgolandicus*: sources, differences between the sexes and consumptive and non-consumptive processes. Limnology and Oceanography (Chapter Four).

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

White, D. A., C. E. Widdicombe, P. J. Somerfield, R. L. Airs, G. A. Tarran, J. L. Maud and A. Atkinson. 2015. The combined effects of seasonal community succession and adaptive algal physiology on lipid profiles of coastal phytoplankton in the Western English Channel. Marine Chemistry 177, Part 4: 638-652 (Chapter Three).

ABSTRACT

Calanus helgolandicus is a key copepod species occurring in the North East Atlantic that is responding to oceanic warming through an expansion of its geographic range. This range extension has led to concerns about how this may affect ecosystem trophodynamics. Here I investigate the interannual variability and seasonality of *C. helgolandicus*, using a ~28 year time-series from the western English Channel (station L4). I focus specifically on the role of mortality, as a key life history process that is challenging to quantify and historically has received little attention. *C. helgolandicus* abundance remained within a narrow ~four-fold interannual envelope, which was a consequence of multiple losses that removed ~99% of the potential population. Loss of early life stages occurred through the incidence of non-viable eggs and abnormal nauplii (both higher in spring), and via predation; egg mortality rates were positively correlated with *C. helgolandicus* copepodite abundance and total copepod biomass, indicative of intraguild predation and cannibalism. By contrast, late-stage copepodite mortality rates were highest in autumn, and were positively related to gelatinous predator abundance and biomass (medusae, ctenophores and chaetognaths). Molecular gut-content analyses revealed that two abundant jellyfish species present during 2015 (*Pleurobrachia pileus* and *Leuckartiara octona*) both preyed on *C. helgolandicus*. Adult male consumptive mortality rates were ~6 times higher than that of adult females; whereas male non-consumptive rates were only ~1.5 times that of females, providing evidence that predation was the primary mortality source in males. Non-consumptive mortality rates contributed 0-54% (median of 4.5%) to total mortality and were positively related to the 72-hour maximum wind speed, implying that turbulence created during extreme weather events may increase zooplankton mortality. I conclude that *C. helgolandicus* population control is modulated via a series of mortality-related losses occurring through the different development stages; from reduced egg viability to predation of copepodites by gelatinous carnivores. Although I find little evidence for changing ecosystem trophodynamics at L4, my results contribute to the knowledge of *C. helgolandicus* population dynamics at a site near the centre of its distribution, and suggest that a future expanding population may be a valuable food source for a variety of predators.

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CHAPTER ONE

General Introduction

Zooplanktonic organisms play a pivotal role in the functioning of the world's oceans, mostly due to their abundance, diversity and wide variety of ecosystem functions (e.g. the transfer of energy from primary production to higher trophic levels and in mediating biogeochemical cycles) (Kaiser et al., 2005). They also represent the first level of integration of the influence of the climate (hydroclimatic forcing) on the pelagic foodweb (Beaugrand, 2005). Zooplankton species are also particularly sensitive to, and so indicative of, hydro-meteorological changes in the ocean environment, and may be particularly useful indicators of climate change (Richardson, 2008). Reasons for this are threefold; firstly most species have a short generation time and so population size can respond very rapidly to environmental conditions; secondly, they are free drifting and sensitive to changes in the ocean environment which can be measured; and thirdly, very few species are commercially exploited, so fishing-related population modification does not occur (Hays et al., 2005), although such impacts may occur indirectly.

The most abundant and biomass dominant mesozooplankton in the oceans are the Copepoda (Milne-Edwards, 1840). Indeed, these animals are considered the most numerous multicellular organisms on the planet (Humes, 1994). Copepods in particular are of global importance due to their role as primary consumers of phytoplankton and their tremendous reproductive capacity, and therefore are pivotal organisms in the transfer of energy from primary production to higher trophic levels (Karleskint et al., 2006). They are also very sensitive to changes in temperature and display interannual changes in numerical density, which often reflect a response to climatic forcing (Hays et al. 2005). Because of this, their relative ubiquity and their numerical dominance in the plankton (Longhurst, 1985), they are also one of the most frequently studied of the zooplankton taxa.

It is now widely recognised that there was a period of hydroclimatic change during the last half of the 20th century, , manifested in markedly increasing sea surface temperatures, which dramatically altered planktonic ecosystems in the Northeast

Atlantic (Beaugrand, 2004; Philippart et al., 2011). This ocean warming has continued and in only 40 years there has been a substantial shift in plankton and fish communities that may have far-reaching consequences (Beaugrand et al., 2009). The Intergovernmental Panel on Climate Change (IPCC) climate projections estimate a mean increase of 0.6°C (RCP2.6) to 2.0°C (RCP8.5) in the top 100 m of the ocean [based on Representative Concentration Pathway (RCP) radiative forcing level scenarios, with RCP2.6 referring to peak mitigation and lowest emissions and RCP8.5, a very high baseline emission scenario], by the end of the 21st century (IPCC, 2013). Around the UK, the greatest increase in sea surface temperatures in the past 25 years has occurred in the eastern English Channel and southern North Sea, with a temperature increase of 0.6° to 0.8°C per decade (MCCIP, 2010).

Much of the research on the effects of the recent environmental change on zooplankton populations in the North Atlantic has focused on copepod assemblages and biodiversity. Beaugrand et al. (2002) provide evidence of a reorganisation of calanoid copepod diversity at the basin scale between 1960 and 1999, where there has been a significant northward movement (10°C of latitude) of warm species, coupled with a decrease in the number of sub-arctic and arctic species in the north. This phenomenon has been related to the increasing trend in Northern Hemisphere temperature (NHT) anomalies. A subsequent study analysed an extended period of data (1958-2005) and concluded that the biodiversity of calanoid copepod assemblages were responding quickly to increasing temperatures and that warm-temperate copepod species were moving northward at a rate of 23.16 km yr^{-1} (Beaugrand et al., 2009).

Calanus helgolandicus () is a key calanoid copepod (Figure 1.1), that is mostly located over the European shelf-edge and in the eastern Atlantic between 40° and 60°N (Helaouët and Beaugrand, 2007). It is a warm, temperate species and typically occupies temperatures between 9^{o} and 20^{o}C (Bonnet et al., 2005). It is an important contributor to the zooplankton biomass in these regions and can contribute 6-93% to the mesozooplankton biomass (Bonnet et al., 2005). It is therefore considered an important prey source for commercially important fish species (Heath, 2007) and indeed Mauchline (1998) classifies *C. helgolandicus* as one of a set of “professional prey species”, whereby the organism functions as a major food source for other

animals and, therefore, must have a reproductive response to match their exploitation patterns in order to maintain the population.

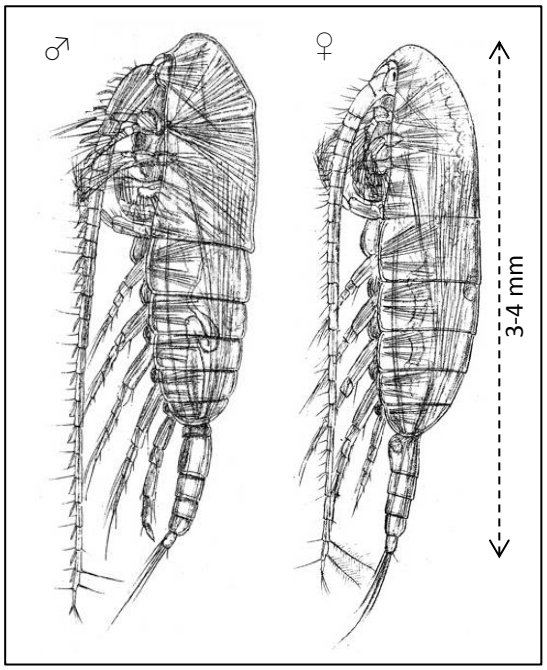

Figure 1.1. *Calanus helgolandicus* morphology, ♂ and ♀ adults. Reproduced from Sars (1903).

The congener *Calanus finmarchicus* (Gunnerus, 1770) by contrast is an indicator sub-arctic species, which is located mostly in the Atlantic Polar Front (Helaouët and Beaugrand, 2007). Its southern-most distribution is approximately 55°N in the North Sea and 50°N in the open ocean (Planque and Fromentin, 1996). There are however, geographic areas of overlap between the two species, for example in the North Sea, the Irish Sea and the Celtic Sea, but it is known that the species occupy distinct thermal niches, have different phenologies and exist at different depths (Williams and Conway, 1984). Together *C. helgolandicus* and *C. finmarchicus* can contribute > 90% to the mesozooplankton biomass of regions such as the North Sea and the Celtic Sea (Bonnet et al., 2005).

Commensurate with the warming of the north Atlantic area, there has been an expanding distributional range of the temperate *C. helgolandicus* coupled with a northward movement of the sub-arctic *C. finmarchicus* (Chust et al., 2013). Therefore, there has been a partial substitution of *C. finmarchicus* by *C. helgolandicus* in some

areas around northern UK waters and the North Sea (Figure 1.2). It is suggested that this may result in long-term changes in predator populations, notably commercial fish species (Barange and Harris, 2003). For example, cod (*Gadus morhua)* recruitment in the North Sea, has been affected by the changing of the timing of the arrival of suitably sized copepod larval stages, due to the different phenology and population dynamics of *C. helgolandicus* compared with *C. finmarchicus* (Beaugrand et al., 2003). Consequently it has become imperative to understand the underlying ecology and population dynamics of this species, to be able to predict the impact of future climate fluctuations.

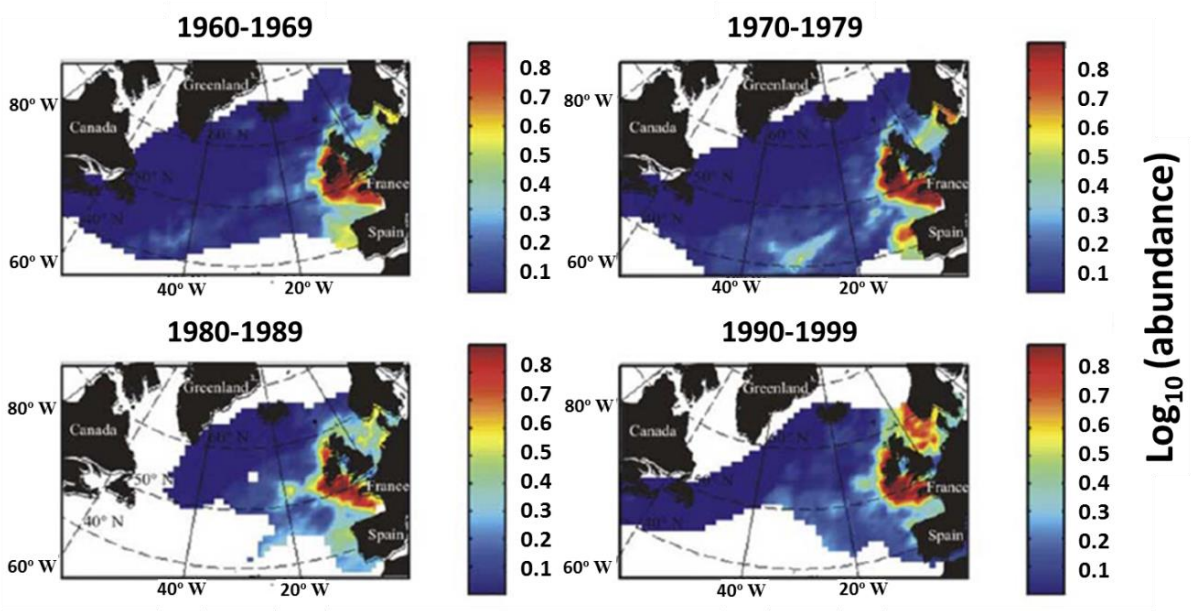

Figure 1.2. Map showing the changing distributions of *Calanus helgolandicus* in the N Atlantic (1960-1999) (reproduced from Bonnet et al., 2005).

Research activity on *C. helgolandicus* has increased substantially over recent decades, but is still a long way from matching that of *C. finmarchicus*, which is the most studied of all copepod species (Helaouët et al., 2011). Investigations of *C. helgolandicus* distribution, biology and ecology, including factors contributing to population gains (i.e. reproduction rates, feeding, growth rates) are numerous (Chapter Three). However, gaps in our knowledge remain, particularly regarding the processes leading to population losses and the causes of mortality (i.e. predation,

starvation, food-limitation, non-viable eggs and naupliar deformities) (Bonnet et al., 2005). Therefore this thesis focuses on understanding the key processes in *C. helgolandicus* population dynamics (Chapter Two), with a special focus on elucidating the importance of mortality,and to investigate mortality rates and the major sources influencing it (Chapters Three to Five).

Predation-related mortality has historically received most attention (Chapter Four), and potential predators include the larvae of commercially important fish species (Daewel et al., 2014) as well as a range of medusae, chaetognaths, siphonophores, euphausiids and carnivorous copepods (Hirota, 1974; Purcell, 1982; Bagøien et al., 2000; Bonnet et al., 2004; Bonnet et al., 2010). However, there has been a more recent move towards understanding non-consumptive mortality and various sources for it including parasites, environmental stress, starvation, disease and injuries have been highlighted (see Elliott and Tang, 2009 and references therein). Efforts to distinguish between living copepods and dead carcasses in the water column have also grown, as it is recognised that this can provide information on the relative importance of consumptive and non-consumptive mortality, and if non-consumptive mortality is not quantified appropriately, this may lead to overestimates of zooplankton population recruitment and secondary recruitment (Tang et al., 2006).

The study area for this thesis is the monitoring station L4, located in the western English Channel, where sampling has been done sporadically since 1888 (Southward et al., 2004). Station L4 is a well-established coastal monitoring station (50°15.00'N, 4°13.02'W) (Figure 1.3), situated in seasonally stratified waters, approximately 10km south of Plymouth Breakwater with a depth of ~55m (Harris, 2010). A weekly zooplankton sampling regime was established in 1988, with phytoplankton added in 1992. A suite of physico-chemical measurements is taken concurrently with the plankton sampling (i.e. temperature, salinity, turbidity, oxygen, pigments, DNA, etc), and weekly CTD (conductivity/temperature/depth profiler) hauls were introduced in 2001.

This site offers several advantages for understanding the response of C. helgolandicus to environmental variability. First, the site is inshore and shows inter-annual variation in temperature, stratification, food quantity, quality and timing (Eloire

et al., 2010; Smyth et al., 2010; Widdicombe et al., 2010; Atkinson et al., 2015). Second, sampling has been ongoing on a weekly basis since March 1988 and has produced a valuable ~28 year time series. Third, *C. helgolandicus* egg production measurements have been made in a standardised manner on a weekly basis since 1992. Finally, there is a background of knowledge on *C. helgolandicus* at the site; Table 1.1 lists studies that have focused primarily on *C. helgolandicus* collected from station L4 and it is evident that most of the research effort occurred some 10-20 years ago.

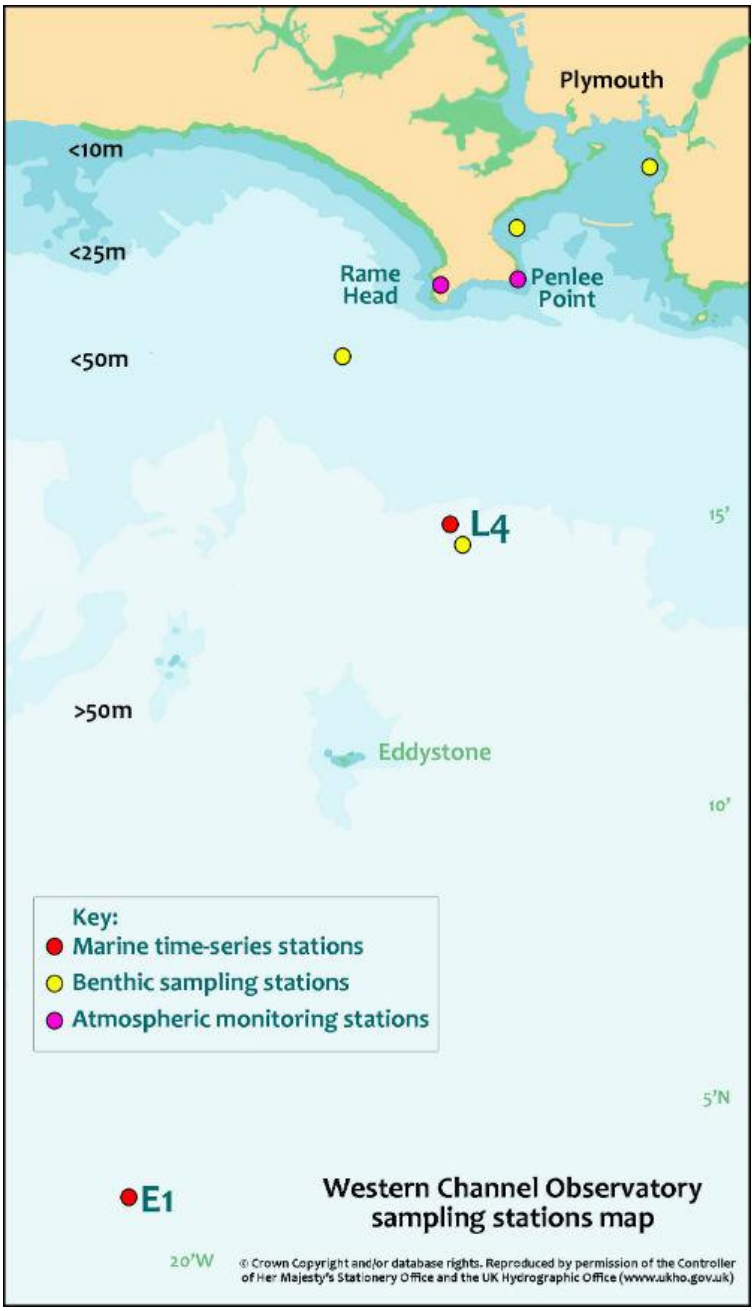

Figure 1.3. Map showing the position of station L4 in the western English Channel (Source: www.westernchannelobservatory.org.uk).

Table 1.1. Published studies of *Calanus helgolandicus* at station L4.

Author	Title	Process
Møller et al. 2012	The effect of changes in temperature and food on the development of *Calanus finmarchicus* and *Calanus helgolandicus* populations	Growth, development
Eloire et al. 2010	Temporal variability and community composition of zooplankton at station L4 in the Western Channel: 20 years of sampling	Various
Bonnet et al. 2010	*Sagitta setosa* predation on *Calanus helgolandicus* in the English Channel	Mortality
Fileman et al. 2010	Grazing by the copepods *Calanus helgolandicus* and *Acartia clausi* on the protozooplankton community at station L4 in the Western English Channel	Feeding
Bonnet et al. 2009	Temperature effects on *Calanus helgolandicus* (Copepoda: Calanoida) development time and egg production	Development, reproduction
Hirst et al. 2007	Seasonal dynamics and mortality rates of *Calanus helgolandicus* over two years at a station in the English Channel	Variability, mortality
Poulet et al. 2006	Influence of diatoms on copepod reproduction. I. Field and laboratory observations related to *Calanus helgolandicus* egg production	Reproduction
Bonnet et al. 2005	An overview of *Calanus helgolandicus* ecology in European waters.	Various
Yebra et al. 2005	Comparison of five methods for estimating growth of *Calanus helgolandicus* later developmental stages (CV–CVI)	Growth
Bonnet et al. 2004	*Calanus* the cannibal	Feeding, mortality
Rey-Rassat et al. 2004a	Secondary production of *Calanus helgolandicus* in the Western English Channel	Abundance, reproduction
Rey-Rassat et al. 2004b	Is weight an important parameter when measuring copepod growth?	Growth
Irigoien and Harris, 2003	Interannual variability of *Calanus helgolandicus* in the English Channel	Variability, reproduction, mortality
Irigoien et al. 2002	Copepod hatching success in marine ecosystems with high diatom concentrations	Reproduction, mortality
Rey-Rassat et al. 2002a	Growth and development of *Calanus helgolandicus* reared in the laboratory	Growth
Rey-Rassat et al. 2002b	Egg production rates of *Calanus helgolandicus* females reared in the laboratory: variability due to present and past feeding conditions	Reproduction
Rey et al. 2001	Influence of algal diet on growth and ingestion of *Calanus helgolandicus* nauplii	Growth, feeding

Table 1.1. contd.

Author	Title	Life process
Harris et al. 2000	Feeding, growth, and reproduction in the genus *Calanus*	Feeding, growth, reproduction
Irigoien et al. 2000a	The influence of diatom abundance on the egg production rate of *Calanus helgolandicus* in the English Channel	Reproduction
Irigoien et al. 2000b	Feeding selectivity and egg production of *Calanus helgolandicus* in the English Channel	Reproduction
Laabir et al. 1998	Comparative study of the reproduction of *Calanus helgolandicus* in well-mixed and seasonally stratified coastal waters of the western English Channel	Reproduction
Shreeve et al. 1998	Moulting rates of *Calanus helgolandicus*: an inter-comparison of experimental methods	Growth
Pond et al. 1996	Environmental and nutritional factors determining seasonal variability in the fecundity and egg viability of *Calanus helgolandicus* in coastal waters off Plymouth, UK	Reproduction, mortality
Laabir et al. 1995a	Measuring production and viability of eggs in *Calanus helgolandicus*	Reproduction, mortality
Laabir et al. 1995b	Reproductive response of *Calanus helgolandicus*. II. In situ inhibition of embryonic development.	Reproduction, mortality
Poulet et al. 1995	Reproductive response of *Calanus helgolandicus*. I. Abnormal embryonic and naupliar development	Reproduction, mortality
Guisande and Harris, 1995	Effect of total organic content of eggs on hatching success and naupliar survival in the copepod *Calanus helgolandicus*	Reproduction
Bautista et al. 1994	Temporal variability in copepod fecundity during two different spring bloom periods in coastal waters off Plymouth (SW England)	Reproduction
Green et al. 1993	The seasonal abundance of the copepodite stages of *Calanus helgolandicus* and *Pseudocalanus elongatus* off Plymouth	Abundance, variability
Butler et al. 1969	On the nutrition and metabolism of zooplankton VI. Feeding efficiency of *Calanus* in terms of nitrogen and phosphorus	Feeding
Lebour, 1922	The food of planktonic organisms.	Feeding
Lebour, 1923	The food of planktonic organisms II.	Feeding

Given the importance of *C. helgolandicus* in the structuring of food webs, its recognised response to warming oceans, and its changing distribution, this study has the following aims and objectives:

1. To explore the L4 time series to understand which processes control the population of *C. helgolandicus* in the western English Channel. I present a broad scale overview of population dynamics, using the basic time series measurements namely abundance, egg production in relation to the physical environment, stratification and taxon-resolved food. I explore the abundance and phenology of *C. helgolandicus* and investigate the evidence for the dominant population control mechanisms, as well as evidence for the effects of climate change (Chapter Two).

2. To investigate rates and sources of *C. helgolandicus* early-stage mortality, with a particular focus on egg hatch success and naupliar health. Here I measure egg viability and the incidence of naupliar abnormalities in relation to the available nutrient resource estimated via the maternal diet. Egg mortality rates are estimated and related to predator abundance and biomass (Chapter Three).

3. To estimate late-stage *C. helgolandicus* mortality rates and establish the main sources of mortality. I estimate total and the constituent consumptive and non-consumptive mortality rates and investigate these in relation to predators and also differences between the sexes (Chapter Four).

4. To collect and analyse the gut-contents of potential predators for physical evidence of *C. helgolandicus* consumption. Using molecular next generation sequencing (NGS) metabarcoding techniques I assess the diets of the major gelatinous zooplankton predators at L4 (ctenophores and medusae), collected during 2015 (Chapter Five).

CHAPTER TWO

How does *Calanus helgolandicus* maintain its population in a variable environment? Analysis of a 25-year time series from the English Channel

Calanus helgolandicus *is a key copepod of the NE Atlantic and fringing shelves, with a distribution that is expanding northwards with oceanic warming. The Plymouth L4 site has warmed over the past 25-years, and experiences large variations in the timing and availability of food for* C. helgolandicus*. Here I examine the degree to which these changes translate into variation in reproductive output and subsequently* C. helgolandicus *population size. Egg production rates (eggs female^{-1} day^{-1}) were maximal in the spring to early-summer period, when diatom blooms and high ciliate abundance also occurred, rather than during the equally large autumn blooms of autotrophic dinoflagellates. Egg hatch success was lower in spring than in other periods, however, there were a greater proportion of naupliar deformities then also. Both the timing and the mean summer abundance of* C. helgolandicus *(CI-CVI) reflected those of spring total reproductive output. However, this relationship was driven by inter-annual variability in female abundance and not that of egg production per female, which varied only two-fold. Winter abundance of* C. helgolandicus *at L4 was much more variable than abundance in other seasons, and reflected conditions from the previous growing season. However, these low winter abundances had no clear carry-over signal to the following season's population size. Overall, the* C. helgolandicus *population appears to be surprisingly resilient at this inshore site, even though other physical and biological conditions were very variable within and between years, with this copepod showing no long-term phenology shift and only a four-fold variation in mean abundance between years. This apparent dampening in population changes may reflect a series of mortality sources, associated with the timing of stratification in the early part of the season, likely affecting egg sinking and loss, plus intense, density-dependent mortality of early stages in mid-summer potentially through predation.*

This chapter is a reformatted copy of my publication: **J. L. Maud, A. Atkinson, A. G. Hirst, P. K. Lindeque, C. E. Widdicombe, R. A. Harmer, A. J. McEvoy, D. G. Cummings (2015). How does *Calanus helgolandicus* maintain its population in a variable environment? Analysis of a 25-year time series from the English Channel. *Progess in Oceanography* 137 (Part B): 513-523**. I conceived, designed and conducted the study, with input from AA, AH and PL; I was lead author on the paper, which received editorial assistance from AA, AH, PL, CW, RH and AM.

This work also contributed to a second paper: **A. Atkinson, R. A. Harmer, C. E. Widdicombe, A. J. McEvoy, T. J. Smyth, D. C. Cummings, P. J. Somerfield, J. L. Maud, K. McConville. (2015). Questioning the role of phenology shifts and trophic mismatching in a planktonic food web. *Progess in Oceanography* 137 (Part B): 498-512.**

2.1 Introduction

Concerns over how climate variability and change may influence *Calanus helgolandicus* distribution have led to considerable research effort into the population dynamics of this species (e.g. Planque and Fromentin, 1996; Bonnet et al., 2005; Helaouët and Beaugrand, 2007; Wilson et al., 2015). Many of these studies emphasise the fact that the main geographic habitat of *C. helgolandicus* is not only warming (Lowe et al., 2009; IPCC, 2013), but is highly variable from year-to-year. This is manifested in major year-to-year changes in both the physics (e.g. temperature, stratification) and the planktonic assemblages (Holt et al., 2010; Widdicombe et al., 2010). Understanding the sensitivity or the resilience of *C. helgolandicus* populations to present-day variation is needed for improved understanding of population processes and the likely responses to future climatic fluctuations.

The *C. helgolandicus* distribution extends from the Mediterranean in the south to the northern North Sea and the species has an optimum temperature of ~15°C (Bonnet et al., 2005; Wilson et al., 2016a). Development time from NI nauplius to adult ranges from 26 to 42 days (Thompson, 1982), and 3-5 generations may be produced per year (Green et al., 1993). Population seasonality is dependent on location, but the average seasonal cycle displays a peak of abundance in April-June, followed by a larger autumn peak (September-October) (Planque and Fromentin, 1996). Bonnet et al. (2005) suggest that seasonality relates to latitude, where southern stations exhibit periods of high abundance in spring, but at the highest latitudes, peak abundance occurs in autumn. At L4, *C. helgolandicus* abundance was reported to peak during June to July with the species occurring all year round (Irigoien and Harris, 2003). Highest densities of up to ~200 individuals m^{-3} have been observed in the Adriatic and off the coast of Vigo (NW Spain); the average *C. helgolandicus* abundance at L4 was was reported to be 20-100 individuals m^{-3} (Bonnet et al., 2005). The species is not thought to undergo diapause, but instead remains in shallow waters through the winter (Bonnet et al., 2005); however, there are as yet no published studies on the duration and timing of *C. helgolandicus* diapause (Wilson et al., 2015).

C. helgolandicus is a broadcast spawning species and the timing of egg production has also been described in relation to latitude, with egg production in

southern populations peaking between February and June, but in late summer in more northern climes (Bonnet et al., 2005). In the English Channel, *C. helgolandicus* appears to have an almost continuous reproductive period lasting from early spring through to winter, and egg production rate has been correlated with chlorophyll-*a* (Bautista et al., 1994; Laabir et al., 1998).

This study aims to explore the *C. helgolandicus* dataset at L4 and provide an overview of the population dynamics. I used data available at the time; namely a 1988-2012 (25 year) time series of *C. helgolandicus* abundance and the 1992-2012 egg production rate data from station L4. My objectives were three-fold; (1) to explore the annual and seasonal variability of *C. helgolandicus* abundance and egg production; I hypothesise that there will be a strong pattern of seasonality, but less obvious interannual variability, (2) to test the hypothesis that environmental variability is a key determinant of year-to-year variability in *C. helgolandicus* abundance at L4, through its effect on egg production rates, and (3) to investigate trends in changes to test the hypothesis that abundance and phenology may have changed in response to a changing marine environment.

2.2 Materials and methods

Table 2.1 presents the data coverage available for this chapter.

2.2.1 Mesozooplankton data collection

Data used for this study span March 1988 to December 2012 and represent a weekly sampling regime. Two WP-2 (200 µm mesh) replicate plankton net vertical hauls were taken from a depth of ~50 m to the surface, at a speed of ~15 m min^{-1} and fixed immediately in 4% formaldehyde. All zooplankton including *C. helgolandicus* adult females, adult males and total copepodites were identified and enumerated under a microscope in the laboratory from sub-samples containing ~200 individuals and converted into abundance (no. m^{-3}). Subsamples were extracted using a Folsom splitter and a Stempel pipette, to identify separately large and small organisms. Values

presented in this Thesis represent the mean of the two replicate hauls. The resultant abundance data were entered into a L4 mesozooplankton database. A WP-2 63µm mesh vertical haul from 50m to the surface was added to the sampling regime in 2010. In the mesozooplankton database animals were assigned to taxonomic groups: Siphonophora, Ctenophora, Chaetognatha, Trachymedusae, Hydromedusae, Echinodermata, Cladocera, Cirripedia, Euphausiacea, Mysida, Decapoda, Copepoda (including Calanoida, Cyclopoda and Harpacticoida). A number of different zooplankton analysts have contributed to the dataset over the years; however the level of expertise with respect to the Copepoda has been consistent (R. Harris, 2014, personal communication, 20th January).

Table 2.1. Time series data availability 1988-2012, Station L4, Western English Channel.

Time series	Data available
Total *C. helgolandicus* (males, females, copepodites) abundance	1988-2012
Female adult abundance	1992-2012 (excl. Aug-Dec 2005)
Male adult abundance	1996-2012 (excl. 2000)
Egg production rate	Feb.1992-2012 (excl. Jul-Dec 2000, 2001, Jan-Sep 2007)
Sea surface temperature	1988-2012
Stratification Index	1993-2012 (excl. Feb-Dec 2000, 2001)
Chlorophyll-*a*	1992-2012
Phytoplankton biomass (microscopy)	Oct. 1992-2012
Microzooplankton biomass (microscopy)	Oct. 1992-2012
Mesozooplankton abundance (including predators)	1988-2012

Over the years of data collection, the level of detail of *C. helgolandicus* identification and enumeration has changed. The specific data collected were as follows; 1988-1991 – total *C. helgolandicus* only (males, females and copepodites); 1992-2000 – female adults, "other" *C. helgolandicus* and total *C. helgolandicus*; 2001-2004 – males, females, "other" *C. helgolandicus* and total *C. helgolandicus;* 2005-2012 – males, females and copepodites CI-CV and total *C. helgolandicus*. Therefore female

adult data were only available for 21 years (1992-2012) and male data for 12 years (2001-2012). Total copepodites (CI-CV) were counted from 2005-2012, and could be calculated from 1996-2004, therefore available for a total of 17 years.

2.2.2 Egg production rate

At each L4 visit between October 1992 and December 2012 live zooplankton were collected with a 710 µm mesh net towed horizontally within the top 10 m layer, at a speed of < 0.5 m s^{-1}. Following return of the live samples to the laboratory in a cool box (typically ~2-3 h after collection), 25 adult (CVI) female *C. helgolandicus* were picked from the sample and 5 replicates of 5 females incubated. To prevent cannibalism of the eggs the animals for each replicate were placed in a 500 µm mesh-bottom Plexiglas chamber inside a 2 L plastic beaker filled with 1.5 L of 0.2 µm filtered L4 seawater (FSW). These were incubated at ambient L4 surface temperature for 24 h in constant darkness. Harvested eggs from each replicate were counted and mean egg production rate (EPR) as eggs $female^{-1}$ day^{-1} was calculated. Total reproductive output (TRO) (eggs m^{-3} day^{-1}) was also calculated as EPR multiplied by the adult female field density.

Calanus finmarchicus sometimes co-occurs with *C. helgolandicus* at L4, albeit in much lower abundance (Lindeque et al., 2013). During a recent series of cold winters since 2010, *C. finmarchicus* abundance has increased slightly in the English Channel (Edwards et al., 2016). However, even during these recent winters *C. finmarchicus* comprised a median of only 4% of *C. helgolandicus* abundance in the top 50 m. Therefore the egg production experiments may have contained on occasion a small admixture of *C. finmarchicus* females picked alongside *C. helgolandicus*, but the proportion overall will have been insignificant.

2.2.3 Egg hatching success and naupliar abnormalities

Eggs obtained from the EPR experiments during 2013 were subsequently used in egg hatching incubations. From each replicate, individual eggs were gently pipetted into the cells of a 24-cell clear Plexiglas multi-well tray, each cell containing 5 mL of 0.2

µm filtered seawater. A maximum of 120 eggs were incubated over the five replicates. The trays were placed in the controlled-temperature room at ambient L4 temperature in constant darkness and examined under a dissecting microscope every 24 h for 5 days. For those eggs that hatched I recorded survival and incidence of deformities in the nauplii.

2.2.4 Phyto- and microzooplankton

Between October 1992 and December 2012 water was sampled from a depth of 10 m using a 10 L Niskin bottle. A 200 mL sub-sample was immediately preserved with a 2% (final concentration) acid-Lugol's iodine solution and a second subsample was preserved with neutral formaldehyde (4% final concentration) for the preservation and enumeration of coccolithophores. Paired samples were stored in the dark (using 200mL glass amber medical bottles) and at room temperature until analysis by light microscopy within ca. 1-4 weeks of collection. Cells with sizes between 2 and 200 µm were enumerated by microscopy, with sample analysis differing according to the specific preservative and undertaken as follows: (a) Lugol's iodine sample, a 50mL sub-sample was examined under phase contrast using a x10 objective (x100 magnification) and using the L4 species list as a reference, density and composition were recorded. Detailed examination of the phyto-flagellates was conducted using a x40 objective (x400 magnification) and phase contrast or differential interference contrast (DIC). Small (ca. 2µm) and medium (ca. 4µm) flagellates were counted in ten fields of view, randomly selected throughout the chamber (ca. 10% error). Large (ca. 8-10µm) flagellates, Cryptophyceae and Choanoflagellates were enumerated on one vertical transect across the chamber. Cells typically >15µm were enumerated using x20 objective (x200 magnification) and phase contrast or DIC. Abundant/blooming species were enumerated using either one (vertical) or two (vertical & horizontal) transects. All remaining cells were enumerated in the whole (or half) chamber. Cells were identified, where possible, to species level according to e.g. Drebes (1974); Dodge (1982); Tomas (1996); Hoppenrath et al., (2009). Species were recorded in a phytoplankton or microzooplankton database and assigned to their different functional groups: phyto-flagellates, diatoms, dinoflagellates, heterotrophic dinoflagellates, ciliates and zooflagellates; (b) neutral formaldehyde solution: One vertical and one horizontal

transect were performed using a x40 objective (x400 magnification) and either phase contrast or DIC for abundant species e.g. *Emiliania huxleyi*. All coccolithophores were identified where possible according to Tomas (1993) and assigned to the 'Coccolithophore' group. The number of cells for each species/taxa was expressed as cells mL^{-1}. Cell volumes, calculated assuming appropriate geometric shapes according to Kovala and Larrance (1966) using average cell length, width and depth measurements of individual taxa, were converted to carbon (pg C $cell^{-1}$) using the formulae of Menden-Deuer and Lessard (2000) and then expressed as mg C m^{-3}. Total biomass for each functional group was thus calculated from the sum of each species/taxon in individual samples. In addition, weekly net samples (20µm), towed vertically from the near-seabed to surface, were analysed live to ascertain dominant species in the near 'real-time'.

2.2.5 Chlorophyll-a

Surface chlorophyll-*a* concentrations were determined using two methods; fluorometry (Turner fluorometer) and reversed-phase high performance liquid chromatography (HPLC). For the period 1992-1999 fluorometry techniques only were used, with both methods used after 1999. In this later period HPLC data were used for analysis. Where available HPLC-derived data were favoured over fluorometry-derived data, however there was 98% agreement of values when they were used concurrently (Smyth et al., 2010).

2.2.6 Temperature and Stratification Index

During the period 1988 to 1992, sea surface temperature (SST) was recorded and determined with a mercury thermometer immediately after collecting a metal bucket full of water from the sampling site. From 1992 a CTD also provided temperature profiles. I have combined these two data sets by using the CTD surface temperature if just the CTD or both CTD and bucket were available, or the bucket value if only that was available. This choice is supported by the fact that both measurements are closely related (R^2 = 95%) and that they show no suggestion of departure from a 1:1 relationship (unpublished data).

Monthly temperature anomalies were calculated by subtracting the overall average of the whole time series for a given month from the observed monthly value, using Equation 2.1:

$$x'_{m,y} = \frac{x_{m,y} - \bar{x}_m}{\sigma(x_m)} \quad [2.1]$$

where m is the month (*m*: 1=January, 2=February, …, 12=December) and y the year; $x'_{m,y}$ is the monthly anomaly of month m in year y; $x_{m,y}$ is the monthly average of month m in year y; $\bar{x}_m$ is the average, and $\sigma(x_m)$ the standard deviation, of month m over the entire time series (Eloire, 2010). Annual and seasonal anomalies were calculated by averaging the relevant monthly anomalies.

A Stratification Index (SI) was calculated according to Irigoien and Harris (2003). This equates to the difference (in °C) between the temperature at the surface and at 30 m. Stratification was said to occur during a temperature difference of ≥1°C. Temperature across depth was only recorded from 1992 onwards; therefore the SI was only available from this time period (Table 2.1).

2.2.7 Phenological indices

Phenological timing indices were calculated based on the 25th, 50th and 75th cumulative percentiles (Figure 2.1), as well as the "centre of gravity" (COG) of the annual population trajectory (see Mackas et al., 2012a). The ''center-of-gravity'' was originally developed by Colebrook and Robinson (1963) to describe average seasonal cycles, and calculates the date of the weighted mean of the entire population curve using the formula:

$$COG = (\sum N_D * D) / \sum N_D \quad [2.2]$$

where N_D is the observed abundance (no. m^{-3}) at time period *D* (Julian Day).

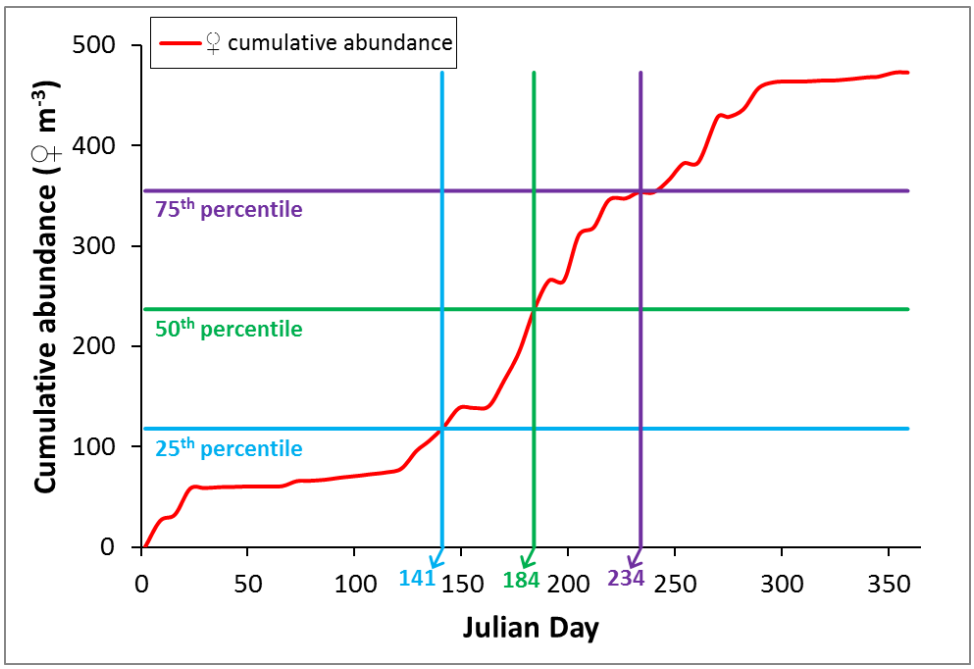

Figure 2.1. Example of calculation of cumulative percentiles, ♀ *C. helgolandicus* (2012). 25th percentile represents the day when 25% cumulative abundance occurs (Day 141) and is used to define the "Start of season"; 50th percentile is the day when 50% abundance is reached (Day 184) and termed "Middle of season", and 75th percentile (Day 234) is the "End of season".

2.2.8 Numerical and statistical analyses

All weekly data were averaged into fortnightly, monthly and annual means. Seasonal means were also determined for each year and were nominally divided into spring (March to May), summer (June to August), autumn (September to November) and winter (December to February). Sea surface temperature values were integrated over the duration of spring-summer warming, i.e. April-August.

Annual and seasonal means for each time series were determined and used in a backward stepwise multiple regression analysis to determine the factors linked to the variability in winter *C. helgolandicus* abundance. Explanatory variables included annual and seasonal mean SST anomalies, microzooplankton biomass, phytoplankton biomass, chlorophyll concentration, the onset and breakdown of stratification, EPR and TRO. Variables were tested for multicollinearity and where variables were found to correlate highly, the variable that correlated most highly with the most number of

variables was included in the analysis, with the aim to include one factor to account for each of food, temperature and stratification. A Durbin-Watson test was performed to detect autocorrelation in the residuals (Durbin and Watson, 1950). Homogeneity of variances and normality of residuals were also examined in the standard regression diagnostic plots. Copepod abundance data were $\log_{10}(x+1)$ transformed where necessary to reduce problems associated with normality. Where heteroscedasticity occurred, residuals versus predictor plots were scrutinised for patterns and variance-covariance structures applied to a generalised least squares model (GLS) as necessary. A GLS is an extended linear mixed-effect model in which errors are allowed to be autocorrelated and/or have unequal variance (Pinheiro and Bates, 2000). The GLS models were compared using the lowest Akaike Information Criteria (AIC) as the decision criterion and for the explanatory variables a significance level of $p < 0.05$ was used. Data manipulation was performed using Microsoft Excel 2010. All statistical analysis was undertaken using the R programming environment (R Development Core Team, 2012).

2.3 Results

2.3.1 Overview of average seasonality at L4

An overall average picture of seasonality at L4 (using a fortnightly averaging period for optimal resolution) is presented in Figure 2.2. Sea surface temperature averaged between 8.5°C in winter to 16.0°C in late summer, with extremes of 6.8°C and 19.9°C. Stratification was typically initiated in May and persisted until September/October (Figure 2.2a), but periodic gales sometimes eroded the main stratification season into several shorter periods. At its peak, the Stratification Index reached 4.5°C, which occurred in July 2006.

While the timings varied substantially between individual years (Atkinson et al., 2015), chlorophyll-*a* concentrations typically increased sharply in spring from 0.5 mg m^{-3} to a peak of over 2.5 mg m^{-3}, with a second smaller peak spanning July to September (Figure 2.2b). Total phytoplankton biomass followed a similar pattern to chlorophyll-*a*, with high values throughout the summer, reflecting successive peaks of

diatoms, dinoflagellates and coccolithophores. Microzooplankton biomass (consisting of ciliates, heterotrophic dinoflagellates and zooflagellates) was substantially less than that of phytoplankton biomass, reaching a maximum typically around July (Figure 2.2b).

Total predator abundance rose sharply in May/June, from less than 50 m^{-3} to ca. 250 individuals m^{-3} (Figure 2.2c). This density persisted throughout the summer and autumn before declining after October. Predator densities followed a progression, with medusae and ctenophores peaking first, then chaetognaths and siphonophores. Fish larvae are not plotted here as they occurred in much lower abundances than the invertebrate predators (Western Channel Observatory, unpublished data).

Mean abundance of *C. helgolandicus* CI-CVI ranged from 6 ind. m^{-3} in December to 204 ind. m^{-3} in August (Figure 2.2d), and usually exhibited a spring-summer peak, with a second larger peak following in the autumn. The trajectory of CVI females was broadly similar, but with a small January peak and a main summer peak rather earlier than that of CI-CVI (Figure 2.2d).

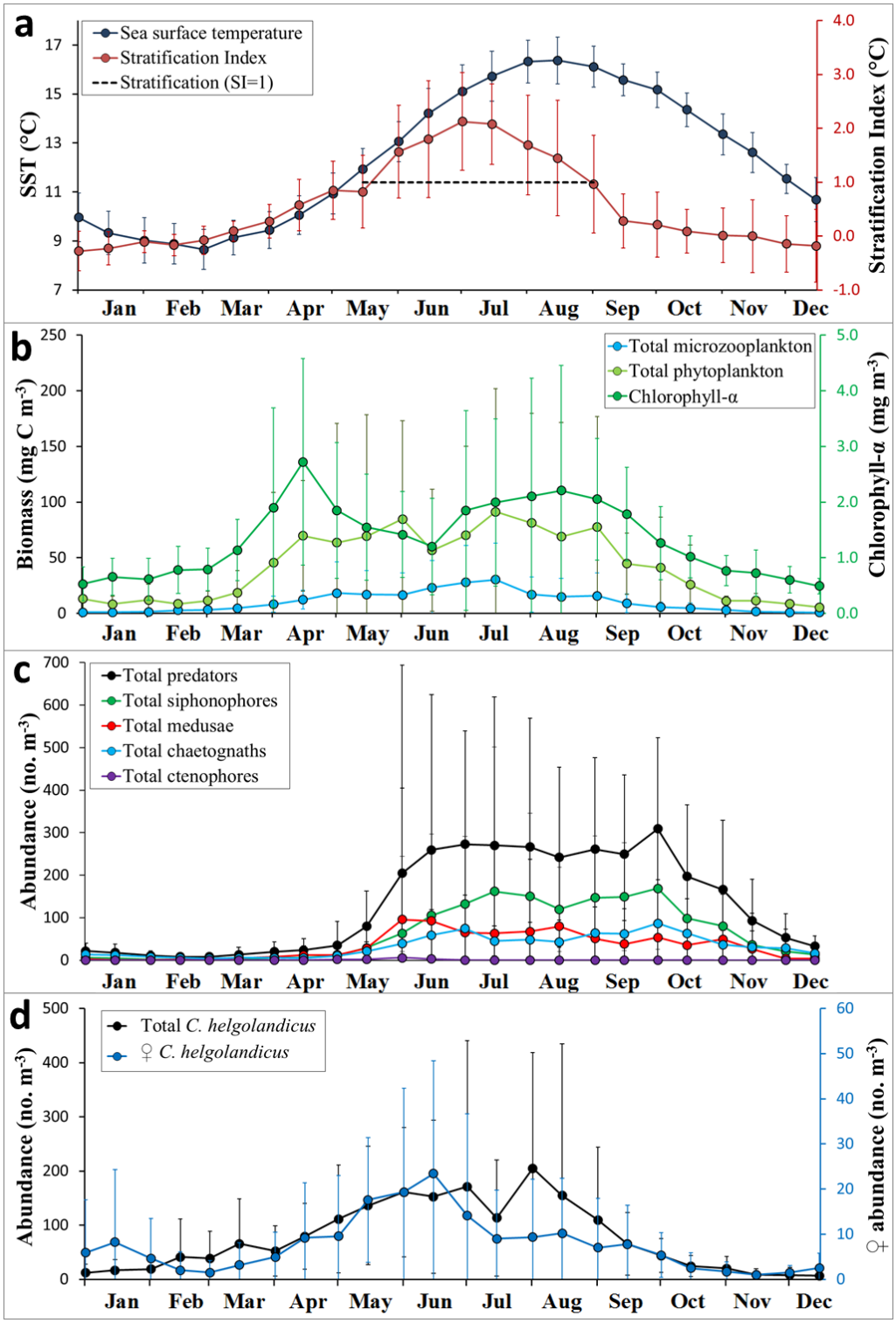

Figure 2.2. Seasonal variation (fortnightly mean and error bars representing standard deviation) of: (a) sea surface temperature and Stratification Index (difference between temperature at the surface and at 30 m), 1992-2012. Stratification is indicated by dashed line as defined by a temperature difference of 1°C or greater; (b) chlorophyll-*a*, total phytoplankton biomass, total microzooplankton biomass, 1992-2012; (c) total predator abundance, 1988-2012; (d) total abundance of *C. helgolandicus* adults and copepodites, 1988-2012, and *C. helgolandicus* ♀ adults, 1992-2012, retained by 200 µm net.

Mean *C. helgolandicus* egg production rate (EPR) ranged from zero (recorded in only 4% of the weeks sampled) to 59 eggs female^{-1} day^{-1}. EPR was maximal from April to June before declining steadily back to winter levels (Figure 2.3a). Total reproductive output, TRO (no. eggs m^{-3} day^{-1}) is a function of both female abundance and egg production per female and showed a strong peak in June (600 eggs m^{-3} day^{-1}). Total healthy nauplii output (THN) (nauplii m^{-3} day^{-1}) represents the output of nauplii that did not display external abnormalities. Despite decreased egg hatch success and concomitantly increased incidence of deformed nauplii in the first half of the year (discussed in the next section) the calculated THN remains almost the same as that of TRO, being driven by the abundance of females and their elevated levels of EPR. All of EPR, TRO and THN decline after June at what would seem intuitively to be a favourable period of the year, given the increasing temperatures and an abundance of prey items.

2.3.2 Egg hatching success and incidence of naupliar deformities

During my detailed study throughout 2013, the success of egg hatching and incidence of naupliar deformity suggested more serious adverse effects in this year than in the previous years where data are available (Figure 2.3b). During 2013, monthly mean hatching success was at its lowest in February at 8%, rising through the spring to a peak in July of nearly 80%. This level was maintained between ~70-80% through to November with a peak of 84% of eggs successfully hatching (Figure 2.3b). Bonnet et al. (2009) also found a dip in hatch success in late winter (2003-2004), but their rates were overall higher in this first part of the year.

Concomitant with the low egg hatch rate I found in winter-spring 2013, the nauplii that did hatch were often deformed (Figure 2.3c). This ranged in severity from a simple shortened or lack of appendage to an indistinguishable mass with no swimming appendages. Almost all of these deformed nauplii died before moulting to the next stage. An earlier study during 1994 also found elevated incidence of deformities in spring, but the effect was not as great as in 2013 (Pond et al., 1996; their data re-plotted in Figure 2.3c). Egg hatch success and the incidence of naupliar abnormalities are studied in greater detail over multiple years in Chapter Three.

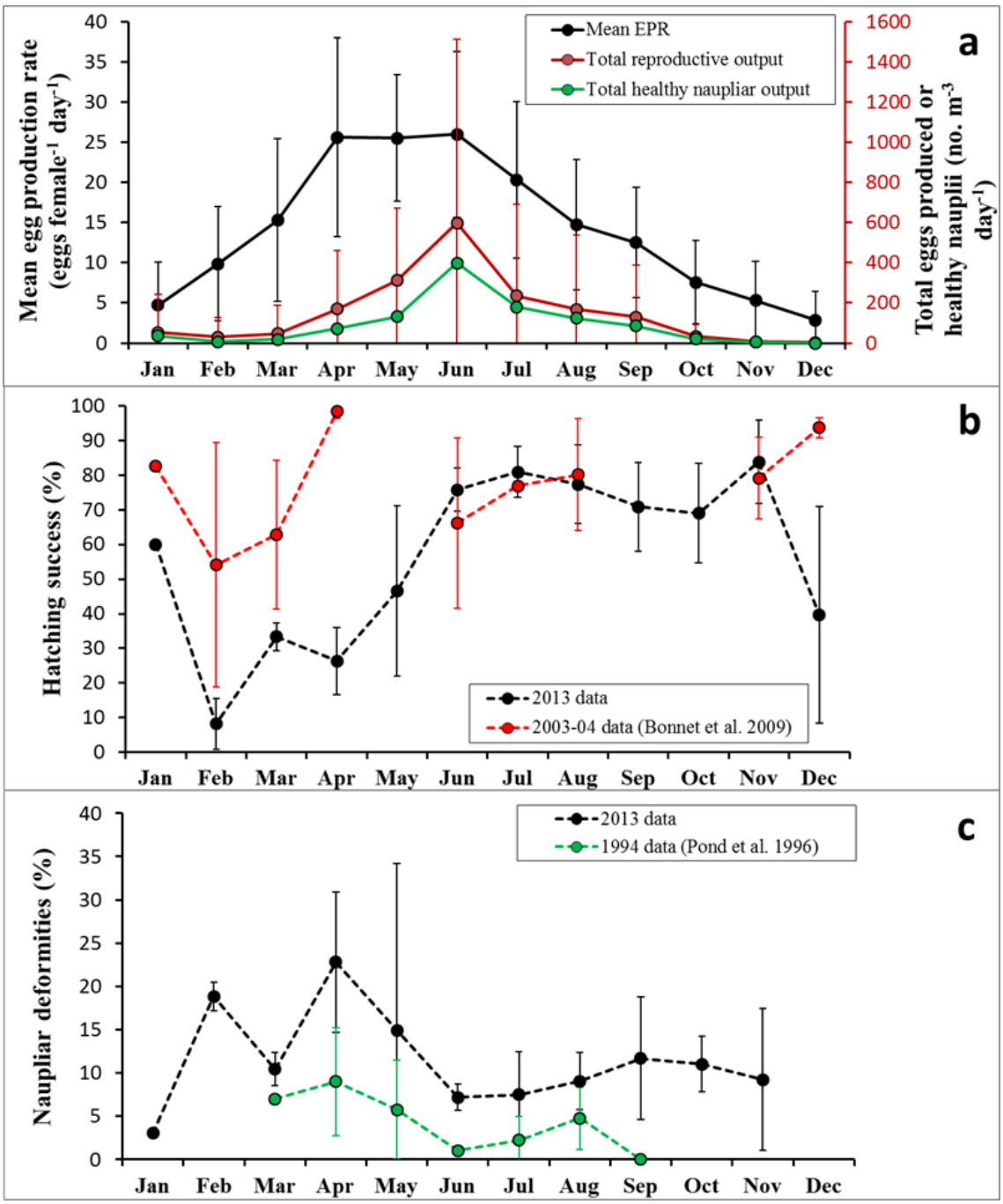

Figure 2.3. Seasonal variation (monthly mean and error bars representing standard deviation) of: (a) mean egg production rate (EPR) and total reproductive output (TRO), 1992-2012; total healthy naupliar output (THN) in 2013; (b) *Calanus* egg hatching success, 2003-2004 data, kindly supplied by Bonnet et al. (2009), alongside the 2013 data (collected by author); (c) abundance of deformed *C. helgolandicus* nauplii (monthly mean and standard error bars) 1994 data (Pond et al., 1996) and 2013 data (collected by author) produced by female adults collected from Station L4.

2.3.3 Inter-annual and longer-term variability

Figure 2.4 summarises the seasonal and inter-annual variability in the *C. helgolandicus* population and its reproductive output, with this variability compared against that of its food environment and predators in Table 2.2. The observation period was characterised by cycles of positive and negative temperature anomalies, and indicated an overall warming of spring-summers (April-August mean: R^2 =0.2, p = 0.038, n = 25; Figure 2.4a). This finding is in line with an overall longer-term warming trajectory in the Western English Channel (Southward et al., 2004; Smyth et al., 2010).

Table 2.2. Mean annual range of plankton variation at L4 over the time series (compared here only for complete years with all data, between 1993 and 2012; see Table 2.1) obtained for the *C. helgolandicus*, microplankton and predator time series; amplitude is the maximum/minimum range value; *n*: number of sampling time-points.

Variable	*n*	Annual mean range amplitude
Total *C. helgolandicus* abundance (no. m^{-3})	867	4.3
Total ♀ *C. helgolandicus* abundance (no. m^{-3})	851	6.4
Mean *C. helgolandicus* egg production rate (eggs $female^{-1}$ day^{-1})	754	1.9
Total *C. helgolandicus* egg production rate (eggs m^{-3} day^{-1})	726	12.4
Microzooplankton biomass (mg C m^{-3})	820	5.9
Phytoplankton biomass (mg C m^{-3})	819	3.8
Microplankton biomass (mg C m^{-3})	814	3.1
Siphonophores (no. m^{-3})	867	14.0
Medusae (no. m^{-3})	867	45.1
Ctenophores (no. m^{-3})	867	26.1
Chaetognaths (no. m^{-3})	867	9.6
Total predators (no. m^{-3})	867	8.0

Mean annual *C. helgolandicus* abundance (Figure 2.4b) varied by a factor of 4.3 over the time series, much less than that of their predators, the siphonophores, medusae and ctenophores (Table 2.2). *C. helgolandicus* mean abundance did not change significantly over the 25 years, in contrast to values in winter (December-February) which increased significantly (R^2 =0.411, p <0.001, n = 25). These winter stocks varied 100-fold over the study period (Table 2.2) with a marked increase

between about 2001 and 2009 (Figure 2.4b). A backward stepwise multiple regression model was developed to attempt to explain this increase in winter abundance. Phytoplankton biomass, chlorophyll concentration and EPR were not found to explain any of the winter abundance variation and were removed from the model entirely. Table 2.3 lists the significant models, with SST anomaly, microzooplankton biomass, TRO and the onset of stratification used as predictor variables. The best model (i.e. lowest AIC) used Apr-Aug SST anomaly and autumn TRO as predictors of winter *C. helgolandicus* abundance.

Mean annual EPR varied less than two-fold (Figure 2.4c), which was much less than the component food sources, and hence was found not to have changed over the 21 years of measurements. Mean annual TRO however, was found to be much more variable (Figure 2.4d) with over a 12-fold range of variation in the number of eggs released. This was a function of the variation in female abundance (factor of 6.4), which again is greater than the total *C. helgolandicus* abundance.

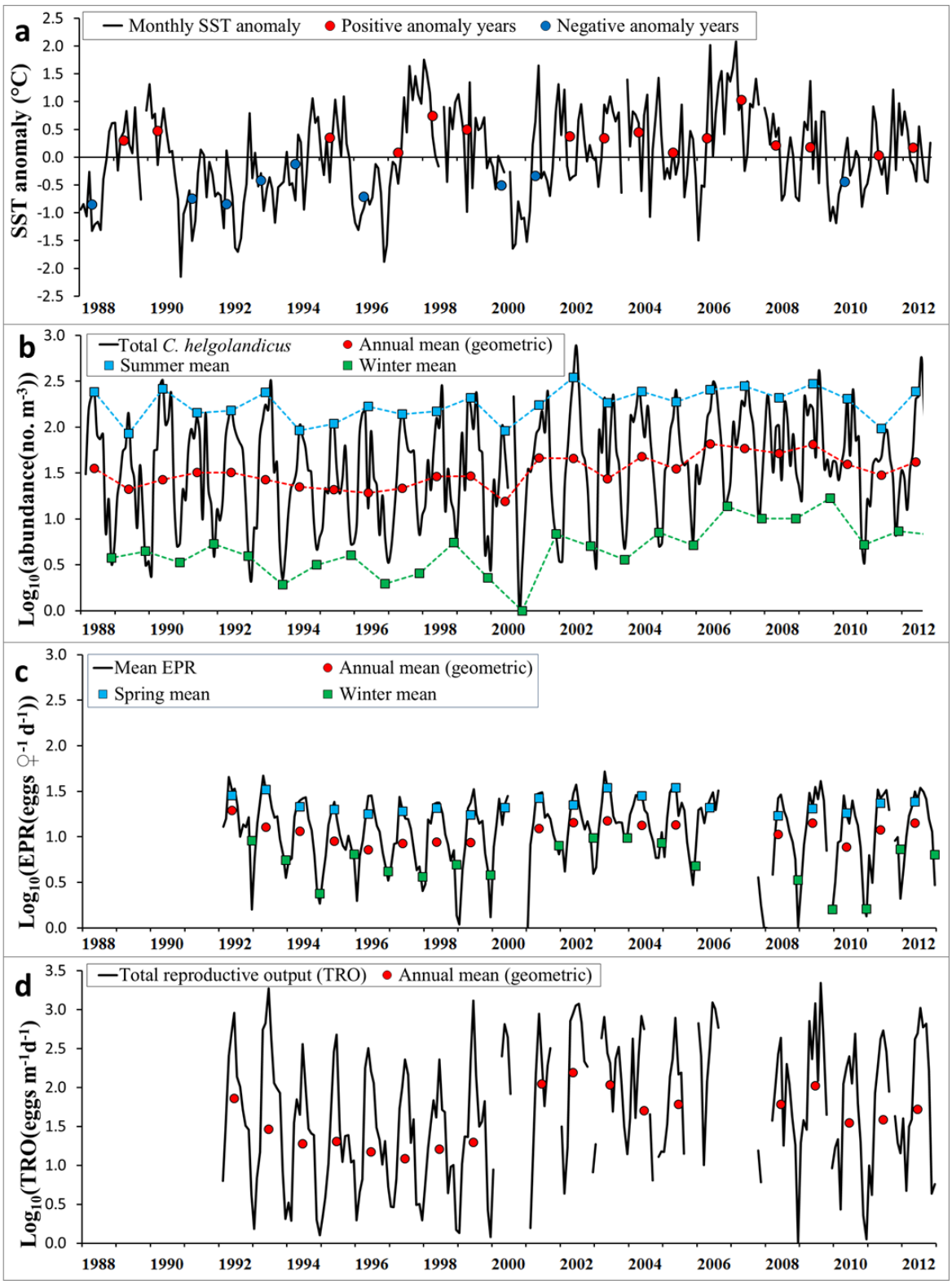

Figure 2.4. (a) Monthly sea surface temperature (SST) anomalies and mean annual anomalies; (b) total *C. helgolandicus* copepodite and adult abundance [$\log_{10}(x+1)$] (monthly mean) (1988-2012), annual (geometric) mean, summer mean (of the 10 highest non-consecutive summer values from June to August) and winter mean (of the 10 lowest non-consecutive weekly winter values from December to February, where for example, winter 1990 consists of data from Dec 1990 and Jan-Feb 1991), plotted for illustrative purposes

only; (c) *C. helgolandicus* EPR - egg production rate [$\log_{10}$(x+1)] (monthly mean) (1992-2012), annual (geometric) mean, spring mean (March to May weekly data), winter mean (December to February weekly data); (d) TRO - total reproductive output [$\log_{10}$(x+1)] (1992-2012), annual mean.

Table 2.3. Generalised least squares analysis of winter *C. helgolandicus* abundance: coefficients, standard error, *t*-value, *p*-value and AIC value for single and multi-variable GLSs; TRO = total reproductive output; SST = sea surface temperature.

Model predictor(s)	Coefficient (slope)	SE	*t*-value	*p*-value	AIC
SST anomaly: Apr-Aug	0.494	0.142	3.484	0.005	5.459
TRO: annual	0.619	0.189	3.277	0.007	
SST anomaly: Apr-Aug	0.404	0.118	3.414	0.009	9.037
TRO: autumn	0.354	0.139	2.549	0.034	
TRO: autumn	0.461	0.200	2.308	0.046	9.056
TRO: annual	0.747	0.257	2.904	0.013	13.844
SST anomaly: Apr-Aug	0.658	0.169	3.889	0.002	17.447
Microzooplankton: spring	1.128	0.358	3.147	0.007	
SST anomaly: Apr-Aug	0.636	0.180	3.530	0.003	20.395
Microzooplankton: Apr-Aug	0.919	0.361	2.545	0.022	
SST anomaly: Apr-Aug	0.628	0.207	3.028	0.008	23.943
Stratification onset (first day SI >1°)	-0.014	0.005	-2.968	0.010	29.340

2.3.4 Variability in phenological timings

The four indices of phenology that I used (Figure 2.5) all show that the timing of EPR (Figure 2.5c) varied much less over the time series than that of total *C. helgolandicus* CI-CVI (Figure 2.5a) or of adult females (Figure 2.5b). For instance the 25% cumulative percentile (a good indicator of the timing of the initial increase) varied from Julian day 65 to 136 for EPR (Figure 2.5c) compared to a range of day 62-169 for total *C. helgolandicus* (Figure 2.5a). Variability in timings of EPR, particularly those of central tendency, tended to reflect variability of the bulk food properties, for example total microzooplankton biomass (R^2 = 0.34, p = 0.018, n = 16), suggesting, perhaps

unsurprisingly, that years of early or late appearance of foods led to respectively earlier or later timings of EPR.

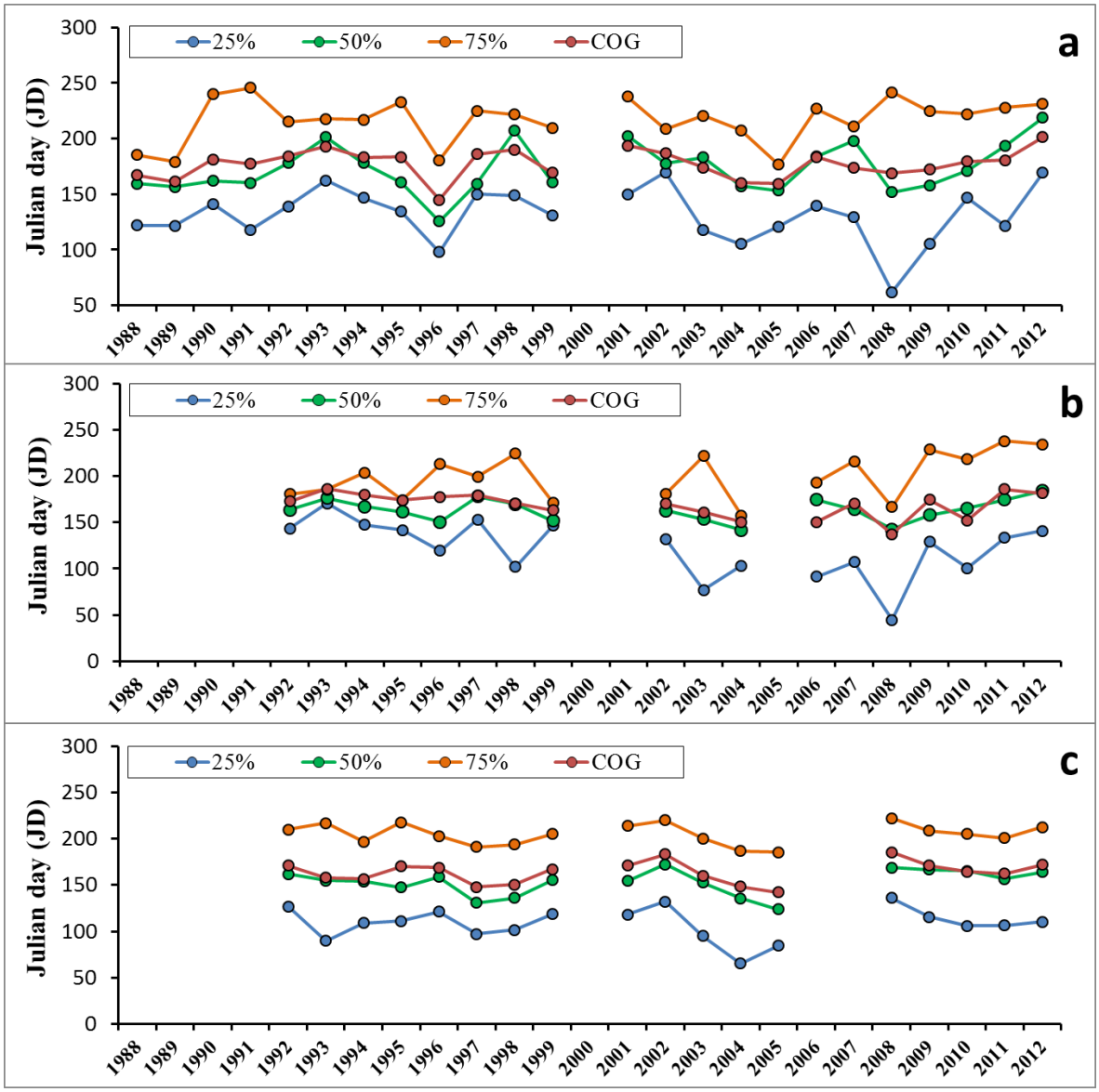

Figure 2.5. Comparison of phenological indices; timing of 25%, 50% and 75% cumulative percentiles and "centre of gravity" (COG) of (a) total *C. helgolandicus* abundance (1988-2012, excluding 2000 due to low sample size); (b) ♀ *C. helgolandicus* abundance (1992-2012, excluding 2000-01 and 2005 due to low sample size) and (c) mean egg production rate (1992-2012, excluding 2000 and 2006-07 due to low sample size).

2.3.5 Causes of inter-annual variability in timing and abundance of Calanus helgolandicus

At the inter-annual scale, the average TRO in the months of greatest EPR (April-May) was a predictor of subsequent mean summer (June to August) total *C. helgolandicus* abundance (Figure 2.6a). Significant positive relationships between the annual timing of TRO and the subsequent timing of *C. helgolandicus* appearance were also found (Figure 2.6b). Together, these findings support my central study hypothesis that variation in reproductive output translates into that of population size. However this relationship was not driven by variation in EPR, which fluctuated only two-fold between years and was unrelated to the total resultant *C. helgolandicus* population, either in terms of timing of increase (Figure 2.6c) or in terms of mean annual values. Instead it reflected the more substantial variation in abundance of egg-laying females (Table 2.2), whose abundance was a strong predictor of the copepodite (CI-CV) population.

Despite the significant increase in mean April-August temperatures, there was no significant phenology shift observed for total *C. helgolandicus* over the 25-year period. The timing of the population growth of this species was also unrelated statistically to April-August water temperature, a finding in common with the majority of zooplankton taxa at L4 (Atkinson et al., 2015). The only clear environmental correlate with the phenology of this species was the timing of stratification (Figure 2.6d). This lends support to the hypothesis of Irigoien and Harris (2003) that stratification may be required for the *C. helgolandicus* population to increase at this site. Further support is provided by the lack of any relationship between timings of increase (25% cumulative percentile) of EPR and timing of increase in *C. helgolandicus* (Figure 2.6c). This suggests that population increases do not follow on predictably from increases in EPR, but that other factors such as stratification may intervene in determining the success of newly recruited individuals.

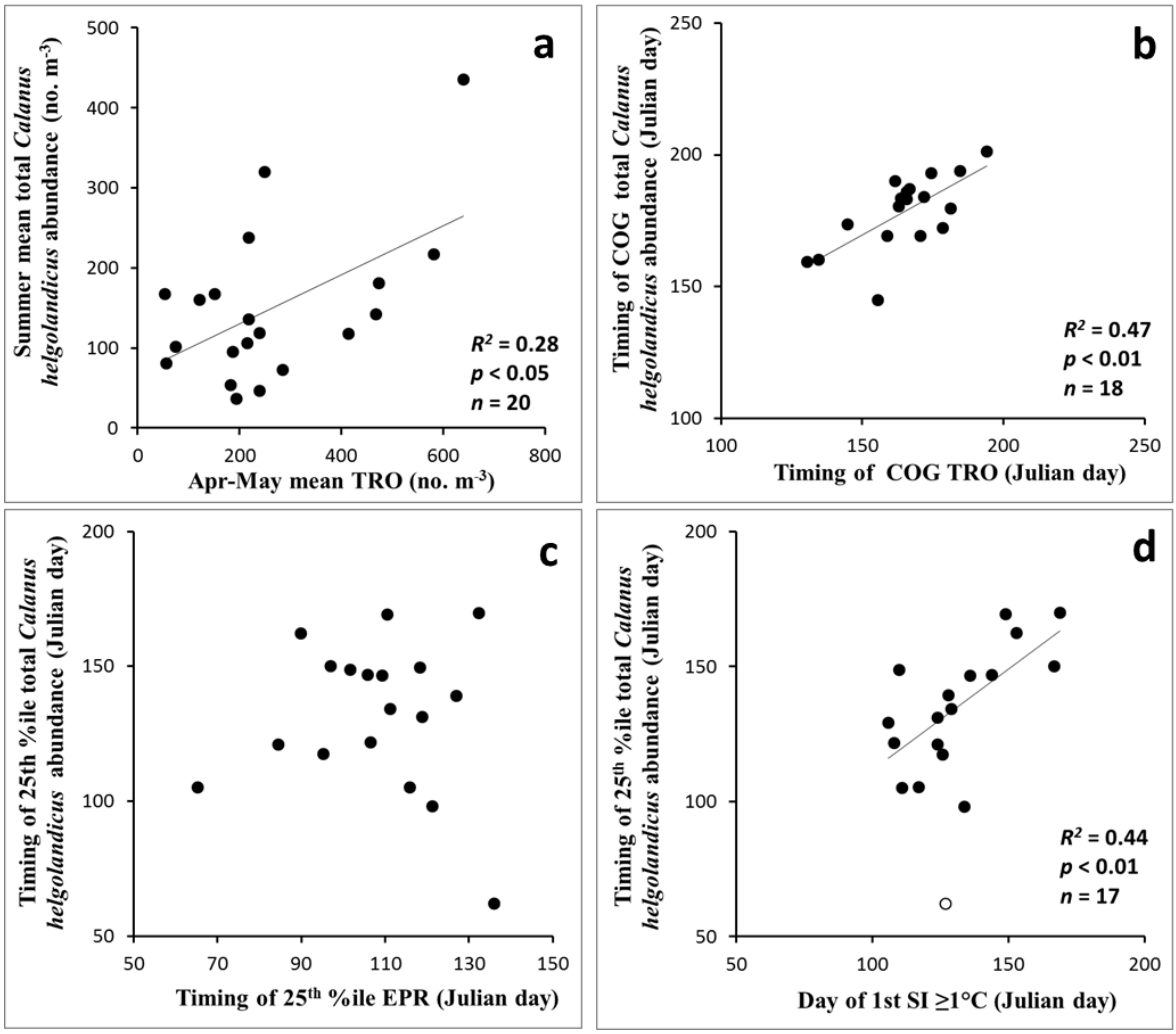

Figure 2.6. Relationship between (a) mean summer (June to August) total *C. helgolandicus* abundance and mean April to May total reproductive output (TRO); (b) timing of "centre of gravity" of *C. helgolandicus* and timing of "centre of gravity" of TRO (other phenological indices, 25th, 50th and 75th percentiles were also positive and excluding the 25th percentile were significant); (c) timing of 25th percentile of *C. helgolandicus* and timing of 25th percentile of egg production rate (EPR); (d) timing of 25th cumulative percentile of total *C. helgolandicus* abundance and the timing of onset of stratification (1st day where Stratification Index (temperature difference between 0m and 30m) ≥ 1°C).

2.3.6 A Calanus helgolandicus *population growth model*

A simple model of *C. helgolandicus* abundance was developed to investigate the degree to which total theoretical *C. helgolandicus* abundance (calculated from the cumulative weekly total reproductive outputs and in the absence of mortality) departed from the actual abundances recorded at L4. This involved calculating the

mean weekly TRO (1992-2012) and "growing" each weekly cohort through from egg to CV copepodite stage within a pre-determined temperature-related and stage-specific development time (Bonnet et al., 2009). The population in each week of the year was calculated by summing the abundances from each cohort. This "theoretical" population trajectory (with simplified assumptions and no mortality) was then plotted alongside the observed population data [using egg to CV stage data collected 2002-04 from Hirst et al. (2007) and 2013 (unpublished data) to compare the deficit between the two (Figure 2.7)], as a relative measure of mortality timing. During the winter and autumn months the theoretical and population densities were 10-25 times that of the observed densities. However from April, this increased dramatically such that in June/July a 100-250-fold deficit was evident. This model is utilised further in Chapter Four (Section 4.3.4), where I develop a mortality index over multiple years.

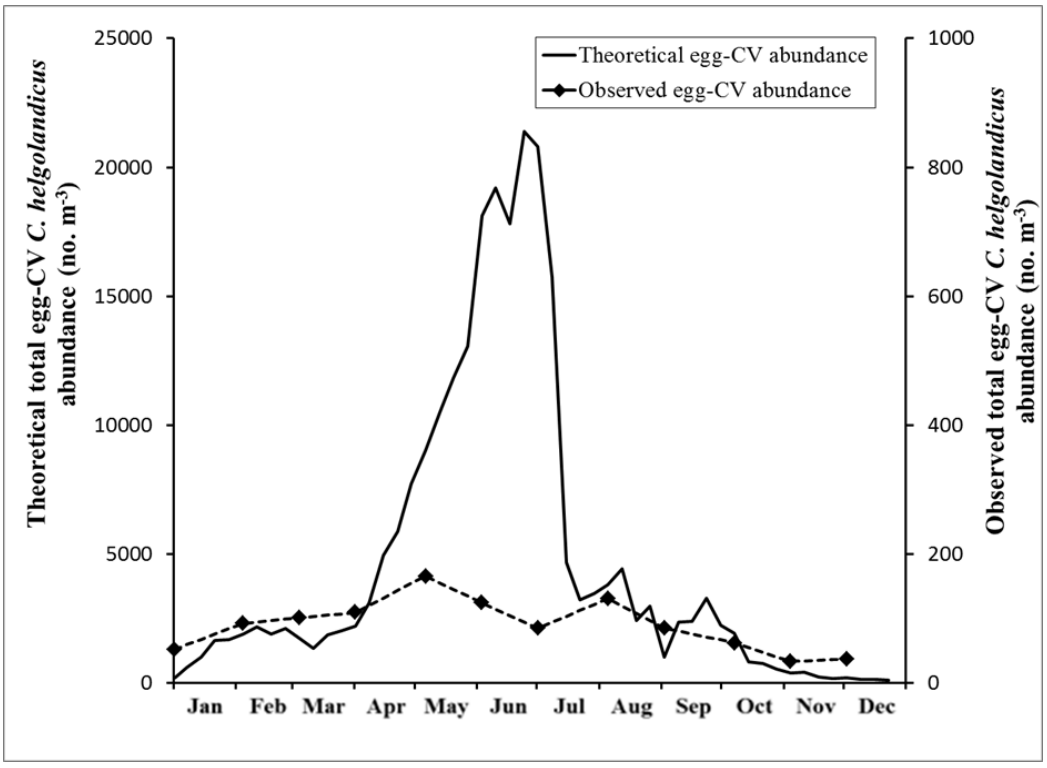

Figure 2.7. Comparison of observed monthly total *C. helgolandicus* abundance (egg-CV) (no. m^{-3}) with a theoretical total abundance calculated from a simple population growth model, summing mean weekly total reproductive output (TRO) at a temperature-specific development rate, from egg production to CV (see Results, 3.6). Note the difference in scales.

2.4 Discussion

At Station L4 the annual mean density of *C. helgolandicus* varied surprisingly little; only ~4-fold over the 25 years, which is remarkable given that many variables show wide ranges, such as SST anomaly (by ~1.8°C), predators (8-fold) and total reproductive output (12-fold). Even where significant increasing trends were found, such as winter abundance, winter stock levels had no obvious carry-over in dictating population density in the following season. Mean summer density of total *C. helgolandicus* reflected variation in spring total reproductive output, but this was driven by changes in female abundance rather than in EPR. In fact EPR varied very little (only two-fold between years), perhaps being buffered by the feeding flexibility of *C. helgolandicus* (Fileman et al., 2010) and the plethora of nutritious foods at the site (Pond et al., 1996).

While *C. helgolandicus* population density was ultimately driven by total reproductive output, I question what further factors control the population, dampening it into a narrow range of densities? I suggest that it is multiple sources of mortality, each probably occurring successively at differing times during the season, that are acting together to limit the exceptionally high values I might expect from the simple matrix model. Here I discuss the various sources of mortality that may impact on the various life stages of *C. helgolandicus* and then extend this to a larger-scale discussion of the spatial distribution and success of the species as a whole.

2.4.1 Food-related factors affecting egg production rate

The seasonal trend in EPR at L4 is surprising in that late season food, dominated by autotrophic dinoflagellates, supported lower EPRs than equivalent biomass concentrations of early season foods (chiefly diatoms and ciliates). The issue of food quality in dictating copepod EPR has received a great amount of attention and debate (Jónasdóttir et al., 2005; Ceballos and Álvarez-Marqués, 2006a; Jónasdóttir and Koski, 2011). At L4 the findings on food quality have been slightly contradictory. On one hand, Pond et al. (1996) found that diatoms, dinoflagellates and particularly ciliates all supported high fecundity, while on the other hand Irigoien et al. (2000) found low EPR associated with autumn blooms of the dinoflagellate *Prorocentrum*

balticum. Interannual variability in the composition of dinoflagellates that dominate later in the season may explain this contrast, since in some years these blooms are strongly dominated by single species which may produce toxins, most notably *Karenia mikimotoi* (Barnes et al., 2015).

During the three seasons in which egg hatch success and naupliar deformities were recorded (Pond et al., 1996; Bonnet et al., 2009 and this study) the values ranged enormously between years. These data revealed that between 30% and 70% of eggs survived to the NII stage, effectively removing a considerable proportion of the potential population within the first few days after egg production. While these studies show differing degrees of egg viability, all suggest that the strongest affect is at the time of the spring diatom bloom.

Cytotoxic compounds from some diatom species have been strongly implicated in inducing teratogenic effects on copepod eggs and nauplii, and diatom degradation products such as polyunsaturated aldehydes (PUA) and oxylipins have been much investigated and debated intensively in the past 20 years (Irigoien et al., 2002a; Ianora and Miralto, 2010). A diet of the diatom *Skeletonema marinoi* was found to reduce egg hatching success and female survival (Miralto et al., 1999; Ianora et al., 2004; Ianora and Miralto, 2010) and alter gene expression (Lauritano et al., 2011a; Lauritano et al., 2011b) specifically in *C. helgolandicus*. The potential dual role of diatoms, in enhancing EPR and reducing viability of these eggs, is studied in greater detail in Chapter Three.

2.4.2 Physical factors affecting egg survival

Egg and early-stage nauplii are known to succumb to the highest mortality rates of any development stage, with less than 10% surviving between egg and NI (Hirst et al., 2007). Mortality can occur as a consequence of many physical, chemical and biological processes. *Calanus* spp. eggs sink at a rate of approximately 36 to 70 m day^{-1} (Irigoien and Harris, 2003). This combined with a shallow depth and a prolonged hatching duration in winter may result in a proportion of eggs reaching the bottom before hatching has occurred. This may potentially expose them to high benthic predation, which may present an important mortality agent (Peterson and Kimmerer, 1994). Irigoien and Harris (2003) suggested that the onset of stratification was an

important factor in allowing the summer increase in *C. helgolandicus* at L4, whereby warmer temperatures lead to a faster hatching rate which reduces the loss of eggs to the sea bed. Additionally, development of the thermocline may prevent the eggs from settling out of the upper layers and suffering predation by the benthos. These suggestions are supported correlatively at least by my analysis of a longer data span, where stratification timing was the only physical variable found to correlate with the timing of increase of the *C. helgolandicus* population.

2.4.3 Predation on nauplii and copepodite stages

The escalating mortality risk from April to July that I predicted from the simple matrix model does not coincide neatly with the elevated abundance of taxa considered as classic predators (Hirst et al., 2007; Bonnet et al., 2010). The latter increase only in June and last longer into autumn, suggesting that additional factors may be involved. For example high autumn water temperatures may allow rapid *C. helgolandicus* development to maximise survival of recruits to adulthood so that they are more likely to avoid predation. Another factor may be that a different suite of predators help to remove the eggs and nauplii, since these early stages show slightly earlier peaks in mortality at L4 than those of later copepodite stages (Hirst et al., 2007). Many copepods are known to predate other copepods or young of their own species (Lebour, 1922; Kleppel, 1993). Ohman and Hirche (2001) further described density-dependent mortality through cannibalism in *Calanus* spp. suggesting that this may be a form of population self-limitation. Indeed, Hirst et al. (2007) found a significant correlation between adult *C. helgolandicus* abundance at L4 and both the rate of removal of their eggs from the water column (ind. m^{-3} d^{-1}) and egg mortality (d^{-1}), implying a density-dependent process at L4. The relative lack of fluctuation in both the upper boundary and annual mean density of *C. helgolandicus* may also suggest that population self-regulation processes operate at this site. Egg mortality and potential predators of early life stages are explored in more detail in Chapter Three.

2.4.4 Population-level responses to a variable and changing environment

Among 46 zooplankton taxa where minimum and maximum annual mean abundances were compared throughout the time series, only three varied less than *C. helgolandicus* in abundance from year to year (Atkinson et al., 2015). My finding that interannual variability in total abundance of *C. helgolandicus* over 25 years at L4 is only 4-fold can also be compared to that of its colder water congener *C. finmarchicus*. Monitoring data from the Continuous Plankton Recorder (CPR) (1997-2009), from the north-east of Scotland demonstrated a 16-fold range of variation in *C. finmarchicus* annual mean abundance (Baxter et al., 2011). The variability that is observed between years reflects the fact that L4 is a fixed point site in an advective environment. However, if fluctuating sources of *C. helgolandicus* were being advected past the site, this would presumably tend to increase the variability that I observed, rather than decrease it.

Evidence of a biogeographic shift occurring over the past 50 years, from cold-temperate to warm-temperate species in the North–East Atlantic, as a result of ocean warming, has been widely reported in the literature (Planque and Fromentin, 1996; Beaugrand et al., 2002; Beaugrand, 2003; Helaouët and Beaugrand, 2007; Helaouët et al., 2013). My emphasis on the relative stability of *C. helgolandicus* populations at a single site, contrasts with many larger-scale studies that stress the sensitivity of *C. helgolandicus* and *C. finmarchicus* to climatic changes. Rapid change affects are particularly well studied within the population gravity centre of *C. helgolandicus* (Helaouët et al., 2013; Maar et al., 2013). These authors found that the large scale distributions of *C. helgolandicus* and *C. finmarchicus* were strongly related to sea surface temperature and suggested a lack of thermal adaptation. That would imply sensitivity to climate change, with dramatic range changes in these species with warming (Hinder et al., 2014). Nevertheless, these authors caution against attempts to project future distribution patterns, for example with climatic envelope modelling based on warming scenarios, since the effect of climatic drivers can change over time (Beaugrand, 2012).

The elucidation of the detailed population dynamics of *C. helgolandicus* is necessary to understand the responses to potentially greater climate variability in the

future. So how do my results, specific to a single site, help to inform the understanding of *C. helgolandicus* populations on wider scales of space and time? On one hand L4 is near the thermal optimum of the species (Bonnet et al., 2005), so climatic effects that may affect a species nearer the edge of its range may not be so acute here. However the overall stability of *C helgolandicus* stocks at L4, (indexed both by lack of phenological shift and low variation in mean density between years) would perhaps not be expected from large scale predictions based on temperature and/or food levels (Richardson et al., 2008). The exact mechanisms for the resilience are still to be clarified, and I investigate the stabilising effects of mortality in Chapters Three (eggs) and Four (copepodites).

CHAPTER THREE

Calanus helgolandicus egg mortality: egg hatch success, naupliar abnormalities and predation

Mortality rates of zooplankton are generally thought to be highest for the early developmental stages, particularly the egg stage. For copepods especially, it is acknowledged that a non-trivial proportion of the eggs produced never hatch and of those that do hatch, a proportion of the nauplii exhibit deformities. Copepod eggs are also vulnerable to ingestion by a variety of predators. Here I investigate the mortality sources and rates of Calanus helgolandicus *in the western English Channel (station L4) using two different approaches; 1) via direct measurement of egg hatching success (EHS) and the incidence of abnormalities over four years, and 2) using a vertical life-table (VLT) method to estimate egg mortality rates over three years. Egg hatch success rates varied from 0-100% (monthly means of 25-80%) and naupliar abnormalities (NA) ranged from 0-54% (mean of 8%). These data imply that 30% to 70% of the potential* C. helgolandicus *population is lost before the NII naupliar stage. Both egg viability and naupliar health were reduced and more variable in the spring, and NA was inversely related to EHS. I found that egg and naupliar health were boosted by the availability of nutritious food for the adult females, but may be reduced by the presence of certain toxic diatom strains. Egg mortality rates (derived using the Basic VLT method and a new Viability Basic method to account for non-viable eggs) were significantly higher in spring and summer than autumn and winter. Total copepod biomass and* C. helgolandicus *copepodite (CI-CV) abundance were positive predictors of egg mortality rates, suggesting that intraguild predation and cannibalism were important sources of egg loss. I conclude that* C. helgolandicus *mortality is a function of three key loss processes, acting through the maternal diet to influence egg and naupliar health, and the strong predatory impact of other copepods.*

This work contributed to a separate paper: **White, D. A., C. E. Widdicombe, P. J. Somerfield, R. L. Airs, G. A. Tarran, J. L. Maud and A. Atkinson. (2015). The combined effects of seasonal community succession and adaptive algal physiology on lipid profiles of coastal phytoplankton in the Western English Channel. Marine Chemistry 177, Part 4: 638-652.**

3.1 Introduction

The fundamental aim of zooplankton ecology is to describe, explain and understand the distribution and abundance of organisms (Begon et al., 2006). The elucidation of the population dynamics of an organism requires the understanding of the processes involved in the production, growth, development and loss of individuals, including the trophic links between prey and predator species. Historically, it is the gain processes that have received most attention and estimation due to the relative ease of experimentation [i.e. reproduction (Poulet et al., 1995; Rey-Rassat et al., 2002a), growth and development (Rey-Rassat et al., 2002b; Bonnet et al., 2009) and feeding (Paffenhöfer, 1976; Meunier et al., 2015]. However, this should be balanced with an equal understanding of the loss processes (i.e. mortality, predation, starvation, advection, etc.) (Hirst and Kiørboe, 2002; Gallego et al., 2012).

Mortality rates are traditionally much more challenging to measure than, for example, egg production rates. Methods and equations for the estimation of zooplankton mortality rates have been available for around half a century (Mullin and Brooks, 1970; Peterson and Kimmerer, 1994; Wood, 1994; Aksnes and Ohman, 1996), although much of the focus of this work has been on copepods, the biomass dominant mesozooplankton in the upper pelagic (Harris et al., 2010). However, much debate still exists on their reliability and accuracy, especially when applied at advective marine environments (Ohman, 2012). Vertical life-table (VLT) methods, where mortality is calculated using a “slice of time” and calculations are made using abundances of zooplankton development stages and their stage duration times, have tended to dominate marine copepod studies.

The mortality of an organism is generally highest for the early life stages, e.g. fish larvae (Sifa and Mathias, 1985; Garrido et al., 2015), birds (Sullivan, 1989; Ridley, 2007) and amphibians (Wilbur and Collins, 1973). This is mainly due to a limited or none existent (in the case of eggs) escape response and an increased vulnerability to predation through their smaller size [through the slow-growth-high-mortality hypothesis (Clancy and Price, 1987), indicating that faster growth rates lead to an increase in size, and a reduced likelihood of being preyed upon]. Highest rates of mortality in zooplankton are also generally found for the early life stages (e.g. Kiørboe

et al., 1988; Eiane and Ohman, 2004). For copepods, this is especially so in freely spawned pelagic eggs (Kiørboe et al., 1988; Hirst et al., 2007). Important sources include predation, cannibalism, sinking (whereby eggs in shallow waters are lost to the benthos before they can hatch) and non-viability. It is recognised that not all copepod eggs produced are viable and that egg hatch success may be much less than 100% (Cook et al., 2007; Maud et al., 2015). The use of standard estimates of mortality rates, if non-viable eggs are not taken into account can lead to overestimates in population dynamic models (Head et al., 2015). In addition, not all viable eggs result in viable nauplii; some may display abnormalities such as missing limbs or evidence of arrested development, and it is unlikely that these nauplii can develop further (Miralto et al., 1998).

Possible explanations for the non-viability of eggs and naupliar deformities are mostly related to the maternal diet (Jónasdóttir et al., 2002). There are two major strands of investigation, the first related to food quality; for example, phytoplankton blooms may lead to monospecific diet that may be lacking essential nutrients that prevent normal reproduction (Huntley et al., 1987; Irigoien et al., 2000b). In addition, the biochemical composition of a bloom may also change with time, such that dietary quality may be compromised, with subsequent effects on reproduction (Diekmann et al., 2009). The second strand relates to the so-called paradox of "toxic" phytoplankton (Ban et al., 1997). Although phytoplankton form a key constituent of the natural diet of copepods, paradoxically, some species or strains are implicated as causal agents of low egg production and non-viability of eggs. These "toxic" species are purported to produce secondary plant metabolites that are detrimental to egg development. Diatoms are much implicated here, and laboratory studies have determined that they may synthesize polyunsaturated aldehydes (PUA) from polyunsaturated FAs (PUFAs) (Miralto et al., 1999) or non-volatile oxylipins (Barreiro et al., 2011). However, there is also evidence for the negative effects of some dinoflagellate species. Following this, research into phytoplankton fatty acid (FA) composition of seston has revealed specific FA biomarkers that indicate the presence of certain phytoplankton groups. For example, diatoms are known to be generally rich in C20:5(*n*-3), whereas dinoflagellates are high in C22:6(*n*-3) (Anderson and Pond, 2000). Certain biomarkers have been utilised to explain egg hatch success/naupliar health; for example C22:6(*n*-3)

(docosahexaenoic acid-DHA) is known to be an important fatty acid for determining fecundity of marine organisms and is present at high levels in dinoflagellates (Pond et al., 1996).

In this study I focus on quantifying and understanding *C. helgolandicus* egg mortality and viability at L4. I derive egg mortality rates over three years using vertical life-table approaches. Egg hatch success was collated over ~four years, mostly through discrete studies, independent of the routine *C. helgolandicus* egg production experiments; now however, since 2015, a routine EHS protocol is in place at L4.

3.2 Materials and method

Time series data availability is provided in Table 3.1.

Table 3.1. Time series data and availability from station L4.

Time series	Data available
Total *C. helgolandicus* (males, females, copepodites)	1988-2015
♀ adult abundance	1988-2015 (excl. August-December 2005)
♂ adult abundance	1996-2012 (excl. 2000)
Egg production rate (EPR)	February 1992-2015 (excl. July-December 2000; 2001; January-September 2007)
Total copepodite (CI-CV) abundance	1996-2015
Copepodite (CI-CV) stage composition	March 2002-March 2004; 2012-2013
Egg hatch success (EHS)	August 2003-August 2004, 2013, March-October 2015 and 2016
Naupliar abnormalities (NA)	March-September 1994, 2013, March-October 2015 and 2016
Mesozooplankton abundance and biomass (incl. predators)	1988-2015
Total fish larvae abundance	1988-2015
Phyto-and microzooplankton abundance and biomass	October 1988-2014
Sea surface temperature (SST)	1988-October 2016
Mean column temperature (MCT)	1993-October 2016 (excl. February-December 2000, 2001)
Stratification Index (SI)	1993-October 2016 (excl. February-December 2000, 2001)
Chlorophyll-α (fluorescence)	1992-October 2016
Particulate organic carbon/nitrogen (POC/PON)	2008-2010, 2012-2015
Seston fatty acid composition	January-December 2013
C. helgolandicus egg fatty acid composition	June-November 2013

3.2.1 Mesozooplankton field sampling

The sampling, identification and enumeration of mesozooplankton [including adult *Calanus helgolandicus* (male and female) and total copepodites (CI-CV)] were undertaken following materials and methods in Chapter Two (Section 2.2.1). During 2013 separate sub-samples of ~100 *C. helgolandicus* CI-CVI stages were taken from one of the weekly 200 µm WP-2 net hauls to enumerate copepodite stage abundances. Counts were converted into abundances (no. m^{-3}). Additionally during 2013, a 63 µm WP-2 net was also deployed in a vertical 0-50 m haul at the weekly L4 sampling. The live sample was returned to the laboratory and preserved in 70% ethanol. Sub-samples of ~100 *C. helgolandicus* eggs and NI-NVI nauplii were staged and enumerated and converted to abundances (no. m^{-3}).

Abundance data of potential predators of *C. helgolandicus* eggs were extracted from the time series including total ctenophore, siphonophore, medusae and chaetognath, copepod and fish larvae abundances. Total meroplankton (larvae of polychaetes, bryozoans, gastropod larvae, echinoderms, cirripedes and decapods) and total other non-copepod holoplankton (cladocerans, hyperiid and mysid shrimps and euphausiids) abundances were also obtained, along with total mesozooplankton and total gelatinous plankton abundance (total of ctenophore, siphonophore, medusae and chaetognaths). Predator biomasses were calculated by measuring lengths of L4 specimens and applying literature length-mass conversions. In total, the characteristic lengths (for example medusa bell height or diameter, copepod prosome length) of 3780 individuals were measured. The length data were first divided into the seasons spring (March-May), summer (June-August), autumn (September-November) and winter (December to February). I then used published length-mass conversions to get a mean individual carbon mass for each taxon in each season, which was multiplied with the respective abundance data to estimate biomass (mg C m^{-3}).

3.2.2 Environmental data

SST, SI and chlorophyll-*a* were determined following materials and methods in Chapter Two (Section 2.2.4 and 2.2.5). Particulate organic carbon (POC) and nitrogen (PON) were quantified by collecting triplicate 250 mL aliquots of surface seawater, pre-

filtering through a 200 µm mesh, before filtering onto 25 mm ashed glass fibre filters (GF/F). The filters were then dried at 60 °C and acidified with sulphurous acid prior to analysis on a Thermoquest FlashEA 1112 elemental analyser.

Microplankton data were sampled and enumerated following the materials and methods in Chapter Two (Section 2.2.4). Phytoplankton data were grouped into total diatoms, dinoflagellates, coccolithophores and phytoflagellates. Microzooplankton data were grouped into total ciliates, heterotrophic dinoflagellates and zooflagellates. Specific "toxic" phytoplankton species were identified from a review of the literature on the effect of diatoms and dinoflagellates on copepod egg viability and potential teratogenic effects, and cross-referenced with those taxa reported at station L4. A list of 35 potential harmful taxa was produced following discussion with an expert taxonomist (C. E. Widdicombe, 2016, personal communication, 14th November) (Table 3.2).

3.2.3 Egg production rate, hatching success and naupliar abnormalities

Mean egg production rate (EPR) was determined weekly from the incubation of 25 adult females over 24 hrs [see Chapter Two (Section 2.2.2)]. Egg hatching success (EHS) was evaluated over the years 2013 and 2015-2016 using two similar methods. During 2013, ~120 eggs (collected during the EPR experiments) were pipetted into individual cells of a multi-well plate, incubated at ambient L4 temperature and observed every 24 hrs for five days [see Chapter Two (Section 2.2.3)]. During the period 2015-16, harvested eggs from the EPR incubations were placed in a 250 ml amber glass bottle with ~200 ml 0.2 µm FSW and incubated at ambient L4 temperature. After ~48 hrs, 10 ml 2% (final concentration) acid-Lugol's iodine solution was added to the incubation to preserve the nauplii and non-hatched eggs. During sample analysis, all nauplii and un-hatched eggs were enumerated. Nauplii were categorised as either healthy or abnormal. Partial hatches were classified as un-hatched or if it was obvious that the nauplius was not healthy, were counted as abnormal nauplii. Egg hatch success was calculated as a proportion of the total number of eggs produced during the EPR experiments. Naupliar abnormality (NA) was calculated as a proportion of the total number of eggs hatched. Egg hatch success data

from April 2003 to August 2004 were kindly provided by D. Bonnet to extend the size of the dataset (Bonnet et al., 2009). Naupliar abnormality data from March to August 1994 were transcribed from Pond et al. (1996).

Table 3.2. Potential harmful phytoplankton species reported at station L4, English Channel, UK and investigated for effects on *Calanus helgolandicus* egg hatch success and naupliar abnormalities; PUA – polyunsaturated aldehyde; NVO – non-volatile oxylipin.

Species	Type	Harmful chemical
Alexandrium tamarense	Dinoflagellate	Saxitoxin
Amphidium spp.	Dinoflagellate	Various
Asterionellopsis glacialis	Diatom	PUA
Cerataulina pelagica	Diatom	NVO
Chaetoceros affinis	Diatom	PUA
Chaetoceros compressus	Diatom	PUA
Chaetoceros curvisetum	Diatom	Unknown
Chaetoceros socialis	Diatom	Weak PUA
Cylindrotheca costatum	Diatom	NVO
Dinophysis spp.	Dinoflagellate	Various
Ditylum brightwellii	Diatom	NVO
Emiliania huxleyii	Coccolithophore	Unknown
Fragilaria spp.	Diatom	PUA
Guinardia delicatula	Diatom	PUA
Guinardia striata	Diatom	NVO
Gymnodinium pygmaeum	Dinoflagellate	Unknown
Heterocapsa niei	Dinoflagellate	Unknown
Karenia mikimotoi	Dinoflagellate	Unknown
Lauderia annulata	Diatom	Unknown
Melosira spp.	Diatom	PUA
Navicula spp.	Diatom	NVO
Phaeocystis pouchettii	Prymnesiophyte	PUA
Prorocentrum spp.	Dinoflagellate	Various
Pseudonitzschia delicatissima	Diatom	PUA
Rhizosolenia setigera	Diatom	NVO
Skeletonema costatum	Diatom	PUA
Thalassiosira anguste-lineata	Diatom	PUA
Thalassiosira eccentrica	Diatom	NVO
Thalassiosira punctigera	Diatom	NVO
Thalassiosira rotula	Diatom	PUA
Total *Chaetoceros*	Diatom	-
Total *Guinardia*	Diatom	-
Total *Pseudo-nitzschia*	Diatom	-
Total *Rhizosolenia*	Diatom	-
Total *Thalassiosira*	Diatom	-

3.2.4 Egg mortality estimation

Stage duration

Egg hatching times (i.e. the period from egg laying until hatching) are required to determine mortality rates. The literature was reviewed to collate all experimentally-derived egg-hatch data. Egg-hatch times over a range of temperatures were fitted to a temperature function (T, ^{o}C) using the Bělehrádek function (Bělehrádek and Mann, 1935; Bělehrádek, 1957):

$$D = a(T-\alpha)^{-b} \quad (3.1)$$

where a is a constant that accounts for the difference in the mean slope, α is the biological zero (the theoretical temperature at which development time is infinitely long) and b is the degree of the curvilinearity of the response (Corkett, 1972). The function was solved using the non-linear least squares (nls) tool in R (R Development Core Team, 2012). The value for b for copepods in the literature has frequently been set at 2.05 [(e.g. see Corkett et al., (1986)] and has been applied here too. Data sources are presented in Table 3.3.

Table 3.3. *C. helgolandicus* egg hatching rates used in derivation of egg stage duration Bělehrádek function.

Source	T (^{o}C)	D (days)
Corkett (1972)	0.7	6.91
"	3.9	4.20
"	7.4	2.41
"	14.2	1.37
Rey et al., (2001)	15.0	1.70
"	15.0	1.50
"	15.0	1.30
"	15.0	1.20
Cook et al., (2007)	8.0	2.21
"	12.0	1.48
"	15.0	1.13
Lopez et al., (2007)	15.0	1.30
Bonnet et al., (2009)	9.0	1.28
"	12.0	1.11
"	15.0	1.16

Vertical life-table mortality methods

Egg mortality rates (instantaneous mortality rates) were ascertained using the Basic vertical life table method [Equation 5 in Hirst et al. (2007)], modified and corrected by Hirst and Kiørboe (2002), originally from Peterson and Kimmerer (1994);

$$\frac{N_{egg}\theta_{egg}}{F \times N_{C6f}} = [1 - \exp^{(-\theta_{egg} D_{egg})}] \quad (3.2)$$

where θ_{egg} is the mortality rate of the egg stage and solved by iteration; N_{egg} is the abundance of eggs (m^{-3}), F is the egg production rate (no. eggs $female^{-1}$ d^{-1}), N_{C6f} is the abundance of females (no. m^{-3}), and D_{egg} is the egg hatching time (d).

From a range of possible vertical methods, the Basic method is recommended as the most suitable for early stages and egg mortality in particular (Gentleman et al., 2012; Head et al., 2015). As it is recognised that egg viability is usually <100%, a modification taking account of egg hatching success has been developed (Head et al., 2015), therefore I have also employed this Viability Basic method (Eq. 7a and 8) from Head et al. (2015) to calculate modified egg mortality rates;

$$\frac{N_{egg}\theta_{egg}}{F \times N_{C6f}} = [1 - v \exp^{(-\theta_{egg} D_{egg})}] \quad (3.3)$$

where v is the proportion of viable eggs obtained from egg hatch success experimentation.

3.2.5 Lipid extraction and fatty acid analyses

Seston

Lipid extraction and fatty acid determination followed the protocol given in White et al. (2015). The method involved the collection of 4-6 L seawater from a depth of 10m, (collected using the CTD), which was pre-filtered through a 200 µm mesh, filtered under light vacuum onto ashed glass fibre filters (Whatman GF/F; 47 mm; *n* =

3), then stored in ca 7 mL of chloroform:methanol (2:1 v/v+0.05% w/v Butylated hydroxytoluene) in combusted glass vials at −20 °C prior to lipid extraction. Extracts were generated using sonication to disrupt cellular material (Folch et al., 1957). Samples were further extracted with 100% chloroform to ensure complete lipid extraction. Lipid-containing layers were pooled and dried under vacuum and stored at −80 °C in 1 mL of chloroform:methanol (2:1).

Fatty acid determination involved the drying of 400 µL of lipid extract under a gentle stream of nitrogen before adding nonadecanoic acid (C19:0; 20 µL, 1 mg mL^{-1}) as an internal standard. Cellular fatty acids were converted directly to fatty acid methyl esters (FAMEs) by adding 1 mL of transesterification mix (95:5 v/v 3 N methanolic hydrochloric acid; 2, 2-dimethoxypropane) followed by incubation at 90 °C for 2 h. After cooling, FAMEs were recovered by addition of 1% w/v NaCl solution (1 mL) and n-hexane (1 mL) followed by vortexing. The upper hexane layer was injected directly into the Gas Chromatography-Mass Spectrometry (GC–MS) system and FAMEs were separated on a fused silica capillary column (30 m × 0.25 mm × 0.25 µm; Omegawax™ 250, Supelco, Sigma-Aldrich, Gillingham, Dorset, UK) using an oven temperature gradient of 75 °C to 240 °C at 4 °C min^{-1} followed by 15 min hold time. Helium was used as the carrier gas (1 mL min^{-1}) and the injector and detector inlet temperatures were maintained at 280 °C and 230 °C, respectively. FAMEs were identified using retention times and qualifier ion response, and quantified using respective target ion responses. All parameters were derived from calibration curves generated from a FAME standard mix (Supelco, Sigma-Aldrich, Gillingham, Dorset, UK). FA data were available in units of µg L^{-1} and as the proportion of total FA.

Calanus helgolandicus eggs

During June-November 2013, the remaining eggs following the egg production experiments (and those not required for egg hatching experiments) were collected in a 2 mL Eppendorf with as small an amount of FSW as possible and frozen at -20°C. The number of eggs preserved was noted. As for seston FA, extracts were generated using sonication and 100% chloroform to disrupt cellular material (Folch et al., 1957) and

FAMES were derived using GC-MS. FA data were available in units of ng egg^{-1} and as the proportion of total FA.

3.2.6 Statistical methods

C. helgolandicus egg hatch success and naupliar deformity proportion data were arcsine-square-root transformed before any regression analyses were performed to linearise the data. Egg and seston fatty acid proportion data were also arcsine-square-root transformed. Phyto-, micro- and zooplankton data were log transformed (as $\log_{10}$ x+1). Both simple and multiple regression models were generated using environmental, phyto/micro-plankton and FA data (see more on FA data below). Homogeneity, normality and independence of model residuals were examined. Where residuals displayed heterogeneity, variance-covariance structures were applied via a GLS (generalised least squares) model. Date was included in the regression models to account for temporal autocorrelation in residuals.

Seston fatty acid data were analysed using principal components analysis (PCA) to reduce the number of variables employed in multiple regression analyses. PCA arranges similar variables on components and calculates the relative loading [eigenvectors on a scale of -1 (negative loading) to 1 (positive loading)], allowing key variables to be selected (Table 3.4). FA with the highest loadings (+ and –ve) from each principal component were included in multiple regression analysis (Table 3.5).

Fatty acid profiles of both egg and seston (relative proportion data) were subject to separate non-metric multidimensional scaling (MDS) analyses to investigate fatty acid composition of each sample. MDS is an ordination technique that represents samples as points in low-dimensional space, so that samples occurring close together in a plot are very similar in composition.

The analysis of the effects of potential toxic species on eggs involved an exploration of toxic species (listed in Table 3.2) reported during the month prior to the timings of low EHS and high NA (using mean proportions as threshold low EHS/high NA levels), to ascertain if there was any co-occurrence.

Egg mortality rates were both positive and negative values, therefore a $\log_{10}[x + (\min(x) +1)]$ transformation was undertaken to convert all data to positive values and normalise the distribution. Following Hirst et al. (2007), mortality rates were LOESS-smoothed (f=0.2) before further analysis for relationships with potential predators (*C. helgolandicus* copepodites, total copepods, total medusae, total meroplankton, etc.) and environmental variables (SST, SI and chlorophyll-*a*, etc.). All regression analyses were performed on the LOESS-smoothed mortality rates.

All regression analyses were performed in the R programming environment (R Development Core Team, 2012). Reduced major axis (RMA) regressions were performed using the RMA Software of Bohonak and van def Linde (2004). All multivariate statistical analyses (MDS and PCA) were performed using PRIMER-E v6 (Clarke and Gorley, 2006).

Table 3.4. Eigenvectors from five principal components of Principal Components Analysis (PCA) of seston fatty acids (proportions); numbers in bold represent highest loadings.

Seston fatty acid	PC1	PC2	PC3	PC4	PC5
C16:0	**-0.36**	-0.02	-0.24	0.07	-0.12
C16:1(*n*-7)	-0.16	**0.32**	**0.31**	0.14	-0.15
C16:1/C16:0 ratio	-0.02	**0.32**	**0.39**	0.10	-0.05
C16:4 (*n*-3)	0.27	0.11	**-0.36**	0.00	-0.18
C16:4 (*n*-1)	0.08	0.30	0.02	**0.34**	-0.06
C18:2(*n*-6)	-0.15	-0.28	0.00	**0.34**	0.19
C18:3(*n*-6)	0.17	0.24	-0.21	-0.29	**0.34**
C18:3(*n*-3)	0.08	**-0.31**	-0.17	**0.33**	-0.26
C18:4(*n*-3)	**0.32**	-0.05	-0.24	0.22	**-0.30**
C20:4(*n*-6)	0.08	0.28	-0.23	0.14	0.14
C20:5(*n*-3)	**0.30**	0.24	0.10	0.22	0.03
C22:6 (*n*-3)	0.14	-0.25	**0.31**	-0.24	0.32
C22:6/C20:5 ratio	0.00	0.12	0.00	**-0.40**	-0.57
n-3/*n*-6 ratio	0.12	**-0.26**	**0.34**	-0.02	**-0.35**
Tot MUFA	-0.28	0.11	0.25	**0.30**	-0.05
Tot PUFA	**0.41**	-0.07	0.06	0.11	0.10
Tot SFA	**-0.37**	0.04	-0.17	-0.24	-0.10

Table 3.5. Seston fatty acids included in multiple regressions with *Calanus helgolandicus* egg hatch success and naupliar abnormalities, following PCA of all seston fatty acids (proportions) and expert knowledge.

Seston fatty acid
C16:0
C16:1(*n*-7)
C16:1/C16:0 ratio
C16:4(*n*-3)
C16:4(*n*-1)
C18:2(*n*-6)
C18:3(*n*-3)
C18:4(*n*-3)
C20:4(*n*-6)
C20:5(*n*-3)
C22:6(*n*-3))
C22:6/C20:5 ratio
n-3/*n*-6 ratio
Tot MUFA
Tot PUFA
Tot SFA

3.3 Results

The average L4 seasonal environment over the time periods analysed in this study is presented in Figure 3.1. Sea surface temperature ranged from ~7^{o}C in spring (low of 7.3^{o}C on 9th March 2013) to ~19^{o}C during late summer and autumn (high of 18.8^{o}C on 9th August 2004) with an annual mean of ~13^{o}C (Figure 3.1a). Thermal stratification occurred between May and September, with peak SI indices recorded August to September when surface temperatures were >4^{o}C greater than at depth (Figure 3.1a). Mean fortnightly chlorophyll-*a* concentrations ranged from 0.3-2.4 mg m^{-3} and peaked from April to May (during the spring diatom bloom), and again in late summer to autumn (with the dinoflagellate bloom). Lowest concentrations occurred during winter and also in the summer stratified period (Figure 3.1b).

Phytoplankton biomass was low throughout November to March, and was superseded by the spring diatom bloom and an increase in flagellates. Dinoflagellates appeared in summer and obtained peak biomass in September (Figure 3.1c). Microzooplankton biomass was elevated from spring though to the end of autumn,

and peaked in the summer (Figure 3.1d). Ciliate biomass increased in mid-winter and constituted between 50-75% of the biomass between February and May. Throughout June and July, heterotrophic flagellates dominated and peak microzooplankton biomass levels were reached. During the autumn, both heterotrophic dinoflagellate and ciliate biomass contributed ~50% to the biomass.

Predator biomass was generally high throughout March to October and was dominated by copepods (Figure 3.1e). Meroplankton biomass was greatest during the spring, whilst gelatinous zooplankton biomass was high during summer and autumn. Non-copepod holoplankton occurred mostly in the spring and fish larval biomass was greatest in spring and summer; both contributed little to total predator biomass.

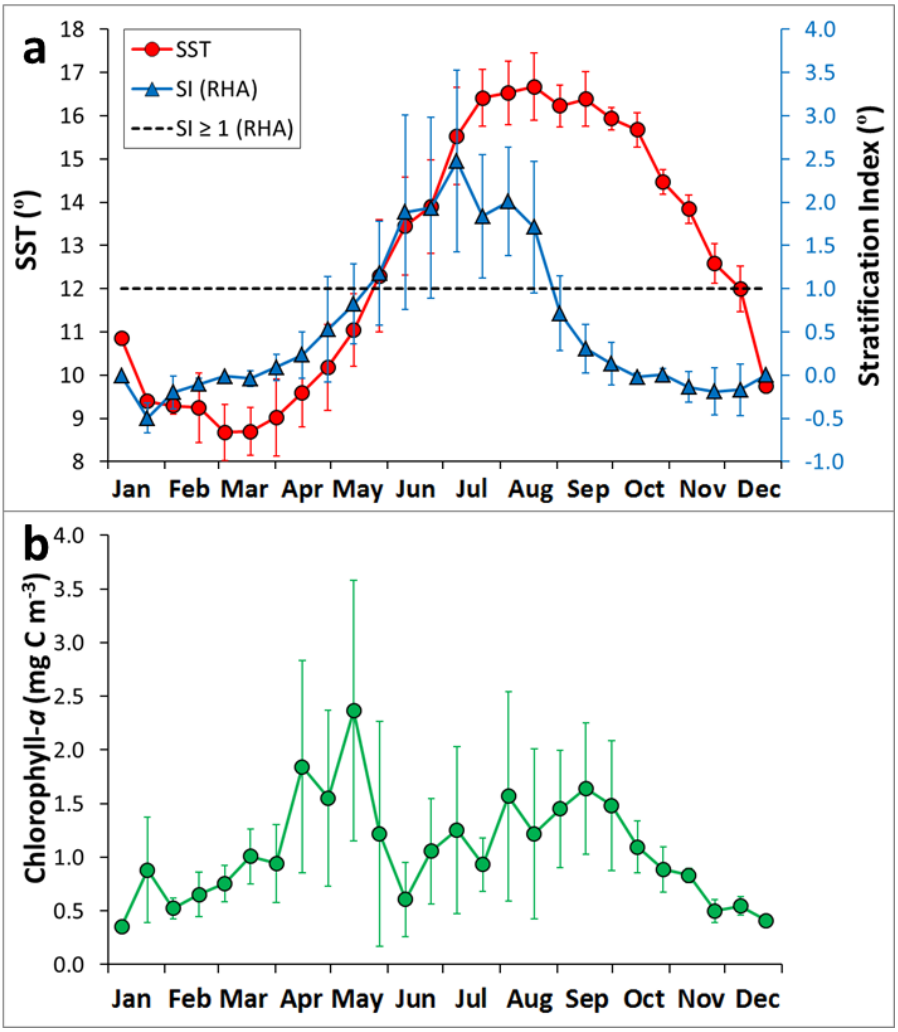

Figure 3.1. Seasonal variation (fortnightly means and error bars representing standard deviation) of (a) sea surface temperature (SST) (°C) and Stratification Index (difference in temperature between surface and 30m) (2002-04, 2013, 2015, Jan-Sep 2016); (b) chlorophyll-*a* concentration; RHA is right hand axis.

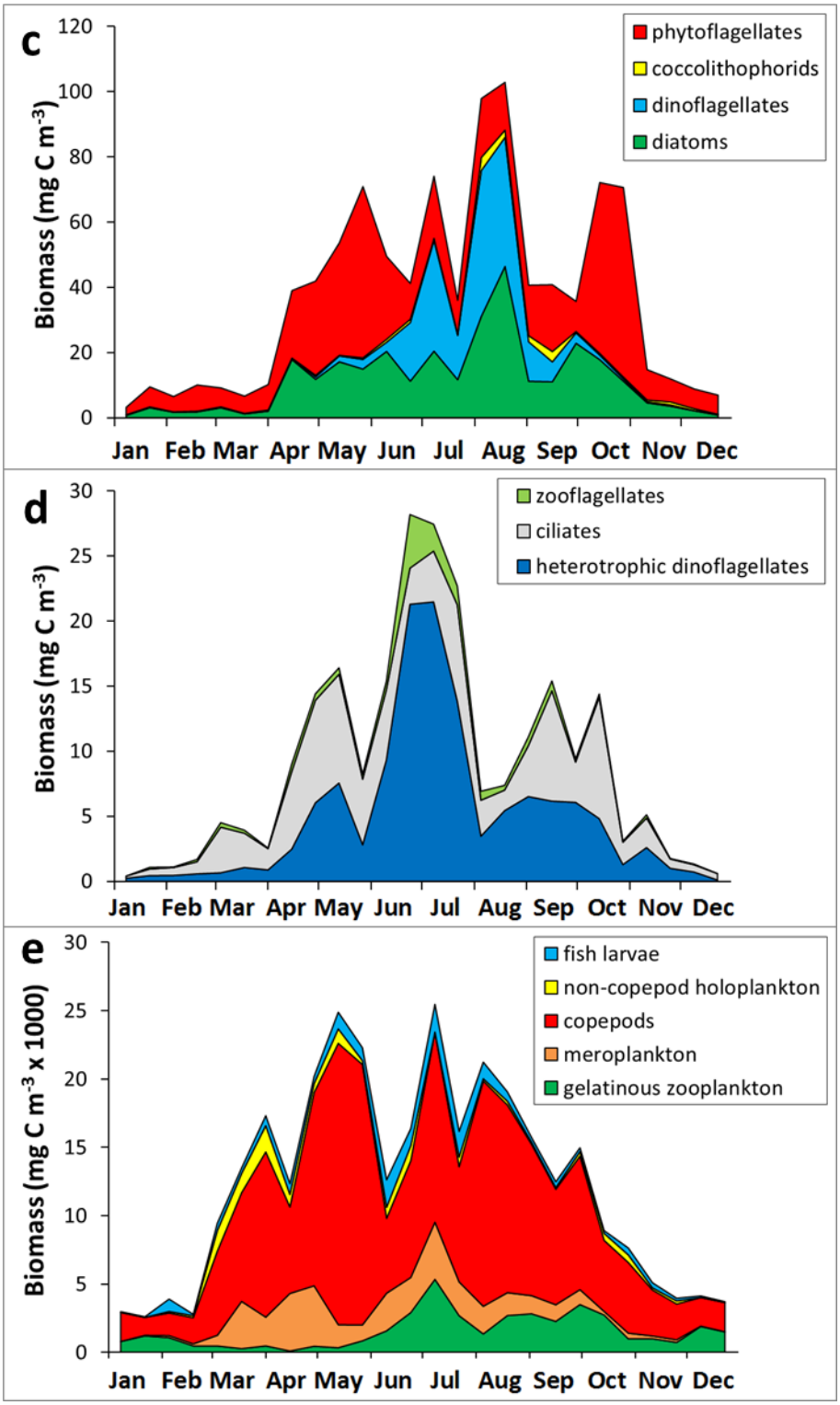

Figure 3.1 contd. Seasonal variation (fortnightly means and error bars representing standard deviation) of (c) phytoplankton biomass (2002–2004, 2013); (d) microzooplankton biomass (2002-2004, 2013), and (e) main predator groups biomass (2002–2004, 2013).

The fatty acid profile of the seston was measured on a near-weekly basis throughout 2013. Total seston fatty acid concentrations ranged from 4 - ~50 µg L^{-1}, with a mean of 15.4 µg L^{-1}. Concentrations rose from background levels from May through to September, with a peak of 48.9 µg L^{-1} occurring in August 2013. The dominant fatty acids included C16:0 – mean of 23%, C22:6(*n*-3) docosahexaenoic acid (DHA) - mean of 12%, C14:0 - mean of 11%, C16:1(*n*-7) - mean of 8% and C20:5(*n*-3) eicosapentaenoic acid (EPA) - mean of 7.5% (Figure 3.2).

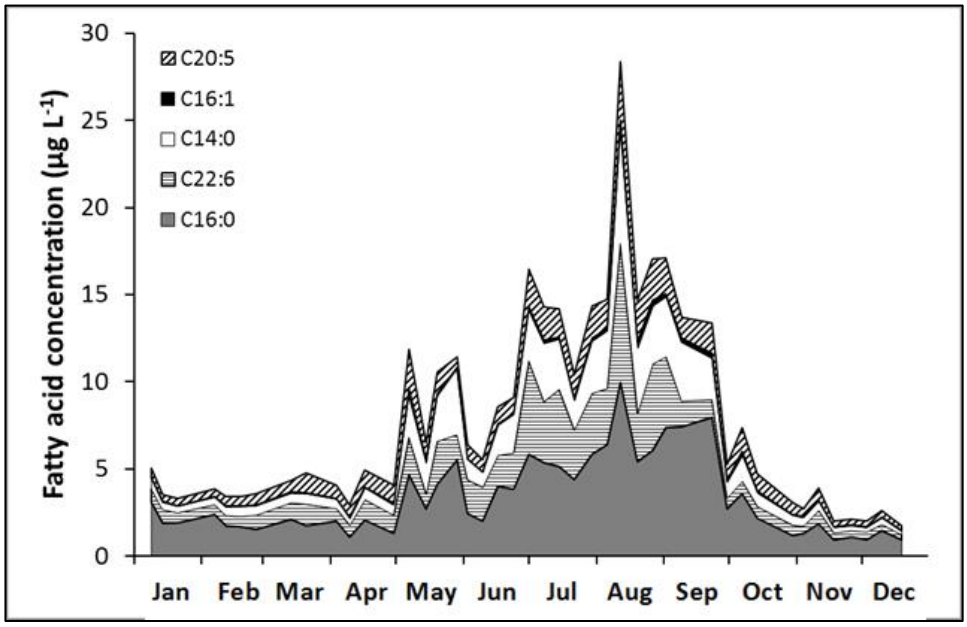

Figure 3.2. Dominant fatty acid concentrations in seston (2013), comprising ~60% of annual FA concentration (C16:0 – 23%; C22:6(*n*-3) – 12%; C14:0 – 11%; C16:1(*n*-7) – 8%; C20:5(*n*-3) – 7.5%).

3.3.1 Calanus helgolandicus *abundance*

Calanus helgolandicus copepodites were continually recorded at L4, albeit at very low levels during December and January (~5-10 CI-CVI copepodites m^{-3}). Total *C. helgolandicus* abundance increased in February and often exhibited two peaks, the first in spring, with a second higher peak in late-summer/early autumn (Figure 3.3a). Abundances ranged from 0.4 to 1251 *C. helgolandicus* m^{-3} with an average of 88.5 m^{-3} individuals (median of 33.3 m^{-3}). Female adult *C. helgolandicus* abundance was very low (~1 m^{-3}) through November and December, but the population increased steadily

from January to mid-summer. The 25-year (1988-2012) L4 time-series showed that female abundance typically reduces dramatically after June (Maud et al., 2015) (Chapter Two), however the truncated dataset analysed during this study showed a second peak in abundance in the autumn (Figure 3.3a). Female abundance ranged from 0-49 m^{-3}, with an average of 7.3 m^{-3} (median of 3.6 m^{-3}).

3.3.2 Egg production and abundance at L4

C. helgolandicus egg production usually continues all year round at this coastal NE Atlantic site. Egg production rates (EPR) were lowest in winter, but started to increase from February, well before the phytoplankton spring bloom. Production rates continued to increase through the spring and peaked between April through June, followed by a decline through autumn into winter. Mean EPR over the time considered here was 13.3 eggs $female^{-1}$ d^{-1} and a maximum of 54.8 eggs $female^{-1}$ d^{-1} was recorded on 22nd April 2003 (Figure 3.3b).

Egg abundance in the water column was very low during October through to January each year (~10 eggs m^{-3}), but also increased from February onwards as EPR increased. The concentration of eggs continued to increase strongly throughout spring and summer as both EPR and numbers of females increased, and declined from October onwards (Figure 3.3b).

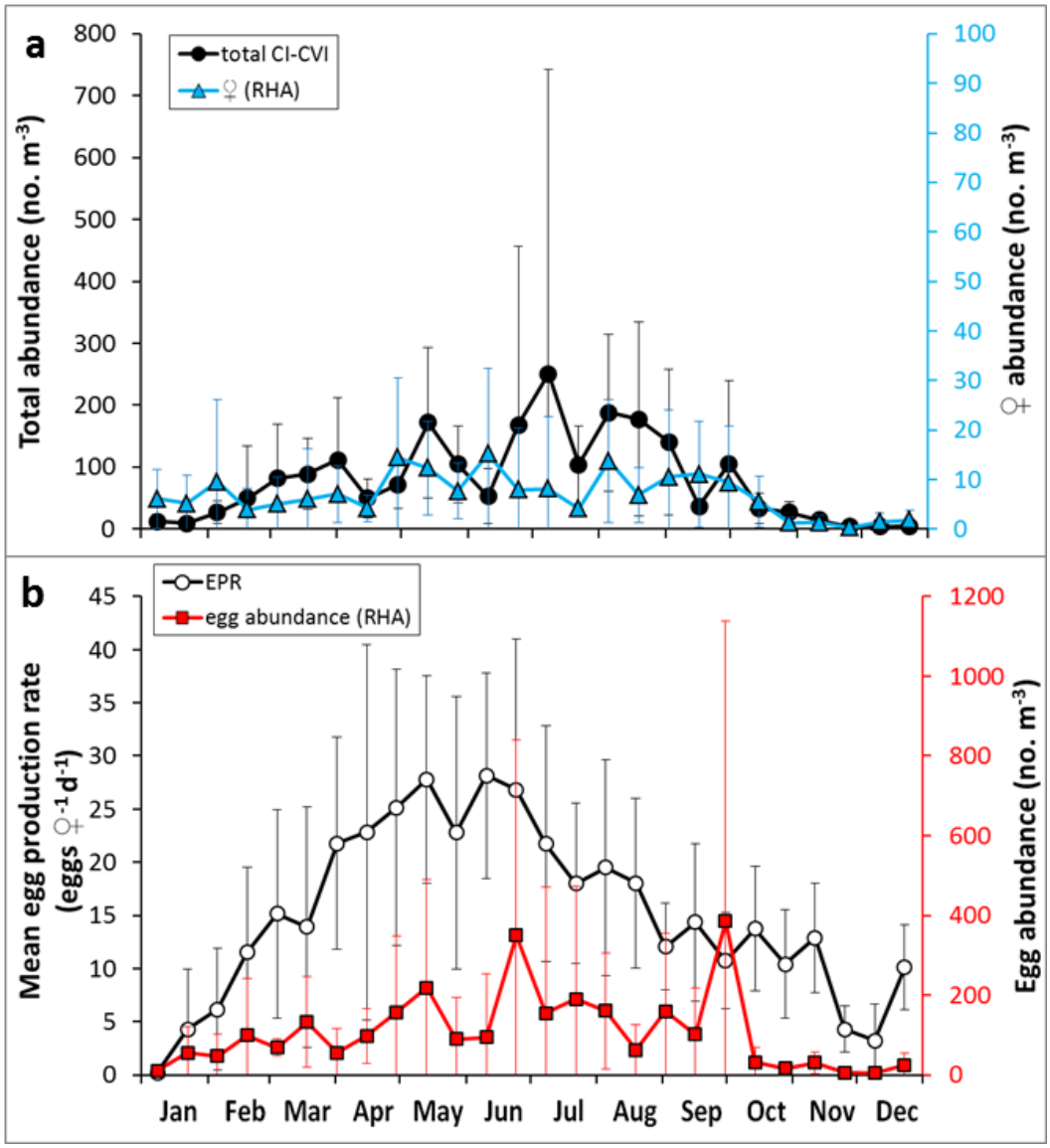

Figure 3.3. *Calanus helgolandicus* seasonality (fortnightly mean and error bars representing standard deviation) (a) total CI-CVI abundance and ♀ abundance; (b) mean egg production rate and egg abundance. RHA is right hand axis.

3.3.3 Egg hatch success and relationships with diet and environment

Calanus helgolandicus EHS was determined for a total of 93 weeks over the study period and ranged from 0-100%, with mean monthly rates of 25-80% (Figure 3.4). Mean winter and spring EHS rates were 47% and 35% respectively. Egg hatch success was much higher during the second half of the year with mean summer and autumn rates of 75% and 68% respectively. This analysis builds upon the evidence presented in Maud et al. (2015), and substantiates a continued pattern of low egg viability in winter and spring followed by consistently high viability in summer and

autumn. Statistical analysis of seasonal EHS rates indicated that spring rates were significantly lower than all other seasons (Kruskal-Wallis H = 24.6, df = 3, p = 0.0001).

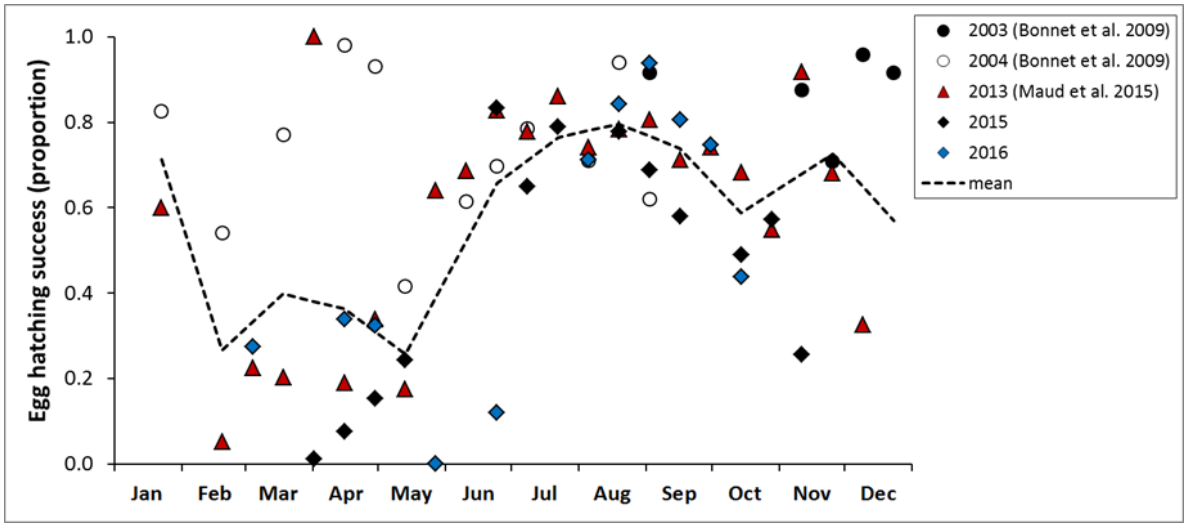

Figure 3.4. Seasonal variation of egg hatch success annual fortnightly means, 2003 and 2004 data kindly supplied by Bonnet et al. (2009), 2013 data taken from Maud et al. (2015), alongside 2015 and 2016 data; black line indicates mean monthly egg hatch success.

Table 3.6 summarises the relationships between EHS and environmental and maternal diet-related factors. There was a strong positive relationship with SST (R^2 = 0.270, n = 89, p <0.000001 with date included as a variable), and also with Stratification Index (R^2 = 0.081, n= 89, p = 0.0097 with date included). There were no relationships with surface chlorophyll-α concentrations or diatom abundance/biomass; however, dinoflagellates, coccolithophores and phytoflagellates (biomass) were positive predictors of EHS. A backwards stepwise regression including all environmental and microplankton biomass factors, resulted in a model where only SST remained as a significant predictor. When microplankton biomasses only were included, dinoflagellates were the only significant variable in a stepwise multiple regression model.

Biomass data from the previous week (1 week lag) also highlighted dinoflagellates and phytoflagellates as important predictors, but in addition, diatoms, ciliates and heterotrophic dinoflagellates also explained EHS. Multiple regressions with

lagged data produced significant models with ciliates + dinoflagellates, and ciliates + coccolithophores (Table 3.7).

Table 3.6. Coefficient of determination (R^2) of linear regressions between egg hatch success rate or naupliar abnormality rate and sea surface temperature (SST), Stratification Index (SI) and microplankton biomasses. All proportions were arcsine-square-root transformed. All biomass data were $\log_{10}(x+1)$ transformed. Significance of regression is indicated by asterisk: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, R^2 value only indicates $p < 0.1$, ns is not significant.

Predictor	Egg hatch success (proportion)	Naupliar abnormalities (proportion)
SST	0.274***	0.119*
SI	0.085**	ns
Diatom biomass	ns	ns
Diatom biomass (-1 wk)	0.093*	ns
Dinoflagellate biomass	0.233***	ns
Dinoflagellate biomass (-1 wk)	0.145**	ns
Coccolithophore biomass	0.071*	ns
Coccolithophore biomass (-1 wk)	ns	ns
Phytoflagellate biomass	0.0722*	0.067
Phytoflagellate biomass (-1 wk)	0.154**	ns
Ciliate biomass	0.0562	ns
Ciliate biomass (-1 wk)	0.152**	ns
Heterotrophic dinoflagellate biomass	ns	ns
Heterotrophic dinoflagellate (-1 wk)	0.090*	ns

Table 3.7. Generalised least squares (GLS) analysis of environmental and maternal diet-related variables predicting *C. helgolandicus* egg hatch success and naupliar abnormalities: coefficients, standard error (SE), *t*-value, *p*-value and AIC value for multi- GLSs; SST = sea surface temperature.

Model predictor (s)	Coefficient (slope)	SE	*t*-value	*p*-value	AIC
Egg hatch success (EHS)					
Log_{10}(dinoflagellate biomass (-1 wk) +	0.175	0.85	2.05	0.046	44.65
log_{10}(ciliate biomass (-1 wk)	0.311	0.144	2.16	0.036	
Log_{10}(ciliate biomass (-1 wk) +	0.343	0.118	2.917	0.005	40.97
log_{10}(coccolithophores biomass (-1 wk)	0.498	0.130	3.832	0.0004	
Naupliar abnormalities (NA)					
SST +	-0.030	0.008	-3.72	0.0005	24.98
log_{10}(ciliate biomass)	0.180	0.069	2.60	0.0123	
Log_{10}(ciliate biomass)+	0.136	0.066	2.057	0.0448	22.81
log_{10}(coccolithophore biomass) +	-0.236	0.099	-2.40	0.0202	
log_{10}(phytoflagellates biomass)	-0.228	0.084	-2.73	0.0086	

The investigation of potential toxic phytoplankton species revealed that there were 17 sample dates (18%) when EHS was below the mean (58%), and of these ~90% could not be associated with any potential toxic species, either during the week of sampling or the previous month. There were two dates when elevated levels of a number of toxic species co-occurred with a low EHS. High (but not peak) levels of *Chaetoceros curvisetus*, *Pseudo-nitzschia delicatissima*, *Skeletonema costatum*, *Thalassiosira rotula* and *Phaeocystis pouchetii* coincided with an EHS of < 30%.

3.3.4 Naupliar abnormalities and relationships with maternal diet and environment

Abnormalities in newly hatched NI nauplii (NA) (Figure 3.5) were determined over a total of 89 weeks. NA ranged from 0-50% of eggs hatched, with a mean of 8%. Higher levels of NA were seen during the first six months of each year, particularly during spring of 2013 and 2015 (Figure 3.6).

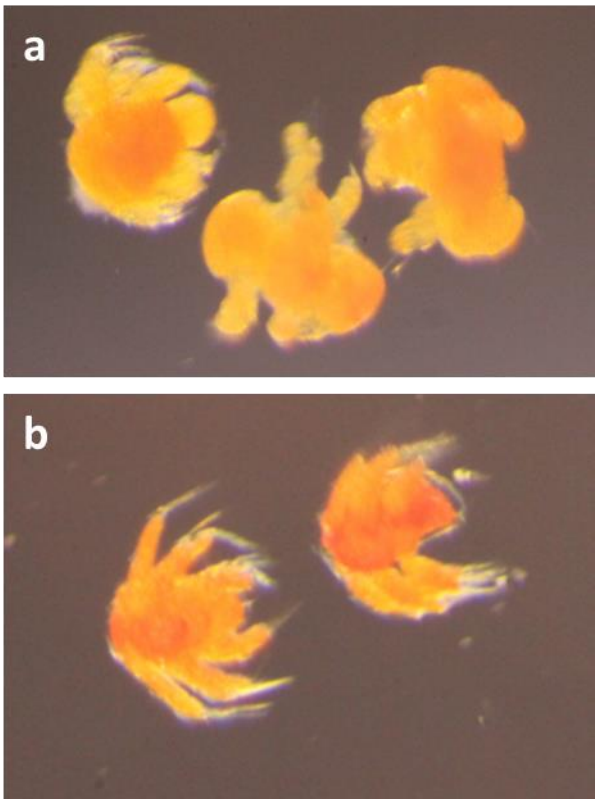

Figure 3.5. ***C. helgolandicus*** **newly hatched (a) nauplii with deformities, and (b) healthy nauplii (stained orange as preserved in 2% (final concentration) acid-Lugol's iodine solution).**

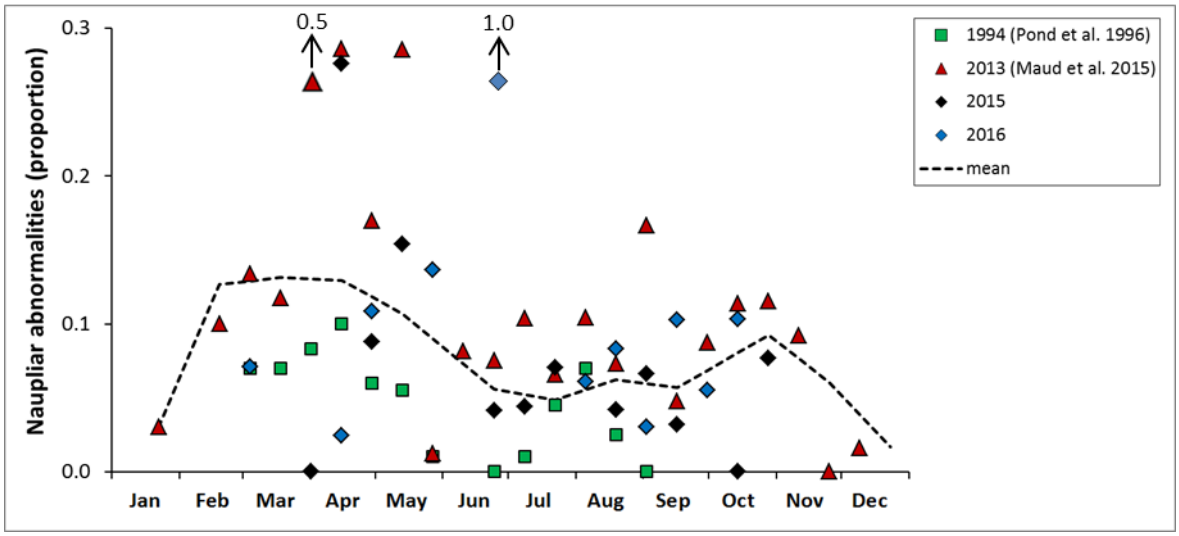

Figure 3.6. ***Calanus helgolandicus*****. Seasonal variation of naupliar abnormalities (annual fortnightly means), 1994 data extracted from Pond et al. (1996), 2013 data taken from Maud et al. (2015), alongside my 2015 and 2016 data; black line indicates mean monthly abnormalities, excluding 100% NA outlier from 20/6/16.**

The general mean seasonal cycle indicates a March-June increase in the rate and variation of naupliar deformities, and spring abnormality rates (mean of 12%) were greater than other seasons, although not significantly. There was an inverse relationship between mean monthly EHS and NA, so that when egg viability was low, there were an increased number of abnormal nauplii [reduced major axis (RMA) regression analysis intercept = 0.22, slope = -0.234, R^2 = 0.606, n = 12, p = 0.0023].

Naupliar abnormalities were inversely related to SST (R^2 = 0.12, n = 86, p = 0.006, with date included as a variable). Phytoflagellate biomass was the only diet-related variable close to being a significant (inverse) predictor of NA (R^2 = 0.067, n = 55, p = 0.056). A multiple backwards stepwise regression starting with all environmental and microplankton data resulted in a model including SST and ciliate biomass. A backwards stepwise regression including microplankton biomass only resulted in a model incorporating coccolithophore, phytoflagellate and ciliate biomass (Table 3.7).

Naupliar deformity rates of greater than 10% were investigated for co-occurrence with toxic phytoplankton. Five dates when NA was between 11% and 17% coincided with multiple potential toxic species, including *Cerataulina pelagica*, *Chaetoceros* spp., *Gymnodinium pygmaeum*, *P. delicatissima, Prorocentrum micans, Guinardia* spp.*, Thalassiosira* spp. and *Karenia mikimotoi.* However, none of the highest NA rates (20-50%) occurred at the same time as toxic blooms.

3.3.5 Calanus helgolandicus *egg and seston fatty acids*

Fatty acid profiles of eggs were determined for only 16 weeks from June to November 2013. Therefore, unfortunately, this only provides insight into the most productive half of the year. Total egg fatty acids were variable throughout this period, and ranged from 14 -~50 ng egg^{-1}, with a mean of 36 ng egg^{-1}. PUFAs accounted for an average of 50% of egg fatty acids; SFAs accounted for ~36% and MUFAs contributed ~13%.

The dominant fatty acids showed similarities to that of the seston and included C16:0, C22:6(*n*-3), C20:5(*n*-3), C16:1(*n*-7) and C18:0 (Figure 3.7). However mean proportions were different, C22:6(*n*-3) and C20:5(*n*-3), in particular were much higher

than in the seston. Linear regression analyses of individual fatty acids determined that C22:6(*n*-3) and C20:5(*n*-3) were more concentrated in the eggs and were not related to seston proportion. The majority of other fatty acids in the eggs also did not mirror that of the seston.

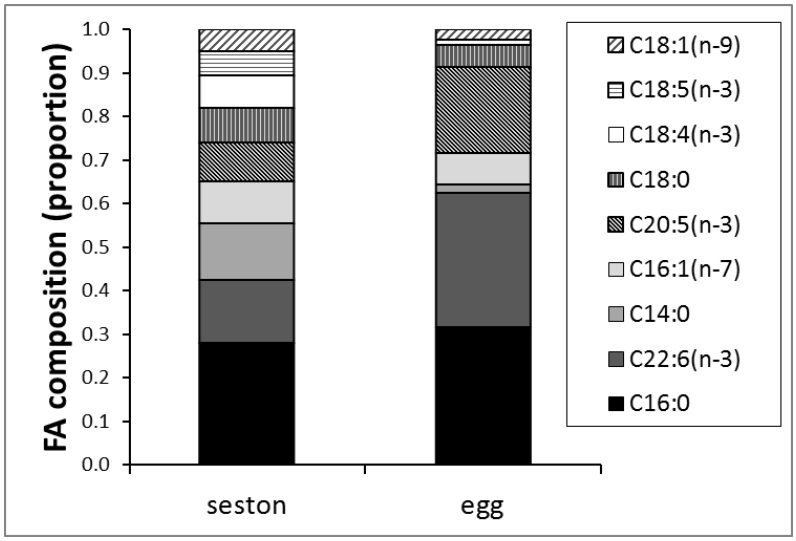

Figure 3.7. Composition of the dominant fatty acids (mean June-November) comprising ~80% of total FA concentration in seston at L4 and *Calanus helgolandicus* eggs laid from wild ♀ collected from station L4.

Non-metric multidimensional scaling (MDS) of the seston fatty acid (proportion) profiles illustrates the weekly variation in composition and provides evidence of some clustering of months, indicating seasonal changes (Figure 3.8a). However, 14 out of 16 egg fatty acid compositions (88%) were clustered in exactly the same position in space on the MDS plot (Figure 3.8b), indicating that the composition of these samples was highly similar.

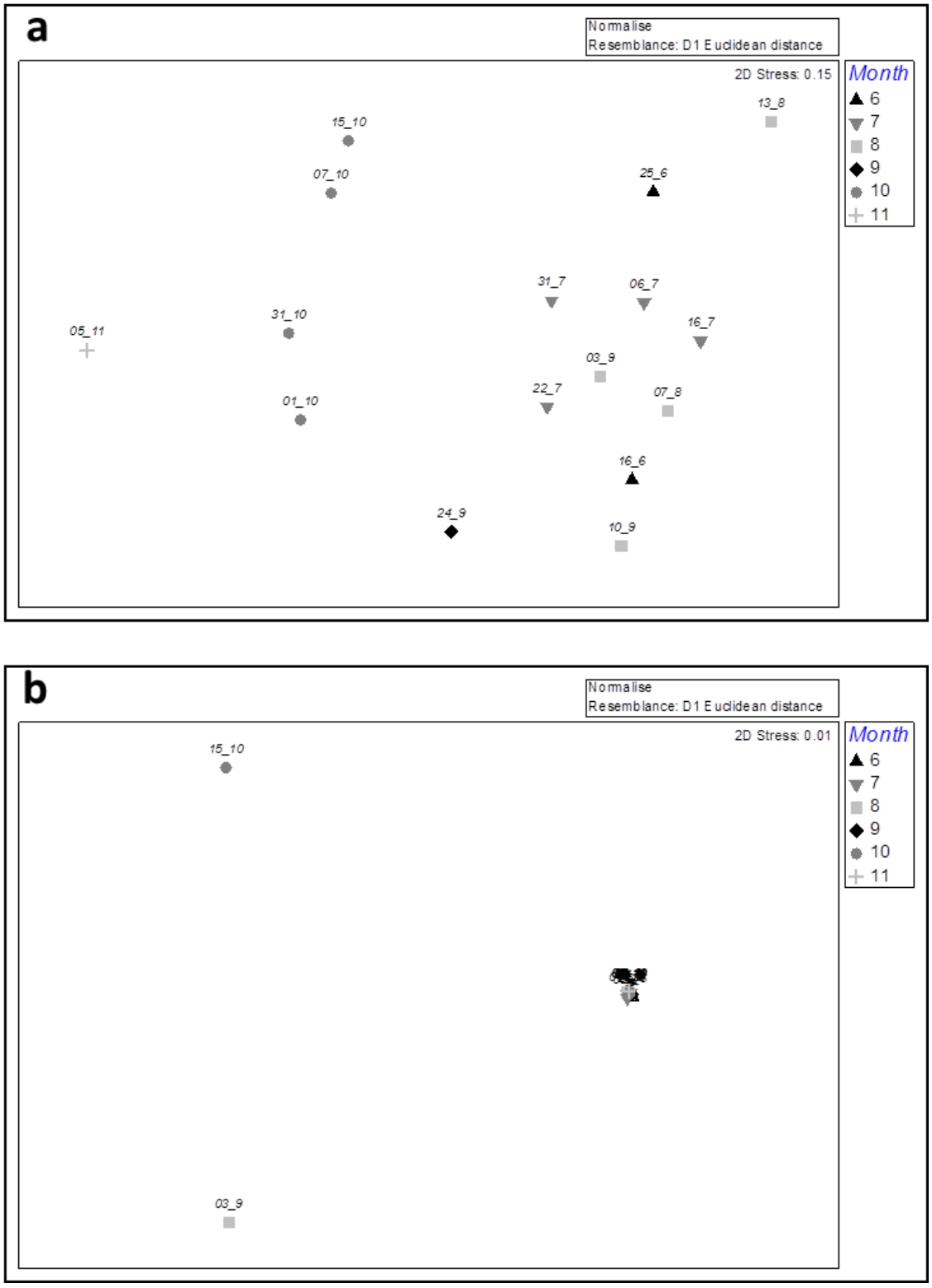

Figure 3.8. Non-metric multidimensional scaling plots representing (a) seston fatty acid composition (proportion) of seawater collected from station L4 (June-November 2013); and (b) *Calanus helgolandicus* egg fatty acid composition (proportion) from ♀ collected from station L4 (June-November 2013), labels in italics represent the date of sampling.

3.3.6 Fatty acids, egg hatch success and naupliar abnormalities

Linear regressions of seston fatty acids with both EHS and NA are presented in Table 3.8. Numerous fatty acids and EHS were positively related, including the diatom biomarkers C16:0 (concentration), C16:1(*n*-7) (concentration), and the dinoflagellate biomarkers C18:2(*n*-6) (proportion), C18:3(*n*-3) (concentration) and C22:6(*n*-3) (concentration). The diatom biomarkers C20:5(*n*-3) (proportion) and C16:4(*n*-1) (proportion) were negatively related to EHS, although only C16:4(*n*-1) significantly (Figure 3.9a and Figure 3.9c). A multiple backwards stepwise regression analysis starting with a reduced set of seston fatty acids (proportions) (following the PCA analysis, Table 3.5), resulted in a model with C18:2(*n*-6) as the only significant variable (R^2 =0.243, n = 34, p = 0.0031). A significant model (starting with environmental and reduced seston fatty acid (proportion) variables) incorporated C16:0, C18:3(*n*-3), C20:5(*n*-3) and SI (R^2 =0.487, n = 34, p = 0.0005; all +ve).

The proportion of NA was positively related to the C16:1/C16:0 ratio, C16:4(*n*-1) concentration (Figure 3.9d) and C20:5(*n*-3) concentration (Figure 3.9b) (Table 3.8). A multiple backwards regression starting with reduced (Table 3.5) seston fatty acid (proportions) produced a model including C16:0 as a negative predictor and C16:4(*n*-1) as a positive predictor (R^2 =0.293, n = 31, p = 0.0079).

As stated earlier, egg fatty acid composition was determined from June to November 2013. There were no significant relationships between any fatty acids (proportion) and EHS or NA. However, close-to-significant, inverse relationships were determined between NA and C16:1/C16:0 ratio (R^2 = 0.218, n = 16, p = 0.0685) and also C22:6(*n*-3) (R^2 = 0.183, n = 16, p = 0.0986).

Table 3.8. *Calanus helgolandicus*. Coefficient of determination (R^2) of linear regressions between weekly egg hatch success rate (EHS) and naupliar abnormality rate (NA) (proportions) and specific seston fatty acids (proportion and µg L^{-1} concentration) and ratios (January-December 2013). All proportions are arcsine-square-root transformed. Significance of relationship is indicated by asterisk: * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$; $n = 34$; SFA saturated fatty acids, PUFA polyunsaturated fatty acids, MUFA monounsaturated fatty acids.

Predictor	Metric	EHS (proportion)	NA (proportion)
C16:0	proportion	0.075	0.177*
"	µg L^{-1}	0.155*	0.014
C16:1(*n*-7)	proportion	0.0044	0.05
"	µg L^{-1}	0.134*	0.0001
C16:1/C16:0 ratio	-	0.034	0.148*
C16:4(*n*-1)	proportion	0.129*	0.135*
"	µg L^{-1}	0.007	0.019
C18:1(*n*-9)	proportion	0.246**	0.077
"	µg L^{-1}	0.176*	0
C18:2(*n*-6)	proportion	0.284**	0.04
"	µg L^{-1}	0.164*	0.017
C18:3(*n*-3)	proportion	0.095	0.04
"	µg L^{-1}	0.128*	0.049
C20:4(*n*-3)	proportion	0.002	0.11
"	µg L^{-1}	0.326**	0
C20:5(*n*-3)	proportion	0.085	0.174*
"	µg L^{-1}	0.078	0.0011
C22:6(*n*-3)	proportion	0.023	0
"	µg L^{-1}	0.121*	0.004
C22:6/C20:5 ratio	-	0.106	0.045
n-3/*n*-6 ratio	-	0.06	0.003
SFA	proportion	0.0064	0.141*
"	µg L^{-1}	0.145*	0.01
PUFA	proportion	0.036	0
"	µg L^{-1}	0.094	0.002
MUFA	proportion	0.126*	0
"	µg L^{-1}	0.162*	0.004

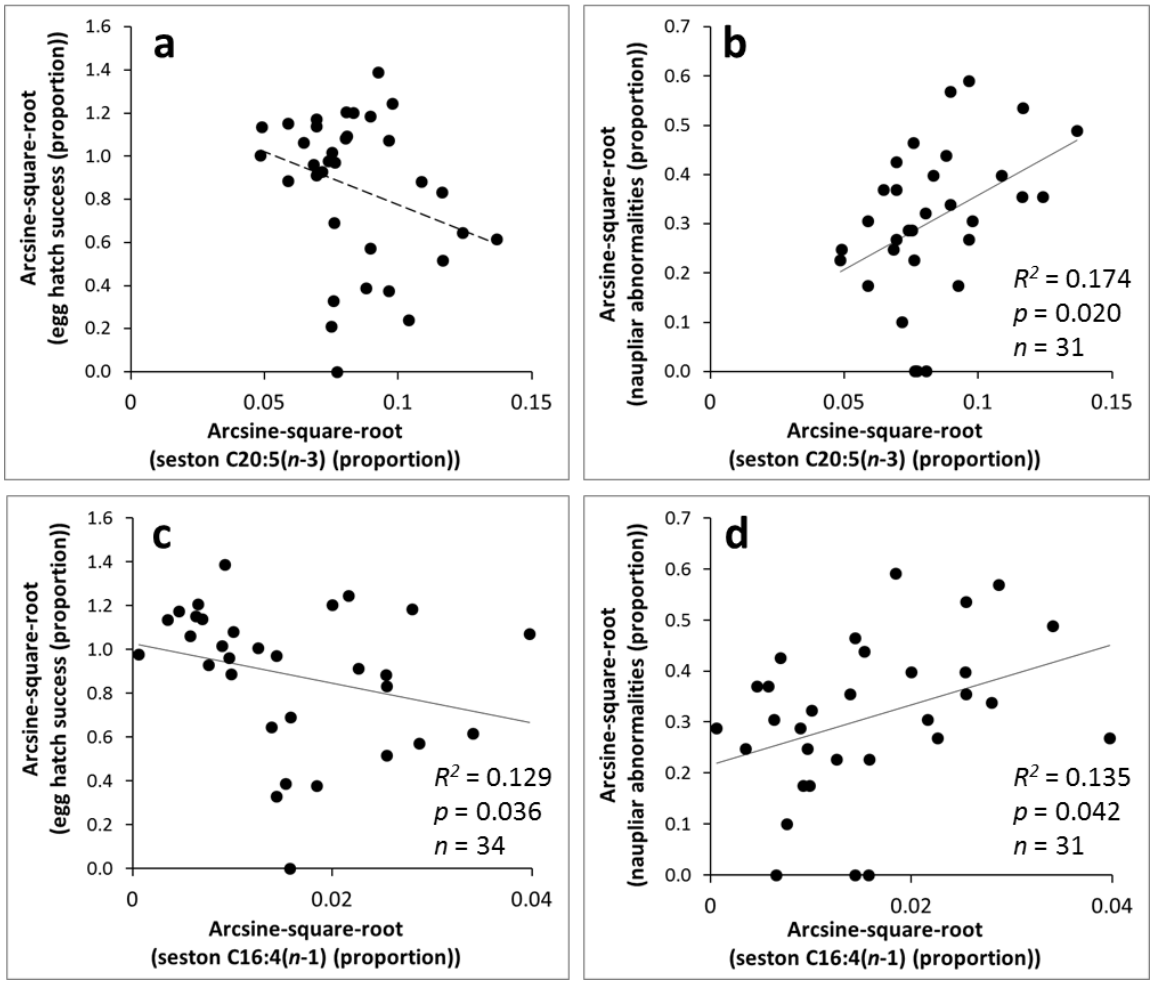

Figure 3.9. Relationship between selected seston fatty acids (proportion) and *Calanus helgolandicus* egg hatch success (EHS) and naupliar abnormalities (NA) (2013); (a) C20:5(*n*-3) and EHS; (b) C20:5(*n*-3) and NA; (c) C16:4(*n*-1) and EHS; (d) C16:4(*n*-1) and NA; (significant regressions indicated by solid black line, close-to significant regressions indicated by dashed black line).

3.3.7 Egg mortality

Calanus helgolandicus egg mortality rates were determined for 107 weeks, and ranged between -2.1 and 236 d^{-1} (median of 0.53 d^{-1}). However, > 90% of the rates fell below 10 d^{-1} (Figure 3.10). The annual mean seasonality confirmed that increased mortality occurred from March to September, with two annual peaks, the first in June and a second lower peak in August. Egg mortality rates were significantly higher in spring and summer than autumn and winter (Kruskal-Wallis H = 33.07, df = 3, p <0.0001).

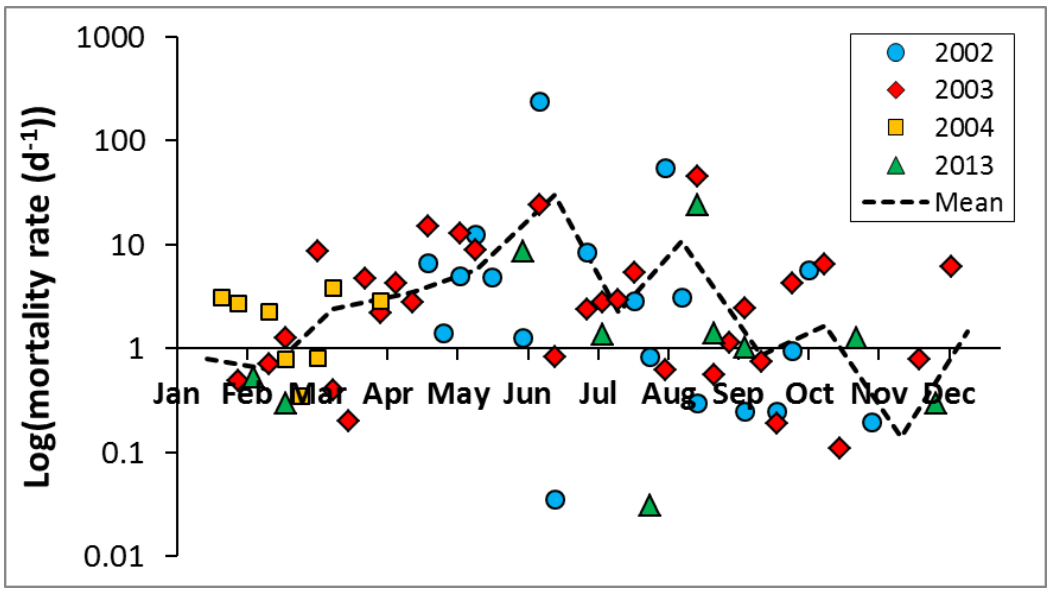

Figure 3.10. ***Calanus helgolandicus*** **egg mortality rates (and mean monthly rates indicated by dashed black line) as determined using the "Basic" vertical life-table method (Hirst et al., 2007), March-December 2002, January – December 2003, January- March 2004 and January-December 2013.**

Egg mortality rates were calculable using the Viability Basic approach for 33 out of a possible 107 sampling dates. Rates very much followed the same fluctuations as the Basic method; however rates ranged from 0.002 – 24.2 d^{-1}, with a median of 0.41 d^{-1} (Figure 3.11). Comparable Basic rates ranged from -1.81×10^{-7} – 24.2 d^{-1}. All Viability Basic rates were positive values and had increased from the Basic rates by 0-~60%. Viability Basic rates were very highly correlated with Basic rates (reduced major axis (RMA) regression analysis intercept = 0.149, slope = 0.993, R^2 = 0.99, n = 33, p < 0.001).

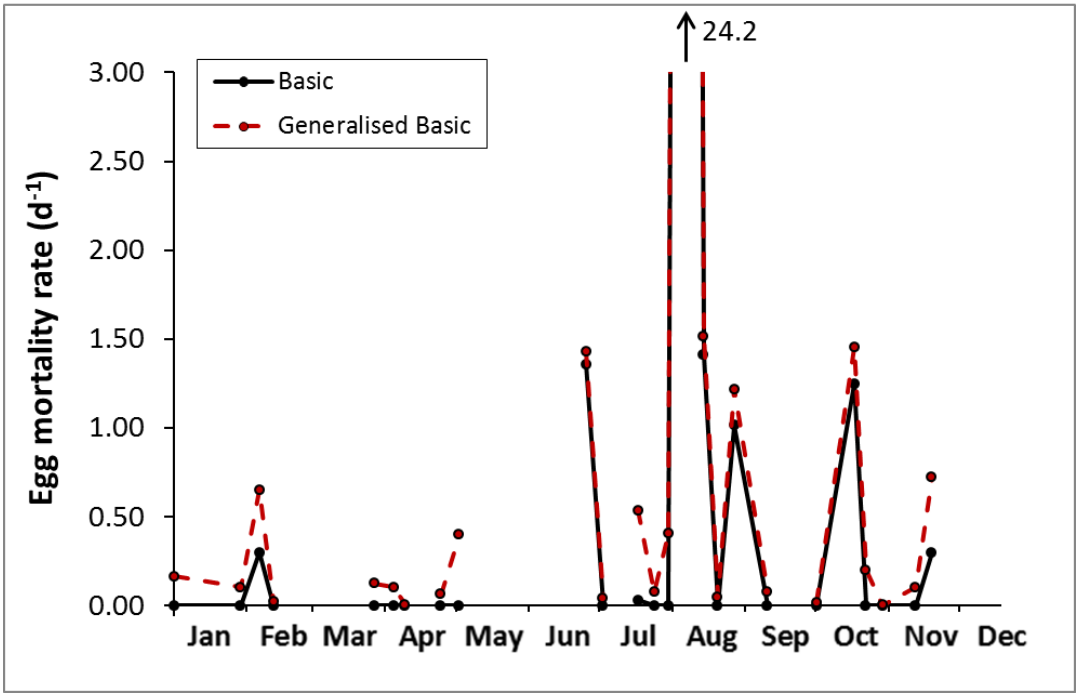

Figure 3.11. *Calanus helgolandicus* egg mortality rates (2013) as determined using the standard Basic vertical life-table method (Hirst et al., 2007) (black line) and Viability Basic method, accounting for egg viability (Head and Gentleman, 2015) (red dashed line).

Causative agents of egg mortality

Table 3.9 summarises all significant simple and multiple regressions. SST was not a predictor of egg mortality rate, although both SI (R^2 = 0.168, n = 106, p < 0.000001) and chlorophyll-*a* (R^2 = 0.072, n = 106, p = 0.0085) were positive predictors.

Of the potential predators, *C. helgolandicus* copepodite abundance (CI-CVI and CI-CV abundances) and total copepod biomass explained egg mortality rates (Figure 3.12). Total meroplankton and total non-copepod holoplankton were also significant predictors; however the typical copepod predator types (medusae, siphonophores, ctenophores, chaetognaths or fish larvae) or total gelatinous zooplankton were not. When a backwards stepwise multiple regression model was applied incorporating all significant environmental and predator variables, CI-CVI abundance was the only remaining significant variable. Including environmental factors only, a model containing SI and chlorophyll-*a* was significant. A model incorporating copepod biomass and SI was also significant.

Table 3.9. Generalised least squares analysis of *C. helgolandicus* egg mortality rates and environmental and predator variables, as derived using the Basic vertical life-table method (Hirst et al., (2007)): coefficients, standard error (SE), *t*-value, *p*-value and AIC value for single and multi-variable GLSs; TRO = total reproductive output; SI = Stratification Index.

Model predictor (s)	Coefficient (slope)	SE	*t*-value	*p*-value	AIC
Log_{10}(SI)	0.784	0.078	10.01	<0.000001	5.27
Log_{10}(total *C. helgolandicus* CI-CVI abundance)	0.143	0.027	5.40	<0.0001	40.93
Log_{10}(total meroplankton biomass)	0.188	0.040	4.74	<0.000001	44.01
SI +	0.049	0.025	1.99	0.049	44.47
log_{10}(copepod biomass)	0.267	0.059	4.49	0.00002	
Log_{10}(total *C. helgolandicus* CIV-CVI abundance)	0.247	0.048	5.18	<0.0001	46.28
Log_{10}(total *C. helgolandicus* CI-CV abundance)	0.280	0.060	4.98	<0.000001	47.44
Log_{10}(copepod biomass)	0.234	0.057	4.10	0.001	48.81
Log_{10}(chlorophyll-*a*)	0.575	0.214	2.68	0.0085	56.02
Log_{10}(non-copepod holoplankton biomass)	0.070	0.033	2.15	0.034	60.40
SI +	0.069	0.027	2.56	0.012	66.71
chlorophyll-*a*	0.073	0.036	1.99	0.049	

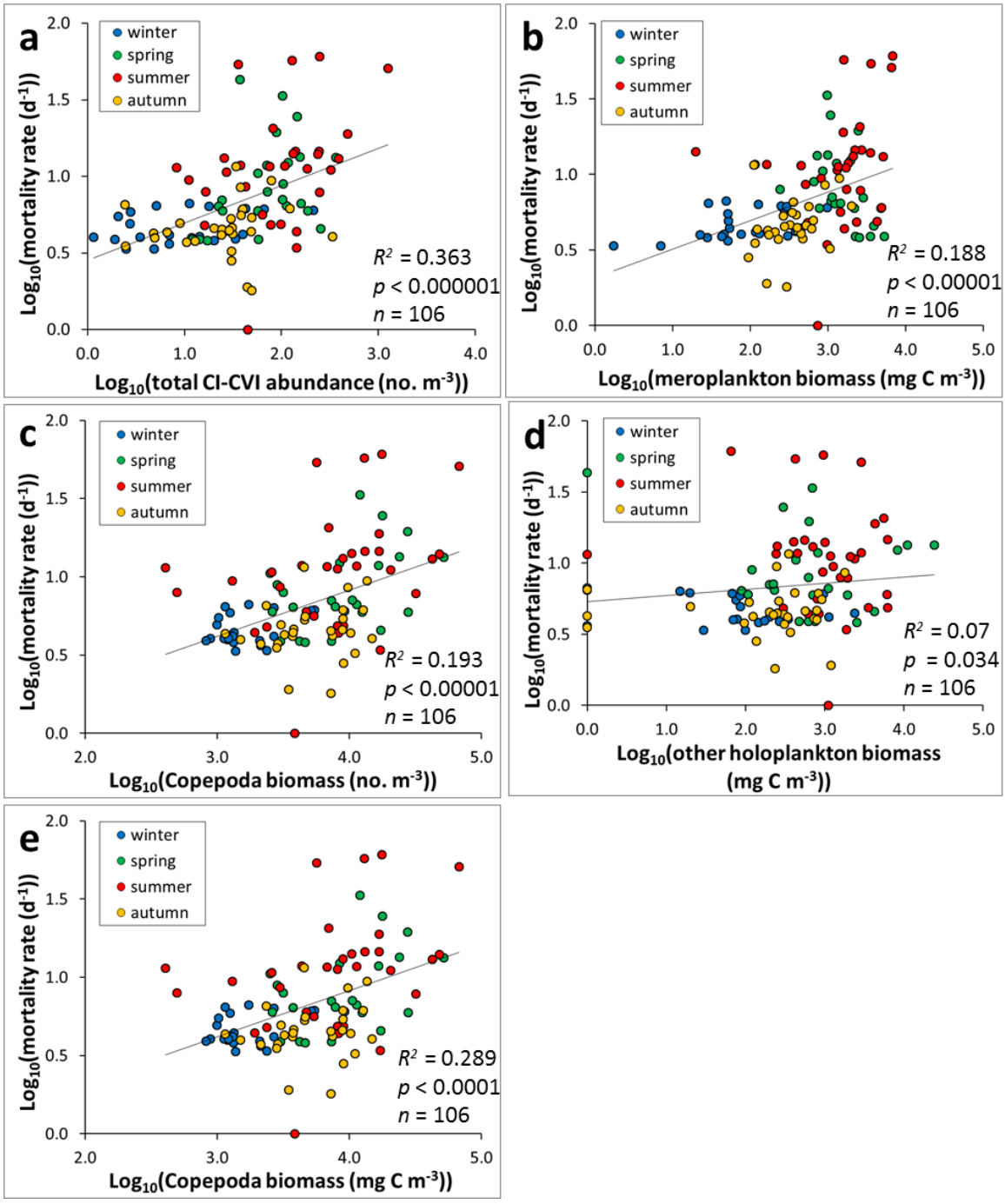

Figure 3.12. ***Calanus helgolandicus*** **egg mortality rates (April 2002 – March 2004, 2013). Relationship with (a) total** ***Calanus helgolandicus*** **copepodite (CI-CVI) abundance; (b) total meroplankton biomass (polychaete larvae, bryozoan larvae, gastropod larvae, echinoderms, cirripede larvae and decapod larvae); (c) total** ***Calanus helgolandicus*** **CI-CV copepodite abundance; (d) other holoplankton biomass (appendicularians, cladocerans, hyperiids, mysids and euphausiids); (e) total copepod biomass.**

3.4 Discussion

3.4.1 Egg hatch success and naupliar health

I have determined *Calanus helgolandicus* egg and naupliar viabilities over ~four years at station L4 and found that NA as a proportion of eggs hatched were inversely related to EHS, so the greater the number of eggs hatched, the lower the proportion of abnormalities. This would suggest that copepod reproduction potential separates into two contrasting scenarios; time periods when population growth potential is likely high (most eggs hatch/few abnormalities) and periods when it is low (few eggs hatch/more abnormalities). Here I discuss causative agents, following three lines of reasoning related to the physical environment, food quality and "toxic" food.

The physical environment

Both egg and naupliar health were lower and more variable in spring. My results concur with other *C. helgolandicus* egg viability studies where spring EHS was low (Laabir et al., 1995b; Jónasdóttir et al., 2005), although one study in the Cantabrian Sea reported a consistently high EHS (> 70%) (Ceballos and Álvarez-Marquéz, 2006b). Studies that measured NA of *C. helgolandicus* in addition to EHS are few. The Laabir et al. (1995b) study reported that "the nauplii that did hatch were abnormal" during low EHS events in summer at Roscoff, indicating a 100% deformity rate. Pond et al. (1996) obtained rates from L4 during 1994, and these data have been consolidated into my study. Abnormality rates of other calanoid species include *Calanus simillimus* in the sub-Antarctic (3-20%) (Miralto et al., 1998); *Centropages hamatus* from Long Island Sound, US (<5%), and *Temora longicornis* (<10%), also from Long Island Sound (Tang et al., 1998). Together these studies indicate typically varied egg viability rates across many calanoid copepods.

I established that both egg viability and naupliar health were positively related to SST, both in simple linear and multiple regressions. EHS data from 1994, also from L4, reported the same association with SST (Pond et al., 1996). However, the 2003-04 L4 EHS data (data incorporated into this study) on its own was not associated with SST (Bonnet et al., 2009), and Ceballos and Álvarez-Marqués (2006b) reported the same lack of relationship. It is likely that any relationship between EHS, NA and SST is an

indirect one, through the effect on the female, or simply that SST is a proxy for the biological/environmental variability that occurs throughout the year. For example, in the colder months, adult females may be lacking in body condition, experiencing starvation episodes, or may simply be older females (Ianora et al., 1996; Jónasdóttir et al., 2005), such that eggs produced are less viable.

Food quality

The results on the effect of maternal food availability and nutrition on EHS were conflicting. Neither chlorophyll-*a* nor diatom biomass were predictors of EHS (in agreement with other *C. helgolandicus* studies [Laabir et al., 1995b; Pond et al., 1996; Tang et al., 1998; Ceballos and Álvarez-Marqués, 2006b)], although EHS was positively related to the diatom fatty acid biomarker C16:1(*n*-7) concentration. Instead, my study highlighted dinoflagellates and ciliates as important explanatory factors.

Both of these microplankton groups might be expected to be advantageous to copepod reproduction. Ciliates have frequently been reported as a particularly nutritious and lipid-rich prey, beneficial to various copepod life processes (Pond et al., 1996; Maud et al., 2015), which may also be selected by copepods, in preference to other microplankton (Nejstgaard et al., 1997; Fileman et al., 2010). Evidence for the nutritional role of dinoflagellates is contradictory. Kleppel (1993) found that dinoflagellates provided more protein, carbohydrate and lipid than diatoms and stated that dinoflagellates were generally viewed as more nutritious for copepods. Jones & Flynn (2005) observed selective feeding of dinoflagellates over diatoms. However, dinoflagellate blooms of species known to produce neurotoxins (i.e. *Alexandrium* spp. and *Karenia* spp.) have been reported to reduce reproduction and egg viability (Gill and Harris, 1987; Roncalli et al., 2016). The potential toxic effects of dinoflagellates are discussed in the following section.

Dinoflagellate fatty acid biomarkers include C22:6(*n*-3), C18:5(*n*-3) (White et al., 2015), linoleic acid C18:2(*n*-6) (Pond et al., 1996) and linolenic acid C18:3(*n*-3) (Jónasdóttir et al., 2005). My study found that *C. helgolandicus* EHS was positively correlated with the PUFA C22:6(*n*-3) and was inversely related to NA, although the relationship was not significant ($p = 0.099$). These results complemented other studies

(Arendt et al., 2005; Jónasdóttir et al., 2005; Evjemo et al., 2008). PUFAs (particularly C22:6(*n*-3), C20:5(*n*-3) and C20:4(*n*-6)) are associated with biological membranes and are precursors of eicosanoids, compounds that are vital for range of invertebrate processes, including egg production and hatching (Brett and Müller-Navarra, 1997). Both C18:2(*n*-6) (Pond et al., 1996) and C18:3(*n*-3) (Jónasdóttir et al., 2005) were also positive predictors of EHS. These fatty acids are important precursors to PUFAs, although it is uncertain if PUFAs are synthesised *de novo* from precursor fatty acids by invertebrates (Monroig et al., 2013). Much is discussed in the literature about the C22:6/C20:5 ratio [where it is reported that a ratio of >1 is beneficial for copepod reproduction (Lacoste et al., 2001)], and although my results did not find that this ratio explained egg or naupliar health to any degree, other studies have reported positive relationships (Jónasdóttir and Kiørboe, 1996; Jónasdóttir et al., 2005; Peters et al., 2007).

C. helgolandicus egg fatty acids did not reflect seston fatty acid composition, although most FAs were detected in both. The most notable difference was the higher concentration of PUFAs, predominantly C20:5(*n*-3) and C22:6(*n*-3). This result corroborates the results of Pond et al. (1996), who suggested that *C. helgolandicus* feeds selectively or is capable of retaining fatty acids. My discovery that egg fatty acid composition remained constant throughout June-November, whereas seston composition varied considerably, suggests that adult females are capable of modifying the content of their eggs in an attempt to produce eggs of optimal quality. Additionally, this suggests that during the population growth period, prey abundance and quality is consistently high to sustain the production of high-quality eggs.

Harmful phytoplankton

I hypothesise that if specific toxic phytoplankton were singularly responsible for low EHS and high NA, I would expect clear indications in the algal data at the timing of the most extreme examples. I found that >90% of the lowest (below mean) EHS rates could not be explained by elevated levels of toxic phytoplankton occurring in the previous month. Furthermore, none of the high NA rates coincided with blooms of those species I recognised as being toxic. There were a limited number of low EHS and high NA events that coincided with or rapidly followed elevated levels of “harmful”

algae, but these involved multiple species, and were not monospecific. Moreover, only one of the elevated algal species (*Cerataulina pelagica*) was a concentrated bloom; the remainder species were generally present at levels above the mean or median biomass.

I specifically discuss *Phaeocystis* spp. as a regular and well-known algal bloomer at L4. *Phaeocystis pouchetii* colonies frequently produce a 2-3 week bloom, usually during the spring, before the diatom bloom. *Phaeocystis* spp. are known to produce PUAs (Hansen et al., 2004) and therefore may affect egg development. However, *Phaeocystis* spp. have also been correlated with C18:3(*n*-3) (White et al., 2015), which in my study was a positive predictor of EHS. As there was no evidence for detrimental effects to eggs in my study, I conclude that *P. pouchetti* blooms at L4 at least, do not compromise copepod egg viability. This is in agreement with a previous laboratory study involving the effect of a uni-algal diet of *Phaeocystis* spp. to *C. helgolandicus*, concluding that *Phaeocystis* spp. do not produce anti-mitotic chemicals (Turner et al., 2002). From the evidence given above, I conclude that there is little evidence for the effect of toxic algae on *C. helgolandicus* EHS or NA at L4.

The seston fatty acid and phytoplankton data provide conflicting evidence for the role of "toxic" diatoms on egg and naupliar health. On the one hand there were no relationships between EHS or NA and diatom biomass. On the other, the fatty acids C16:4(*n*-1) and C20:5(*n*-3) were found to predict both egg and naupliar health. Diatom abundance has been correlated with C16:4(*n*-1) (White et al., 2015) and C20:5(*n*-3) (Anderson and Pond, 2000), implying that certain diatom strains may contribute to a decreased egg and naupliar health. Previous investigations have found both positive (Jónasdóttir et al., 2005) and negative (Arendt et al., 2005) impacts of C20:5(*n*-3) on EHS. One might expect both positive and negative effects of diatoms on egg and nauplii viability, as it is acknowledged that only certain diatom strains are detrimental to copepods, whilst most others are likely beneficial.

The literature reveals numerous laboratory (and mesocosm) studies of toxic diatoms and dinoflagellates, but rather fewer *in situ* investigations. From a review of the available literature, I compiled a list of ~45 references where effects of phytoplankton were tested on copepod EHS (see Appendix B: Table 3.10). These

references contained data on 125 separate experiments involving 50 different "toxic" factors. Of the *in situ* studies (accounting for ~20%), 45% reported negative effects on copepod EHS and included species such as *Pseudo-nitzschia delicatissima* (Miralto et al., 1999), *Skeletonema costatum* (Miralto et al., 1999), *Thalassiosira weissfloggii* (Campbell and Head, 2000), *Thalassiosira* spp. (Halsband-Lenk et al., 2005), diatom non-volatile oxylipins (NVOs) (Ianora et al., 2015) and polyunsaturated aldehyde (PUA) concentration (Wichard et al., 2008). Only 13 references on phytoplankton effects on naupliar deformity rates were collated. Two experiments pertained to natural studies and investigated the effect of total PUAs (Wichard et al., 2008) and diatom biomass (Ban et al., 2000); neither of these affected NA.

3.4.2 Calanus helgolandicus *egg mortality*

Egg mortality rates were estimated over three years using the Basic method, incorporating data from the only published study of *C. helgolandicus* egg mortality (Hirst et al. 2007), and ~one year using the Viability Basic method. I discuss the potential link with predators (intraguild and other mesozooplankton), and also compare the results of the two mortality approaches.

My three-year dataset of egg mortality estimates (including January – December 2013) determined a median rate of 0.53 d^{-1}, with elevated rates in summer (peaks in June and August) and lows from September to February. Egg mortality rates have been derived for the congener species *C. finmarchicus*, albeit using different methods. Ohman and Hirche (2001) reported a median *C. finmarchicus* mortality rate of 1.76 d^{-1} with a peak in April [using the Population Surface Method (Wood, 1994)] in the Norwegian Sea. An Index of Daily Loss (calculated from VLT mortality rates) for *C. finmarchicus* eggs peaked in May on Georges Bank (Ohman et al., 2008). Other copepod species also suffer highest egg mortality during the spring months; *T. longicornis* in Long Island Sound (median rate = 2.72 d^{-1}) (Peterson and Kimmerer, 1994) and *Acartia clausii*, *T. longicornis* and *Centropages hamatus* in the Kattegat, Denmark (Kiørboe and Nielsen, 1994).

Egg-only mortality rates are relatively rare in the literature; more usually they are reported in combined development stages (egg-NI, egg-CI, etc.) when Ratio-type

methods have been employed. This merging of stages is particularly problematic for drawing conclusions on egg mortality, as this approach averages mortality across the stages and assumes that mortality risk is the same for eggs and nauplii (Head et al., 2015; Gentleman and Head, 2016). Mortality sources of eggs include non-viability, sinking and loss to the benthos, predation and advection (Hirst and Kiørboe, 2002). Nauplii may be lost if they are deformed, during the moulting-process, via predation or through advection. In addition eggs, as non-motile stages, will have a higher predation risk than nauplii, as they have no escape response (although one could counter-argue that they also avoid detection because of their lack of mobility). Therefore egg mortality is likely to be much higher than other stages, and so where possible should be estimated as a single stage.

Basic vs Viability Basic egg mortality rates

There has been a significant movement to improve egg mortality rate calculations that involve eggs, as there is a growing recognition that a non-trivial proportion of eggs spawned are non-viable and so will not contribute to copepod recruitment. The assumption that all eggs are viable has been criticised and a handful of attempts have recently been made to generate new models that account for the presence of non-hatching eggs. Here I have employed the Viability Basic equation of Head et al. (2015) and utilised my experimentally-derived *C. helgolandicus* egg hatch success data to calculate new mortality rates and compared them with the conventional Basic rates. Viability Basic mortality rates were 0-60% higher than Basic rates and all were shifted to positive rates, complementing the results of Head et al. (2015). The extent to which Viability Basic egg mortality rates mirrored the Basic rates (R^2 = 0.99) was somewhat surprising. In my study, the variable experimental egg viability rates were used for each Viability Basic mortality rate calculation. The same 80% viability rate was employed for all estimations in Head et al. (2015), therefore I expected the variability in my egg viability rates to affect the Viability Basic mortality rates much more significantly than they did. Because of this, all relationships between potential mortality sources were the same for both Basic and Viability Basic mortality rates (data not presented here). There has been no attempt here to carry out any detailed sensitivity analysis and I have only carried out a very simple exercise in

comparison to investigate the effect of accounting for viability with a small dataset. However, it is recognised that copepod population models should utilise the most realistic data and attempt to account for as many loss scenarios as possible.

Basic egg mortality in relation to environmental variables

My investigations over three years determined that SST was not significantly correlated with egg mortality rates. This was surprising, especially as SST is frequently stated to be an important predictor of egg mortality. For example Hirst and Kiørboe (2002) performed a meta-analysis on egg mortality rates and reported that SST was a positive predictor. However, egg mortality was related to Stratification Index, both on its own and with copepod biomass. *Calanus helgolandicus* abundance was found by Maud et al. (2015), to be a function of stronger stratification and as suggested later in my study, higher copepod abundances lead to greater egg mortality, I can hypothesise that increased egg mortality through predation is commensurate with increased stratification. I note however, that this result is in contrast to that of Irigoien and Harris (2003) and Maud et al. (2015), who related the date of onset of stratification with the timing of the 25% cumulative percentile, indicating that stratification is necessary for egg survival [see Chapter Two (Section 2.3.5 and 2.4.2].

Egg mortality was related to total *C. helgolandicus* copepodite (CI-CVI) abundance, CI-CV abundance, CIV-CVI abundance and also total copepod biomass, providing evidence for both cannibalism and intraguild predation. This is not the first time results suggestive of cannibalism in copepods have been reported (Ohman and Hirche, 2001; Ohman et al., 2002; Ohman et al., 2004; Plourde et al., 2009a; 2009b). In addition, Ohman et al. (2008) calculated predation potentials using published prey clearance data and compared these rates with mortality rates, concluding that *C. finmarchicus* was a dominant source of *C. finmarchicus* egg predation. Filial cannibalism (that is eating one's own offspring) has been reported in almost all major groups of egg-laying animals, including insects, spiders, crustaceans, amphibians and birds (Polis, 1981), and ecological theory purports a number of population effects of cannibalism, including density-dependent population regulation, population destabilisation, population stabilisation, bistability, where a population converges on

one of two possible states, and modification of the population size-structure (Claessen et al., 2004). Cannibalism of eggs by copepods is suggested to be a density-dependent population control mechanism (Ohman et al., 2002) and a stabilising mechanism, by which population cycles caused by other density-dependent effects, can be dampened by cannibalism. The validity of such density-dependent results in copepods however, has been called into question for a number of reasons.

Firstly, such relationships with female abundance should be treated with caution, due to female abundance values being an integral part of some egg mortality equations (Equation 2); therefore the two axes are not independent of each other. In these cases, the employment of more general variables such as total copepodites and total CI-CV reduces the potential for spurious significant relationships.

Secondly, and more importantly, the focus on female copepods as dominant consumers of copepod eggs may also be erroneous. *C. helgolandicus* female biomass contributes a mean of 7% (ranging from 0 – 85%) to total copepodite biomass at any one time of sampling, so generally the bulk of the predation potential is ignored if only female *C. helgolandicus* are considered. *C. helgolandicus* eggs measure 0.16-0.19 mm in diameter (Conway, 2012a) and *C. helgolandicus* copepodites CI-CVI range 0.9-3.9 mm in length (Conway, 2012b). Therefore it is highly likely that eggs could be eaten by earlier-stage copepodites and in fact clearance rates of *C. helgolandicus* eggs have been reported for every *C. helgolandicus* copepodite stage (Bonnet et al., 2004). Furthermore, a simple calculation of total *C. helgolandicus* CI-CVI predation potential in the L4 water column (depth of 55 m) can be made using the maximum female adult abundance (203.7 m^{-3}) and maximum total CI-CV abundance (1790.1 m^{-3}), and assuming clearance rates of 0.32 L d^{-1} for females and 0.11 L d^{-1} for CI-CV (from Bonnet et al., 2004). Total females (11, 203 m^{-2}) would be capable of clearing 3.56 m^3 m^{-2} d^{-1} and CI-CV copepodites (total of 98,454 m-2) capable of clearing 10.83 m^3 m^{-2} d^{-1}; giving a total of 14.39 m^3 m^{-2} d^{-1}, which translates to 26% of the water column.

In addition to cannibalism, intraguild predation has been estimated to account for ~30% of egg and naupliar mortality (Boersma et al., 2014). Omnivorous copepods can display increased predatory behaviour in the absence of other food (Daan, 1988), and Bonnet et al. (2004) suggested that copepods may actively target eggs even when

phytoplankton is not limiting. It is acknowledged that copepods have a wide range of prey-predator ratios (Fuchs and Franks, 2010), and even small copepods can ingest very large prey items (Atkinson, 1996). Hansen et al. (1994) presented an optimal copepod prey-predator ratio of 0.056, although the maximum stated was 0.33. A recent study of L4 copepod grazing found that some species could tackle items up to half their size; *i.e*. *Oithona similis* [length 0.5-0.96 mm (Conway, 2012a)] ingested prey with a prey-predator ratio of 0.52, and were also capable of clearing *Calanus* nauplii, suggesting that a *Calanus* egg would not pose a problem (Djeghri et al., 2017, manuscript in preparation). In my study I also observed that newly-laid eggs were soft, misshapen and malleable for a number of hours after spawning, which may increase the manageability for smaller stages/species.

Thirdly, plausible copepodite egg ingestion rates have been investigated using the relationship with instantaneous egg removal rates (I_e) and copepodite abundance (I_e = egg mortality x egg abundance (no. m^{-3} d^{-1})). I_e are a more useful metric of actual quantity of eggs lost per day than instantaneous mortality rates. Hirst et al. (2007) indicated that each female *C. helgolandicus* would have to consume 48 eggs each day; a feat that was unlikely following work on ingestion rates by Bonnet et al. (2004). I have recalculated I_e and related them with total CI-CVI abundance, generating the equation I_e = [12.48 x CI-CVI abundance] + 11.68. This indicates that each *C. helgolandicus* copepodite was capable of ingesting ~12 eggs per day. This rate is much more plausible, given that Bonnet et al. (2004) reported a maximum rate of ~20 eggs per day for female *C. helgolandicus.*

In summary, I conclude that *C. helgolandicus* egg mortality is a function of three key loss processes, acting through egg and naupliar health and predation. Egg hatch success and nauplii health are boosted by the availability of nutritious food for adult females, but may be reduced by the presence of toxic diatom strains (although I found no correlation with specific species in this study). Predation on eggs is likely widespread throughout the zooplankton and I have provided evidence for the predatory influence of copepods, meroplankton and other non-copepod holoplankton.

CHAPTER FOUR

Mortality of *Calanus helgolandicus*: sources, differences between the sexes and consumptive and non-consumptive processes

Mortality losses are as important as reproductive gains in zooplankton population dynamics, but are challenging to quantify. I used three approaches to provide complementary insights into the mortality of Calanus helgolandicus *at Station L4 in the western English Channel. Using a neutral-red staining method, I found that dead carcasses averaged 9% of the* C. helgolandicus *copepodites sampled. The resulting non-consumptive mortality rates are the first ever derived for* C. helgolandicus*, contributing 0-54%, with an average of 11% to the total mortality. Consumptive mortality dominated for most of the year, particularly in summer and autumn, whereas non-consumptive mortality increased during summer and winter. The non-consumptive mortality rates were positively correlated with maximum wind speed during the preceding 72 hours, indicating that extreme weather events may lead to increased mortality. Mortality rates across the CV-adult male stage pair were ~2.5 times greater than that of CV-adult females over four years. This reflected higher male mortality, both from consumptive and non-consumptive sources. Summer CV-adult total mortality rates were positively correlated with sea surface temperature, chaetognath and siphonophore abundance. The long-term weekly measurements of copepodite abundances, female abundance and their egg production rates allowed us to construct simple matrices that budgeted mortality loss directly. Over 13 years, the year-to-year variability in mortality over all life stages related to chaetognath, ctenophore and medusae abundance. My results, based on a variety of timescales and methods, all point to the gelatinous predator assemblage as the dominant agent for population control of* Calanus helgolandicus *at L4.*

This chapter is a reformatted copy of a manuscript currently in review: **J. L. Maud, A. G. Hirst, A. Atkinson, P. K. Lindeque and A. J. McEvoy. (In review). Mortality of *Calanus helgolandicus*: sources, differences between the sexes and consumptive and non-consumptive processes. *Limnology and Oceanography***. I conceived, designed and conducted the study, with input from AH, AA and PL; I was lead author on the paper, which received editorial assistance from AA, AH and PL and AM.

4.1 Introduction

The mortality of an organism is one of the key life history processes impacting on an individual, and along with reproduction, immigration and emigration forms the major ecological processes involved in population dynamics (Jorgensen and Fath, 2014). The loss of individuals from a population over time can result from various processes; in pelagic marine species, losses can be from advection, emigration and the death of individuals. The consumption of a copepod by a predator typically involves the removal of the entire animal from the water column. Such consumption can be from predators such as fish, fish larvae, jellyfish and chaetognaths (Bonnet et al., 2005; Bonnet et al., 2010), but also other copepod species (Daan, 1988; Boersma et al., 2014), and even via cannibalism (Bonnet et al., 2004). Predation has been ascribed as the dominant cause of mortality in copepods (Hirst and Kiørboe, 2002; Hirst et al., 2010; Daewel et al., 2014), however, other causes may dominate at times (Elliott and Tang, 2009). Indeed, there may be a substantial proportion of zooplankton carcasses present in the water column, whose death cannot be ascribed to consumptive predators (Wheeler, 1967; Elliott et al., 2010; Elliott and Tang, 2011a; Daase et al., 2013). Non-consumptive mortality; that is mortality not caused by predation, tends not to lead to the immediate removal of the body of the animal, and rather usually results in a carcass. Non-consumptive mortality can result from death from old age (Rodríguez-Graña et al., 2010), disease and parasitism (Kimmerer and McKinnon, 1990), the ingestion of toxic prey (Kâ et al., 2014), exposure to environmental pollutants (Cohen et al., 2014; Wendt et al., 2016) and challenging environment-related hydrodynamics, including extreme weather events (Dubovskaya et al., 2005; Bickel et al., 2011; Tang et al., 2014). Determining the causes of death can be difficult, and therefore mortality is poorly understood for most zooplankton species. Fortunately, the combination of mortality estimates and the identification of carcasses in the water column provide an excellent opportunity for separating some major causes of mortality, specifically consumptive and non-consumptive causes (Elliott and Tang, 2011b).

A topic of particular interest relates to the strong sex skew observed in the adults of many copepod species (Hirst and Kiørboe, 2002; Hirst et al., 2010); with the females at times outnumbering males by 5 or even 10 to 1 (Hirst et al., 2010). This

skew may arise from greater mortality of the males than females, especially when the sex ratios of the previous stage (CV) are near equal (Hirst et al., 2010). Differential mortality of the sexes in adults can result from shorter physiological longevity of males; this may for example result from the reduced feeding rates or absence of feeding in CVI males. Alternatively, or additionally, sex skew may also be attributable to differential predation in the field, with greater predation rates on males than females. Males may show riskier behaviour when searching for the females, and at times they perform extravagant courtship behaviour (Kiørboe, 2008; Hirst et al., 2010), thereby increasing encounter and detection by predators. While these issues can in part be explored through incubation experiments with predators and prey, an *in situ* approach would certainly be useful in further testing this predation hypothesis. Since death from non-consumptive sources results in a carcass, while death from predation typically does not, the use of vital staining methods to separate live and dead copepods presents an opportunity to explore any differences in the causes of mortality between the sexes.

This study aims to elucidate the mortality rates, and sources and temporal dynamics of the copepod species *Calanus helgolandicus.* While a recent study of *C. helgolandicus* population dynamics highlighted the dual role of reproduction and mortality (Maud et al., 2015), only one previous study has specifically examined mortality rates of *C. helgolandicus* at station L4 (Hirst et al., 2007). Since mortality rates are difficult to quantify, and some controversy exists in the application of the particular methods (Ohman, 2012), I have combined three complementary methods to reveal different facets of the topic. These comprise a Vertical Life Table (VLT) approach, a carcass staining method to evaluate non-consumptive mortality, also using VLT methods, and via a budgetary approach to compare the difference between my measurements of total egg production rate and observed copepodite population size. Here I address the questions: (1) What are the main sources of mortality of *Calanus helgolandicus* at the L4 site? (2) How do these mortality sources vary between the sexes? (3) Which agents can be identified as determining these non-consumptive and consumptive components of mortality?

4.2 Materials and methods

4.2.1 Calanus helgolandicus *stage composition*

The *C. helgolandicus* stage composition was determined from the L4 mesozooplankton weekly samples from March 2002 to March 2004 [as previously published by Hirst et al. (2007)], and supplemented with new data from January 2012 to December 2013. Composition was obtained from the staging and sexing (of CVI adults) of ~100 *C. helgolandicus* copepodites from one of the two WP-2 200 µm vertical hauls chosen at random. A 200 µm sampling mesh was considered the most appropriate for copepodites due to the recommendation by Skjoldal et al. (2012) for large calanoid copepods including *Calanus* spp., although it was recognised that CI copepodites may be under-represented. I therefore determined mortality across four years of weekly copepodite stage data from L4, albeit that the two periods were separated by a decade.

4.2.2 Predator abundance and biomass

Mesozooplankton abundance data were extracted from the weekly L4 dataset [sampling and identification as described in materials and methods, Chapter Two (Section 2.2.1)]. Data were pooled into broad groups, namely medusae, siphonophores, chaetognaths and ctenophores, with total gelatinous zooplankton predators being their sum. Due to a change in analysis method I have only used ctenophore data from 2008. Data on total fish larvae abundance were also extracted. Fish larval data were also made available from the Marine Biological Association's weekly Young Fish Trawl (YFT) survey (2005-2014). Predator biomass (mg C m^{-3}) was estimated using the methods detailed in Chapter Three (Section 3.2.1).

4.2.3 Physico-chemical measurements

Mean water column temperature (MCT), SST, SI, salinity and O_2 were determined from the weekly CTD profile data. Chlorophyll *a* concentrations were determined via fluorometry or HPLC. Chapter Two (Section 2.2.4 and 2.2.5) details the

specific methods. All data sources and time periods for which the data were available are given in Table 4.1.

Table 4.1. Time series data available within the period 1988-2015, at Station L4, western English Channel, UK.

Time series	Data available
Total *C. helgolandicus* (males, females, copepodites)	1988-2015
♀ adult abundance	1988-2015 (excl. August-December 2005)
♂ adult abundance	1996-2012 (excl. 2000)
Egg production rate (EPR)	February 1992 – 2015 (excl. July-December 2000; 2001; January-September 2007)
Total reproductive output (TRO)	February 1992 – 2015 (excl. July-December 2000; 2001; August-December 2005; January-September 2007)
Total copepodite (CI-CV) abundance	1996-2015
Copepodite (CI-CV) stage composition	March 2002 – March 2004; 2012-2013
Mesozooplankton abundance (including predators)	1988-2015
Total fish larvae abundance	1988-2015
Fish larvae abundance (Marine Biological Association)	2005-2014
Sea surface temperature (SST)	1988-2015
Mean column temperature (MCT)	1993-2015 (excl. February – December 2000; 2001)
Salinity	
O_2 concentration	1992-2015
Chlorophyll *a*	1992-2015

4.2.4 Calanus helgolandicus *stage duration*

Stage-specific development times are required to determine mortality rates using the approaches applied here. The literature was reviewed to collate all experimentally-derived copepodite stage duration data (Table 4.2). These data were obtained across a range of temperatures from 1^{o} to 15^{o}C; however the most frequent temperature incubations were 8^{o}, 12^{o} and 15°C. Available stage duration data were fitted to a temperature function (T, ^{o}C) and used to determine stage duration (D) (days) for each development stage using a Bělehrádek function (Bělehrádek and Mann, 1935; Bělehrádek, 1957) [see Chapter Three (Section 3.2.4)]. The results and data sources are presented in Table 4.2.

Table 4.2. *Calanus helgolandicus*. Bêlehràdek functions applied in this study to determine stage-specific development times (*D*, h). Egg hatching times fitted to the equation $D = a(T - \alpha)^{-2.05}$, α was determined to be -9.523. This value was then subsequently used when fitting the equation to data for all other individual and grouped life stages to determine *a*. Curve-fitting was performed using R (R Development Core Team, 2012).

Stage(s)	*a*	α	Data sources
Egg	19488	-9.523	Corkett (1972), Rey et al. (2001), Lopez et al. (2007), Cook et al. (2007), Bonnet et al. (2009)
CI	113024	-9.523	Shreeve et al. (1998), Cook et al. (2007), Bonnet et al. (2009)
CII	43106	-9.523	Shreeve et al. (1998), Rey-Rassat et al. (2002), Bonnet et al. (2009)
CIII	52488	-9.523	Shreeve et al. (1998), Rey-Rassat et al. (2002), Bonnet et al. (2009)
CIV	58131	-9.523	Shreeve et al. (1998), Rey-Rassat et al. (2002), Bonnet et al. (2009)
CV	101421	-9.523	Rey-Rassat et al. (2002), Bonnet et al. (2009)
Egg – CI	289911	-9.523	Rey et al. (2001), Rey-Rassat et al. (2002), Cook et al. (2007), Lopez et al. (2007), Møller et al. (2012)
Egg – CV	530544	-9.523	Diel and Klein Breteler (1986), Rey-Rassat et al. (2002), Bonnet et al. (2009), Møller et al. (2012)

4.2.5 Vertical Life Table mortality rate calculations

The stage-ratio Vertical Life Table (hereafter "VLT") method was used to estimate mortality rates over four seasons (March 2002 – March 2004 and January 2012 – December 2013). This method determines total (defined as both consumptive and non-consumptive sources) mortality rates across stage pairs. I calculated mortality across the CV-adult female and CV-adult male stage pairs using the equation given by Aksnes & Ohman (1996):

$$\beta = \frac{\ln\left(\frac{N_{CV}}{N_{adult}} + 1\right)}{D_{CV}} \qquad [4.2]$$

where β is the mortality rate across the CV-adult stage pair (days^{-1}), *D* is the stage duration (days) estimated here from the Bělehrádek functions in Table 4.2, using the mean temperature (*T,* ^{o}C) across depth (MCT), as measured on the day of sampling, while *N* is the abundance of the stage (no. per m^{-3}) as quantified from the depth integrated WP-2 nets. When deriving mortality for individual sexes, I assumed a 1:1 sex

ratio in the abundance of the CV stage, and applied the abundance of adult males or females [see Discussion (Section 4.4.3)].

The use of VLT methods require a number of assumptions to be fulfilled. The first is that any transport processes equally influence stage pairs over the period of the total duration of the two stages. As the duration of *C. helgolandicus* stages is typically a few days, this seems a reasonable assumption. The second assumption requires that there is no trend in recruitment to a stage over the duration of the combination of stages for which mortality is being determined, (i.e. no stage trends or strong cohorts). Whilst there was some evidence of population peaks, as expected, in spring and autumn, the data do not generally suggest strong cohorts (Figure 4.4b). However, there was evidence of a strong trend in CV accumulation during the winters of 2002 and 2012. I have not omitted these data, but am aware that my mortality estimations during the winter months may be impacted. I used two time periods in further analyses; i) all months and ii) the subset of May-September, which corresponds to the main population growth period and is also when the water column is typically stratified.

A few negative mortality rates were calculated during this study. These can be expected using this method, but these data were not removed, as this would subject the data to positive bias (Hirst et al., 2007). As recommended for the VLT approach (Aksnes and Ohman, 1996), I have averaged mortality across several sampling time-points. This was done by the application of a LOESS (locally weighted) smooth using the loess and predict functions within R (R Development Core Team, 2012), set to an f-value of 0.2. This value was chosen to produce a smoothing in which major seasonal patterns were still evident. Further analysis of VLT-derived mortality rates used the LOESS-smoothed rates at each sampling point, rather than the actual mortality rates. It is recognised that the traditional VLT method estimates total mortality rates using abundance data that includes intact carcasses, which creates a bias in the abundance ratios (Elliott and Tang, 2011b), resulting in the under- or overestimation of mortality rates. However, this frequently used VLT approach allows us the increased temporal coverage of four years.

4.2.6 Total, consumptive and non-consumptive mortality rates

From February 2013 to January 2014 on the same days that the quantitative vertical net tows were taken, I also undertook additional zooplankton collections at L4 to determine the incidence of *C. helgolandicus* carcasses. Collecting live samples required slow (~0.2 m s^{-1}), gentle oblique trawls, which I performed with a 63 µm ring net to a depth of ~50 m. These catches were immediately stained with neutral red following the method of Elliott and Tang (2009). For this 1 L of sample-water from the net cod-end was poured into a plastic container and 1.5 mL of neutral red solution (10 g L^{-1} conc.) was immediately added. The container was incubated in a water bath at sea surface temperature on deck for 15 mins and afterwards sieved through a detachable 63 µm mesh. The mesh was stored in a petri-dish and flash frozen with Freeze Spray to instantly preserve all the zooplankton in the sample. The petri dishes were stored on ice in a cool box for the time taken to return the samples to the laboratory (~two hrs), and then stored in a -20°C freezer until sample processing.

Chilled 0.2 µm-filtered seawater (FSW) was used to thaw the frozen sample on the mesh and rinse the sample into a conical flask. The sample was then collected on a 63 µm sieve and washed into a sorting dish with ~10 ml 0.2 µm FSW and 0.5 ml 0.1% hydrochloric acid (HCL) was added to enhance the brightness of the pink stain (Elliott and Tang, 2009). Samples were examined under a dark field dissecting microscope and *C. helgolandicus* were staged, counted and assigned to a live or dead category (bright pink/patchy bright pink areas – live; dull/pale pink – dead (Figure 4.1). In some instances various sub-samples were pooled so that a total of ~100 copepodites (stage CI to CVI) were identified.

Figure 4.1. *Calanus helgolandicus* female adults collected from station L4. "Alive" bright pink neutral red-stained copepod indicating was alive when captured; "dead" pale pink copepod indicating non-staining copepod carcass in water column.

The carcass turnover time (τ) is an important parameter in the mortality rate estimation calculations, and is a function of carcass decomposition rate and sinking losses (Elliott et al., 2010). Sinking losses may be ignored if the turbulence within the sampling site is enough to retain carcasses in the water column and enable resuspension of those that have settled (Elliott and Tang, 2011a). Due to lack of data on sinking losses of *Calanus* at L4, I assumed for simplicity in my calculations that these were zero. Since any sinking will reduce the calculated carcass turnover time and thus increase the estimate of non-consumptive mortality, my estimates thus provide a lower boundary of this mortality source.

Carcass decomposition time was examined by incubating freshly-killed copepodites at a range of temperatures and recording the rate of decomposition. Live *C. helgolandicus* copepodites were collected from L4, stages CIV-CVI were sorted from the catch, and placed into a petri dish in a sealable plastic pouch. An AnaeroGen sachet (Oxoid Atmosphere Generation System) was introduced into the pouch to generate an anaerobic atmosphere and the copepods were incubated for four hours to ensure that all were killed. They were sorted into batches of ~80 carcasses and placed in a petri dish with ~10 ml 5 µm FSW from L4; this level of filtration was selected to allow microbial colonisation and decomposition of the carcass, but to exclude bacterivorous protozoa that may alter the microbial community composition. The samples were incubated at one of four temperatures across the range usually experienced at L4 (8^{o},

12°, 15° or 18°C) for a period of 10-14 days. A sub-sample of five carcasses were removed from the incubations each day and photographed (therefore *n* = 20), each carcass was then categorised on a scale of 1-8 (where 1 was near transparent with no decomposition and 8 was an almost completely empty exoskeleton with minimal residual tissue; as soon as they are dead, the copepods no longer take up the neutral-red stain) (Figure 4.2).

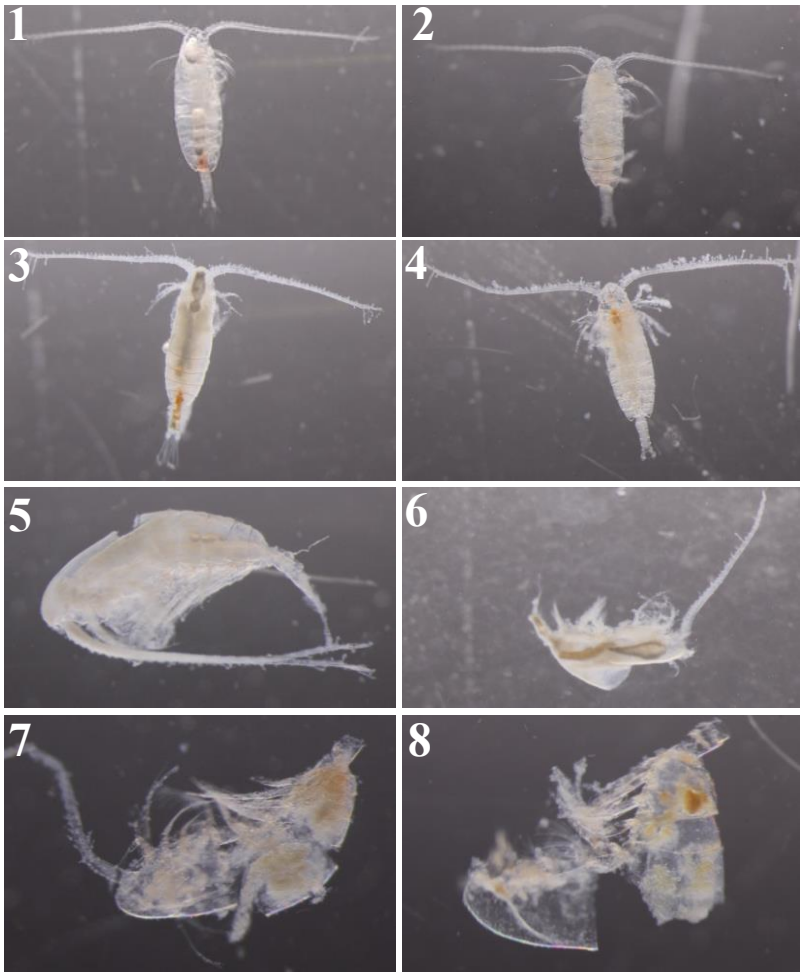

Figure 4.2. Stages of *Calanus helgolandicus* carcass decomposition, (1) transparent, no decomposition; (2) opaque; (3) opaque and brown colouration; (4) bacteria apparent on external carapace; (5) metasome fractured, mostly in tact; (6) metasome split; (7) metasome completely split, internal material spilling out or depleted; (8) split carcass and most of internal material depleted.

A carcass decomposition time-temperature function was fitted using the mean time taken to reach decomposition category 8 (τ, days) (any exoskeletons collected with no residual tissue were treated as copepod exuviae, and were not counted as a carcass), against temperature (T, ˚C), and is described by the equation:

$$\tau = e^{-0.114T+3.531} \quad [4.3]$$

where R^2 = 0.79, n = 20, p-value < 0.00001 (Figure 4.3).

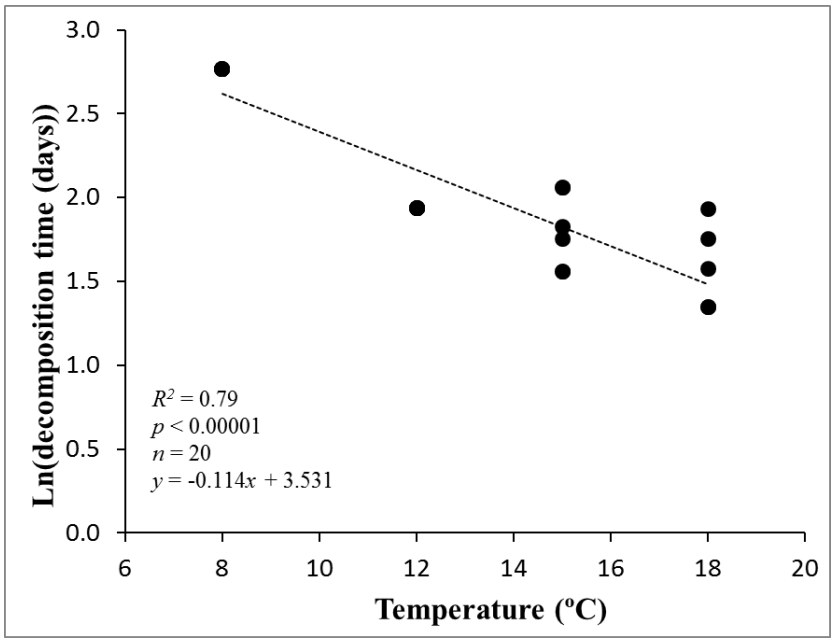

Figure 4.3. *Calanus helgolandicus* carcass (CIV-CVI) decomposition time as a function of temperature, as measured in the laboratory; dotted line indicates fitted linear model and equation used to calculate τ, as used in consumptive mortality rate equation (Elliott and Tang, 2011a).

Consumptive mortality rates (β_c, d^{-1}) were derived by iteration following the equation of Elliott and Tang (2011b), see their Appendix 1:

$$\frac{N_{\text{CI-CV}}}{N_{\text{CVI}}} = \frac{1 - \pi_1 e^{-\beta_c D_{\text{CI-CV}}} - \pi_2 e^{-\beta_c \tau}}{\pi_1 (e^{-\beta_c D_{\text{CI-CV}}})(1 - \pi_4 e^{-\beta_c \tau})} \quad [4.4]$$

where π_1 is the relative proportion of CV alive, π_2 is the proportion of CV dead, π_4 is the proportion of stage CVI dead (where dead carcasses fall within the stage categories of 1 to 8 described above, and which can be discerned from live animals by the general lack of the neutral red stain), *N* is the total abundance of the stage(s) collected in the WP2 nets (no. m^{-3}), and *D* is stage duration time (days).

Total mortality (β_t) was calculated using the ratio VLT method, but using corrected abundances so as they represent only those copepods identified as being alive at the time of capture (hereafter "corrected" mortality) (Elliott and Tang, 2011b), where in addition to Equation 4.4, π_3 is the relative proportion of stage CVI alive:

$$\beta_t = \frac{\ln\left(\frac{\pi_1 N_{\text{CV}}}{\pi_3 N_{\text{CVI}}} + 1\right)}{D_{\text{CV}}} \quad [4.5]$$

This corrected mortality rate removes the potential bias introduced by including copepod carcasses in abundance ratios, and is a more robust calculation, but requires additional sampling effort and analysis.

Finally, the non-consumptive mortality rates were calculated as total corrected mortality minus consumptive mortality rates (Elliott and Tang, 2011b). Equations 4.4 and 4.5 were applied across the stage pair of CV and adult, deriving these separately for males and females (assuming a sex ratio of 1:1 in CV).

I explored correlations between a range of variables and mortality rates, including SST, stratification, salinity, fluorescence (as a proxy for chlorophyll-α), tidal

height, tidal state (state of spring-neap cycle) and wind speed. Environmental factors and their data sources included are listed in Table 4.3.

Table 4.3. Explanatory variables and data sources collated for use in regression analyses with non-consumptive *C. helgolandicus* mortality rates. PML is Plymouth Marine Laboratory, BODC is British Oceanographic Data Centre, and BADC is British Atmospheric Data Centre.

Explanatory variable	Source of data	Categories
Sea surface temperature (SST) (˚C)	PML	n/a
Stratification Index (SI) (˚C)	PML CTD	n/a
Salinity	PML CTD	n/a
Fluorescence	PML CTD	n/a
O_2	PML CTD	n/a
Tidal height	BODC	n/a
Tide	BODC	Ebb or flow
Tidal state	BODC	Spring, neap, intermediate
Mean wind speed:		
in preceding 24 hrs	BADC	n/a
in preceding 48 hrs	BADC	n/a
In preceding 72 hrs	BADC	n/a
Maximum wind speed:		
in preceding 24 hrs	BADC	n/a
in preceding 48 hrs	BADC	n/a
in preceding 72 hrs	BADC	n/a

4.2.7 Matrix mortality model

This alternative method is based on the simple population growth model developed in Chapter Two (Section 2.3.6), and was used to provide a different perspective on mortality and to provide inter-annual comparisons. Data were available from 2002 to 2015 (excluding 2007). This method is based on the good fortune of L4 having concurrent weekly time series of adult female abundance and their egg production rate over many years, and from which I could calculate total egg supply to the system.

Egg production rate methodology is detailed in Chapter Two (Section 2.2.2), but briefly, this involved incubating 25 adult females each week in filtered seawater within

~four hrs of capture at the L4 site. The matrix method involved the creation of a matrix in an Excel spreadsheet covering one year, consisting of 52 rows and 52 columns, with each cell representing one week in the calendar year. I then used the mean egg production rates (no. eggs female^{-1} day^{-1}) and female abundance data (no. m^{-3}) to calculate a daily Total Reproductive Output (TRO) (a product of mean egg production rate and the density of females; in no. eggs m^{-3} day^{-1}). The daily TRO was then multiplied by seven to derive a weekly TRO (no. eggs m^{-3} week^{-1}). Mean water column temperature (MCT) was used to estimate the stage durations (weeks) to "develop" each individual egg up to a CV copepodite at the onset of moult to CVI (by inserting the weekly TRO value into consecutive weeks, representing the stage duration, along each row). Because temperature varies throughout this growth period, the MCT applied for naupliar stage growth was that recorded at egg production, while for copepodite growth, the MCT during the week when NVI moulted to CI was applied in the stage duration calculations. The cumulative abundance of growing individuals was then calculated at the bottom of each column, so in weekly time-steps. By integrating each week's egg output and growing them without mortality I could calculate a predicted density of stages (NI-CV) that would be present in the water column each week, if no mortality had occurred. The predicted copepodite (CI-CV) abundance was compared with the weekly observed L4 copepodite (CI-CV) abundance. The rationale of comparing across the CI-CV time window of development was first because nauplii of *C. helgolandicus* are not consistently enumerated from the L4 samples, and secondly the CVI longevity is unknown. In any given week, the difference between the predicted abundance in the absence of mortality and that observed was termed the "absolute mortality" (no. m^{-3}) and the proportion difference (1 minus observed, as a proportion of predicted abundance) provided the "proportional mortality" (PM).

4.2.8 Statistical analyses

Data analyses of VLT, non-consumptive and consumptive mortality rates were performed on the weekly values. Predator abundance and biomass data were $\log_{10}(x+1)$ transformed before being used to minimise problems associated with non-normality. Proportion mortality data derived from the matrix mortality model were arcsine-squareroot transformed. Simple linear and backwards stepwise multiple linear

regression techniques were used initially to gauge the strength and direction of any relationships (using the lm function in R). Models were validated by an examination of the residuals. Where residuals were non-normal the response variable was subject to a square-root or fourth root transformation. If there was evidence of curvilinearity, a polynomial regression was applied. Heterogeneity of residuals was accounted for via the implementation of a variance-covariance structure within a generalised least squares regression model (gls function in R). Non-independence of data was addressed by the addition of an appropriate correlation structure within a gls model. The annual matrix model proportional mortality (PM) indices were used in inter-annual analyses of environmental and predator abundance and biomass relationships. Data manipulation was undertaken using Microsoft Excel 2010. All statistical analyses were performed using the R programming environment (R Development Core Team, 2012). Reduced major axis (RMA) regressions were performed using the RMA Software provided by Bohonak and van def Linde (2004).

4.3 Results

4.3.1 Overview of the L4 marine environment

Figure 4.4 summarises the L4 environment as monitored during two separate 24-month periods separated by a decade; March 2002 to March 2004 and January 2012 to December 2013 {see Results of Chapters Two (Section 2.3.1) and Three (Section 3.2.1) for a more detailed overview of the complete time series]. *Calanus helgolandicus* reproduction occured throughout the year at L4, as CI to CV stages were present through most of the year, except for the winter months when there was an increase of CV stages and adult females (Figure 4.4b).

Strong seasonal variation of *C. helgolandicus* CV and adult abundance occurred in each of the four years of observations (Figure 4.4c). The peak abundances of the older stages (CV-CVI) varied through March to October, and in 2013, three peaks were apparent. Total *C. helgolandicus* abundance (CI-CVI) (shown on red secondary axis; Figure 4.4c) followed the same general seasonality as the CV and adult stages, but was

approximately four to eight times greater. Total *C. helgolandicus* abundance was greater during the period 2012-2013 than between 2002-2004.

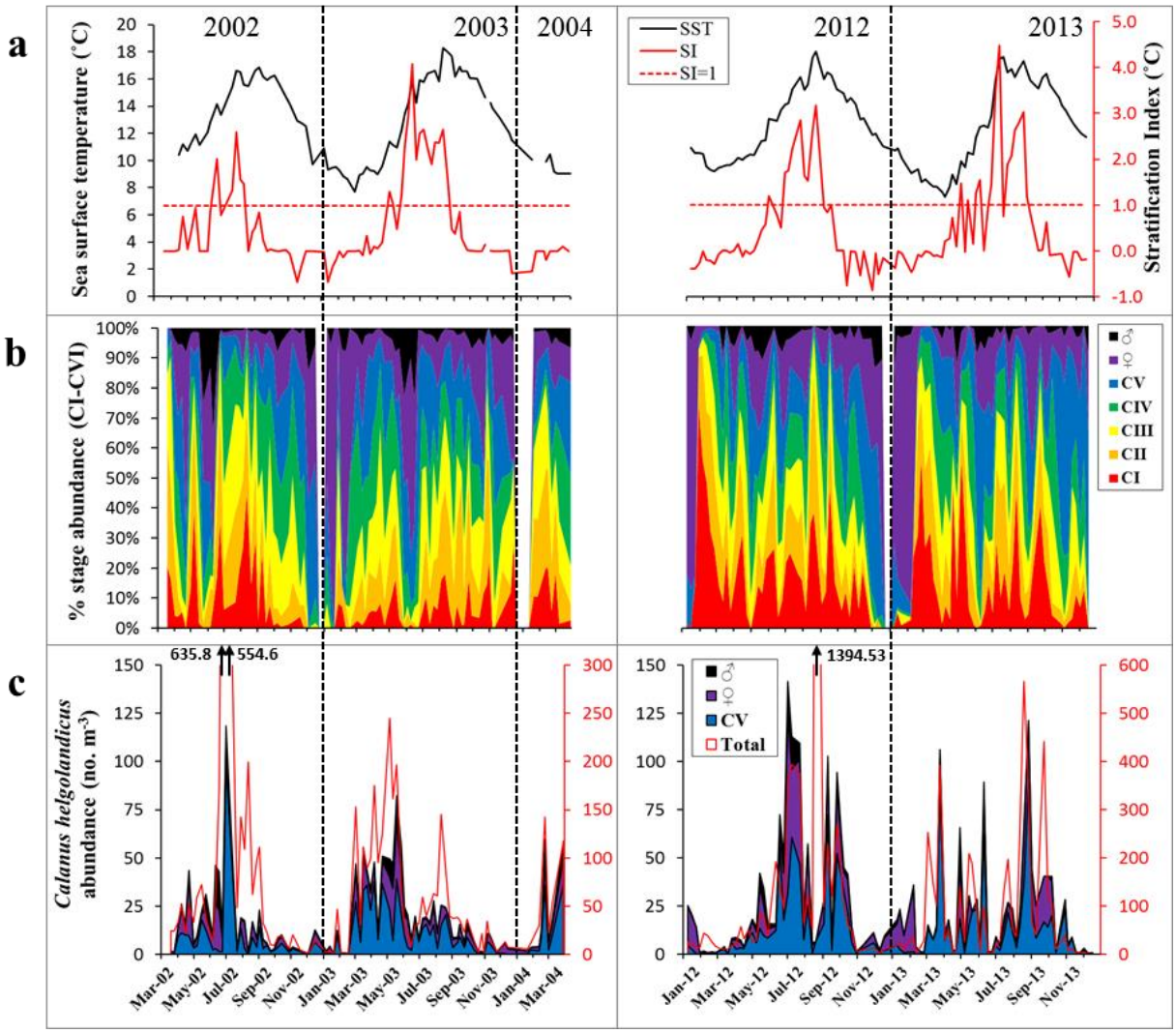

Figure 4.4. The L4 environment March 2002 – March 2004 and January 2012 – December 2013, (a) sea surface temperature (SST) and Stratification Index (SI) (difference between temperature at the surface and 30m). Stratification is indicated by the dashed line and defined as a temperature difference of 1˚C or greater (SI = 1). (b) *Calanus helgolandicus* copepodite stage composition (CI-CVI); (c) *C. helgolandicus* stage CV, female adult and male adult abundance.

The main planktonic predators large enough to consume CV-CVI *C. helgolandicus* are presented in terms of both their abundance (Figure 4.5a) and estimated biomass (Figure 4.5b). It is noteworthy that the 2012-2013 period had both

a greater abundance and biomass of predators than during the 2002-2004 period. Siphonophores and chaetognaths dominated all years in terms of predator abundance, although medusae became more numerous during 2012-13. Predator biomass patterns were somewhat different; siphonophores and chaetognaths dominated the predator biomass, but the contribution of ctenophores (2012-13 only) was much more marked than numerical abundance alone suggests. In addition, fish larvae biomass contributed much more to the predator biomass than to abundance, indicating that peaks occurred earlier in the year compared to the gelatinous predators. The Young Fish Trawl dataset revealed that larval fish predators at L4 were dominated by the Clupeidae (herring), *Callionymus lyra* (common dragonet), the Gadidae (codfishes) and the Pleuronectidae (flounders) (Figure 4.6).

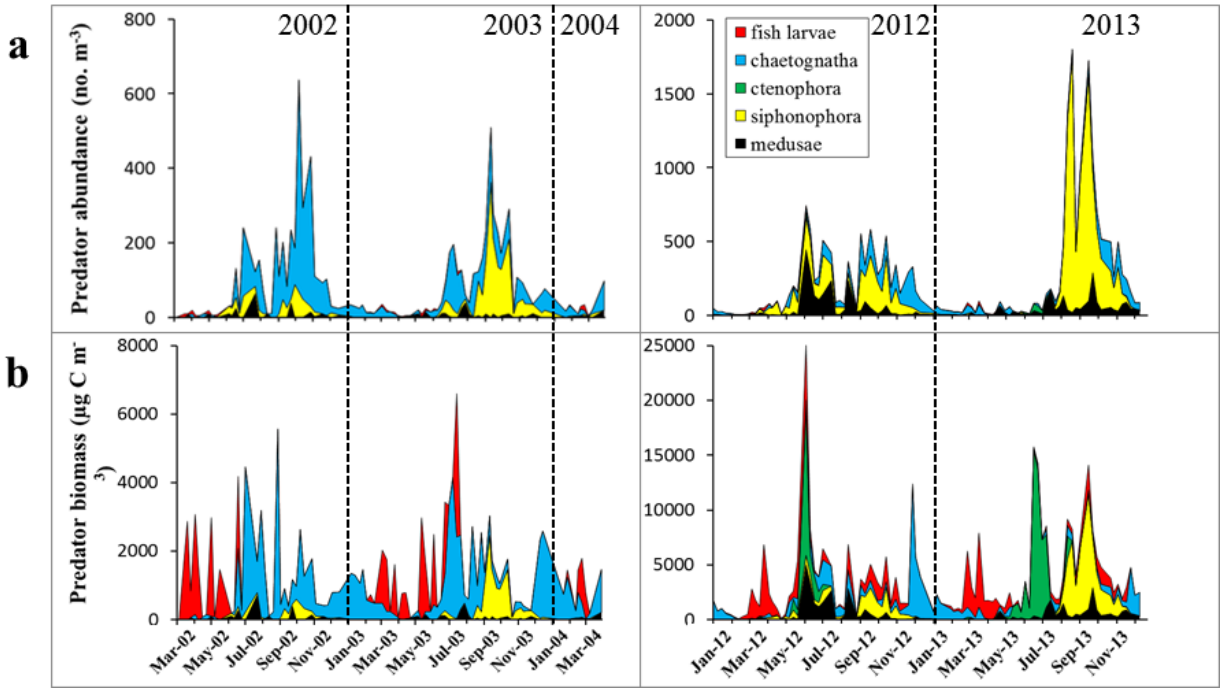

Figure 4.5. The L4 environment March 2002 – March 2004 and January 2012 – December 2013, (a) main predator abundance; (b) main predator biomass.

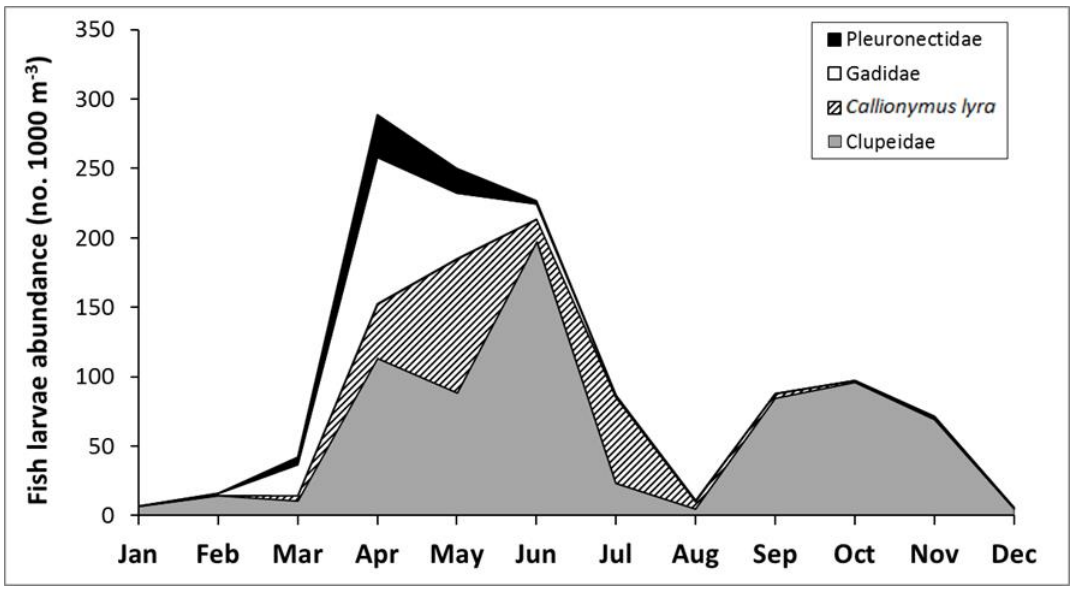

Figure 4.6. Mean seasonality (2012-13) of dominant fish larvae at station L4 (data courtesy of N. Halliday, MBA).

Here I provide some context regarding trends in gelatinous zooplankton populations using the complete L4 time series (1988-2015) (Figure 4.7). Mean monthly chaetognath abundance was elevated between May and the following January (so for ¾ of the year), with highest abundances occurring from October to December. Mean annual chaetognath abundance increased over the 28 years (R^2 = 0.179, p = 0.025, n = 28). Siphonophores appear at L4 between May and November, with greatest abundances occurring between July and October. Mean annual siphonophore abundance was relatively stable over the time-series (R^2 = 0.007, p = 0.674, n = 28). Medusae are generally present at L4 during May to November, with peak abundance levels occurring in May-June. It is difficult to identify medusae population trends as "total medusae" incorporates so many different species, but whilst there was no trend in mean annual medusae abundance from 1988 to 2006, numbers have increased in the past decade. The Ctenophora are usually present for a short period between May and July, with a peak in June. The truncated ctenophore time-series (2008-2015) did not suggest a change in annual mean ctenophore abundance over this timescale (R^2 = 0.067, p > 0.05, n = 8).

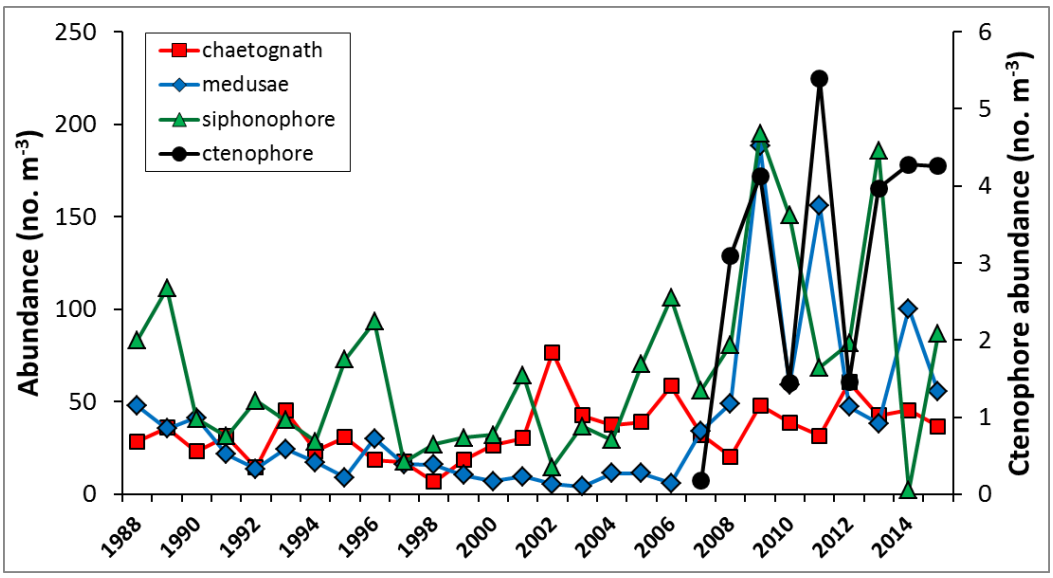

Figure 4.7. Annual mean abundance of gelatinous predators at station L4 (1988-2015).

4.3.2 VLT stage-ratio CV-CVI total mortality

January to December

There were no differences in *C. helgolandicus* CV-adult total mortality rates (where abundance values did not differentiate live animals from carcasses) between the four years (Kruskal-Wallis *H* test statistic = 3.93, $p = 0.269$, $n = 4$). However, there were clear differences between the sexes. CV-female mortality rates ranged from 0.038 to 0.150 d^{-1} (mean of 0.091 d^{-1}) and CV-male rates varied between 0.044 and 0.446 d^{-1} (mean of 0.223 d^{-1}). CV-male mortality rates were ~2.5 times greater than CV-female rates (Mann-Whitney test statistic $W = 3621$, $p < 0.00001$, $n = 138$). Both CV-females and CV-males typically demonstrated fluctuating mortality rates, with summer to autumn peaks (Figure 4.8). Peaks of mortality for CV-females were less well-defined however, and also exhibited larger spring peaks during 2012 and 2013. Despite these differences, CV-female and CV-male mortality rates were highly related (reduced major axis (RMA) regression analysis intercept = 0.022, slope = 0.318, $R^2 = 0.542$, $p < 0.00001$, $n = 123$) (Figure 4.9).

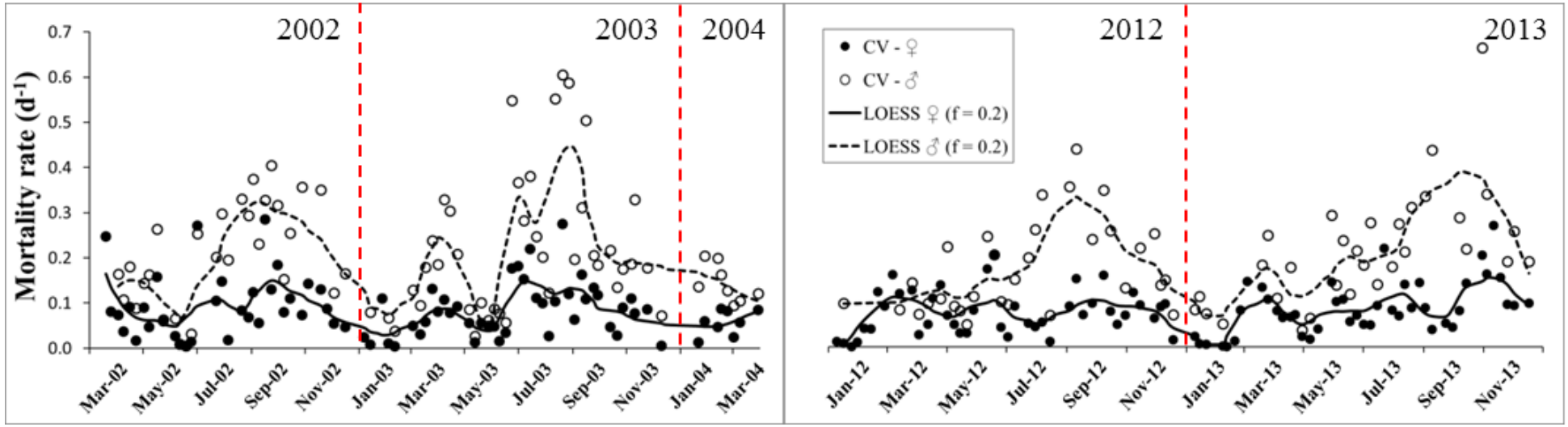

Figure 4.8. *Calanus helgolandicus* mortality rates for the CV-♂ and CV-♀ stage pairs derived using the vertical life table (VLT) method (over the periods 2002-04 and 2012-13).

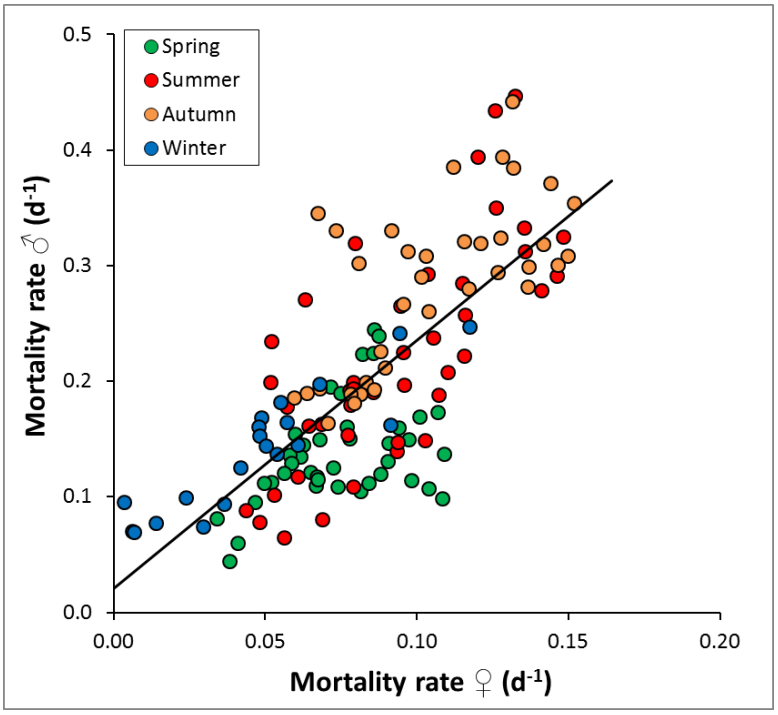

Figure 4.9. Relationship between CV-♂ and CV-♀ total mortality rates, as derived using the vertical life table (VLT) method (over the periods 2002-04 and 2012-13); symbol colour indicates seasons to illustrate that the relationship was not driven by a seasonal difference in mortality rates.

The CV-female summer (June-August) and autumn (September-November) mortality rates were higher than spring (March-May) mortality, which in turn were higher than winter (December-February) mortality (Kruskal-Wallis *H* statistic = 30.06, *p*-value < 0.0001, *n* = 169). CV-male mortality showed a similar pattern, but here winter and spring mortalities were lowest and autumn mortalities were highest (Kruskal-Wallis I statistic = 41.78, *p*-value < 0.0001, *n* = 138).

Total corrected mortality rates (excluding copepod carcasses) for 2013 were on average 1.5% less than the uncorrected rates (including carcasses), with rates ranging from 21% higher to 36% lower. Corrected rates were not significantly different from uncorrected rates (paired *t*-test: $T = 0.98$, $p = 0.336$, $n = 35$).

Regression analyses of the entire twelve months LOESS-smoothed VLT mortality values (2002-04 + 2012-13) with predators yielded no relationships, bar that between medusae abundance and CV-females ($R^2 = 0.06$, $p = 0.084$, $n = 167$).

May to September

Using mortality data from the main growth period of May to September only (see Table 4.4), SST was a significant predictor of both CV-female and CV-male mortality rates (Figure 4.10).

Table 4.4. Generalised least squares analysis of mean summer (May-September) *C. helgolandicus* CV-adult VLT total mortality rates (2002-04 + 2012-13): coefficients, standard error (SE), *t*-value, *p*-value and AIC value for CV-♂ and CV-♀ stage pairs, single and multi-variable GLSs; SST = sea surface temperature; all abundances were $log_{10}(x+1)$.

Stage and Sex	Model predictor(s)	Coefficient (slope)	SE	*t*-value	*p*-value	AIC
2002-04 + 2012-13						
CV-♀						
	SST	0.006	0.001	7.254	0.00001	-454.8
	Total gelatinous zooplankton abundance	0.007	0.002	2.932	0.004	-426.8
	Chaetognath abundance	0.006	0.002	2.667	0.009	-425.6
	Siphonophore abundance	0.009	0.002	5.141	0.00001	-443.0
	Siphonophore biomass	0.008	0.001	5.615	0.00001	-466.1
	SST +	0.004	0.001	4.176	0.0002	-449.2
	ctenophore abundance	0.006	0.002	2.417	0.021	
	SST +	0.005	0.001	5.009	0.00001	-216.4
	siphonophore abundance	0.006	0.002	3.332	0.001	
	SST +	0.003	0.001	4.517	0.0001	-208.6
	ctenophore abundance +	0.006	0.002	2.703	0.011	
	siphonophore abundance	0.004	0.001	2.957	0.006	
CV-♂						
	SST	-0.252	0.041	-6.189	0.0000	-288.5
	Total gelatinous zooplankton abundance	0.025	0.007	3.475	0.001	-252.6
	Chaetognath abundance	0.018	0.006	2.769	0.007	-248.3
	Siphonophore abundance	0.026	0.005	5.673	0.00001	-269.9
	SST +	0.013	0.002	5.536	0.00001	-283.0
	siphonophore abundance	0.016	0.004	3.837	0.0003	

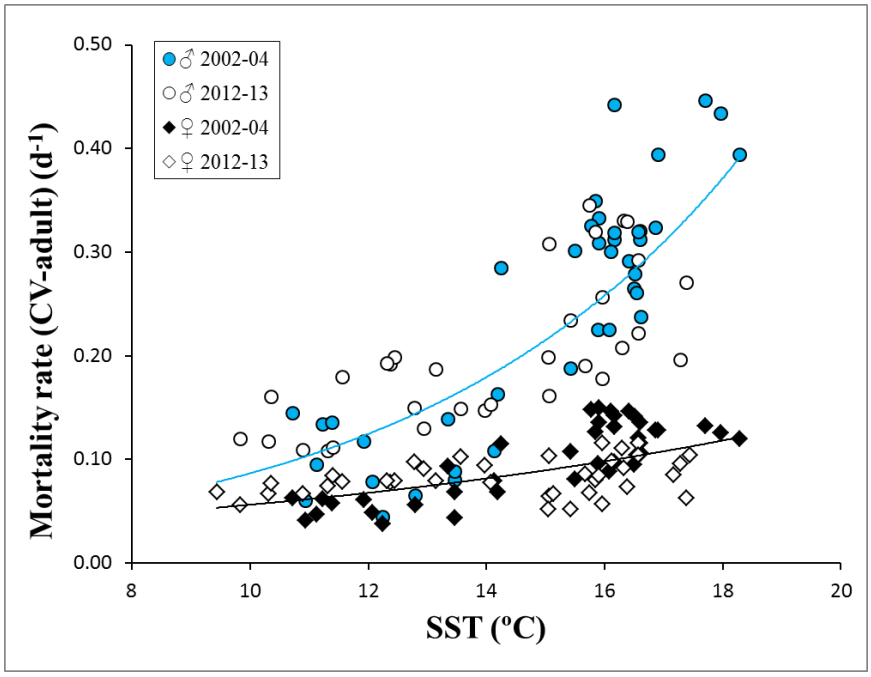

Figure 4.10. Relationship between mortality rates of CV-♀ and CV-♂, and sea surface temperature (SST), (covering the period May-September in the years 2002-2004 and 2012-2013); Q_{10} temperature coefficient for CV-♀ is 2.53 and CV-♂ is 6.17.

Both CV-female and CV-male mortality rates were positively correlated with chaetognath abundance (Figure 4.11a) and siphonophore abundance (Figure 4.11b). Total gelatinous zooplankton abundance (the sum of chaetognath, siphonophore, ctenophore and medusae abundances) was also a highly significant predictor of mortality rates for both sexes (Figure 4.11c). A stepwise backwards multiple GLS regression analysis indicated that only chaetognath abundance was a significant explanatory variable, again for both sexes. When SST was included with the predator groups in multiple regression analyses, a different suite of predators was highlighted. Here, CV-female mortality was related to SST with siphonophores, SST with ctenophores and SST with both. CV-male mortality was related to SST and siphonophores only. Predator biomass data were also used as predictor variables, however they provided little insight beyond that based on the abundance data.

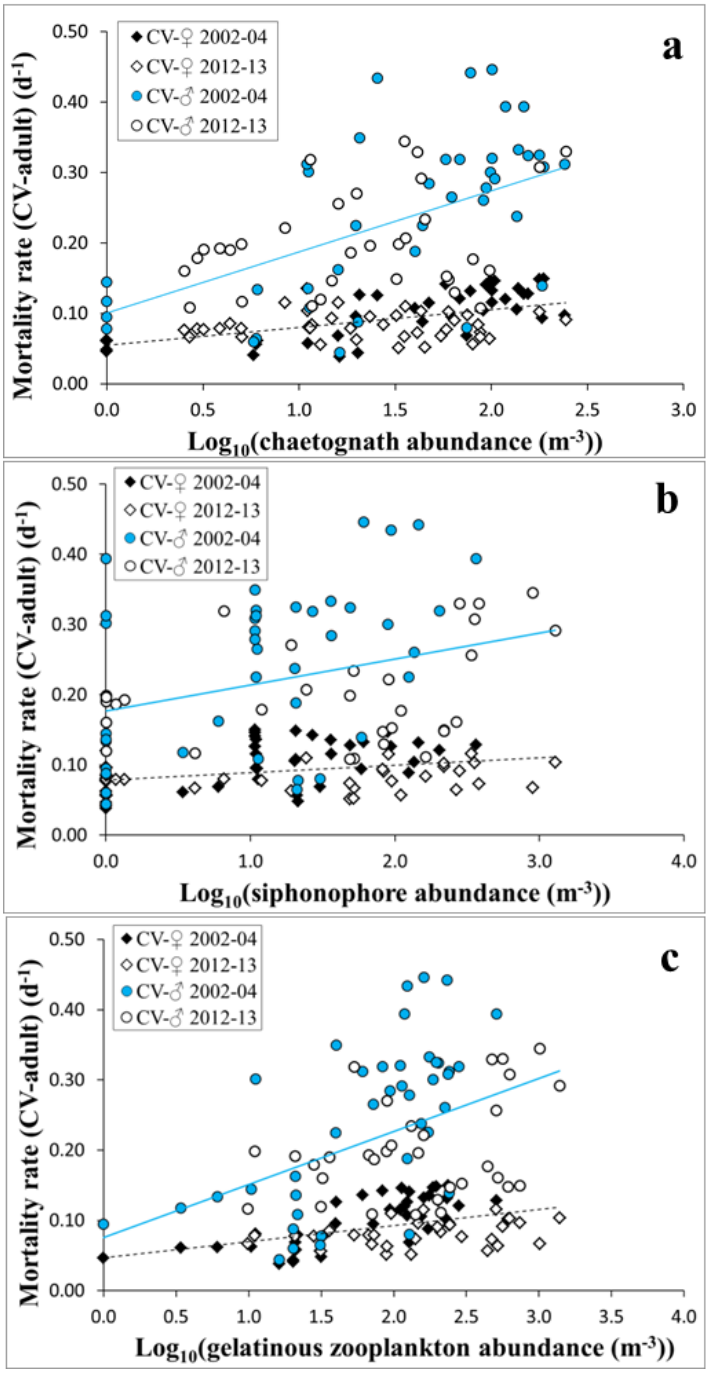

Figure 4.11. Relationship between mortality rates of CV-♀ and CV-♂, and (a) chaetognath abundance; (b) siphonophore abundance; (c) total gelatinous zooplankton abundance (including chaetognaths, siphonophores, ctenophores and medusae), (over the period May-September in the years 2002-2004 and 2012-2013). It should be noted that if I account for the strong relationship with SST, by performing a multiple gls regression with SST + predators, the relationship with chaetognaths is lost. The relationship with siphonophores remains and a relationship between female mortality and SST + ctenophores emerges (Table 4.4).

Separate analyses of 2002-04 and 2012-2013

Separate analyses of the two datasets (2002-04 and 2012-13) revealed that there were different predator effects between the two periods (Table 4.5). The 2002-04 time period showed relationships between both CV-female and CV-male mortality rates and chaetognath and siphonophore abundance, and a multiple regression analysis found that both chaetognath and siphonophore abundances together were significant predictors of CV-female mortality rates. During 2012-13, no predator group was found to significantly influence *C. helgolandicus* CV-male/female mortality and medusae emerged as the only predators with any suggestion of a positive relationship with CV-female mortality (R^2 = 0.16, p = 0.218, n = 38).

Table 4.5. Generalised least squares analysis of mean summer (May-Sept) *C. helgolandicus* CV-adult VLT total mortality rates (separate 2002-04 and 2012-13 time periods): coefficients, standard error (SE), *t*-value, *p*-value and AIC value for CV-♀ and CV-♂ stage pairs, single and multi-variable GLSs; SST = sea surface temperature; all abundances were $\log_{10}(x+1)$.

Stage and Sex	Model predictor(s)	Coefficient (slope)	SE	*t*-value	*p*-value	AIC
2002-04						
CV-♀						
	SST	0.111	0.0159	6.967	<0.00001	-28.7
	Chaetognath abundance	0.005	0.0037	1.372	0.1780	-192.2
	Siphonophore abundance	0.0121	0.0031	3.931	0.0003	-204.1
	Total gelatinous zooplankton abundance	0.0138	0.0025	2.652	0.0116	-191.2
	Chaetognath abundance + siphonophore abundance	0.0093 0.0131	0.0033 0.0032	2.843 4.033	0.0072 0.0030	-195.7
CV-♀						
	SST	0.0713	0.0275	2.593	0.0134	-22.6
	Siphonophore abundance	0.0191	0.0059	3.226	0.0026	-143.9
2012-13						
CV-♂						
	SST	0.104	0.0195	5.315	<0.00001	-18.6
	Total gelatinous zooplankton abundance	0.0386	0.0141	2.733	0.0104	-103.8

4.3.3 Non-consumptive vs consumptive mortality

Total copepodites (CI-CVI)

A total of 38 *C. helgolandicus* neutral-red stained samples were collected throughout 2013. All copepodite data were pooled and samples with a low abundance (< 30 live/dead individuals) were excluded from further analysis. A total of 31 weeks of data were available for further analyses (with a median of 95 copepodites enumerated each week).

Carcasses constituted 0-22% (mean of 9%) of the total copepodite (CI-CVI) abundance (including live and dead individuals) (Figure 4.12a). No carcasses were collected during 7 out of the 38 sampling events and these tended to occur in the winter months of December to February.

Consumptive mortality rates were typically much greater than non-consumptive rates (Figure 4.12b) and varied from 0.003 to 0.123 d^{-1} with a mean of 0.062 d^{-1}. Consumptive rates contributed on average 89% to total mortality, with a range of 46 to 100%. Non-consumptive mortality rates were often over an order of magnitude less than consumptive mortality, ranging from 0-0.02 d^{-1}, with a mean of 0.005 d^{-1}. Non-consumptive mortality was responsible for an average of just 11% of the total, but varied between 0-54% (Figure 4.12c). Seasonal patterns indicated an upturn in the contribution of non-consumptive mortality to total mortality during late spring and summer, with a decline during autumn, before a sharp increase in winter.

Simple linear regressions indicated that non-consumptive mortality rates were positively correlated with all three of the maximum wind speed variables (24, 48 and 72-hr), although only the maximum 72-hour wind speed was significant and explained around 13% of the variation (Figure 4.13). None of the mean wind speed variables indicated any relationships. None of the environmental factors (SST, SI, O_2 concentration, salinity, and fluorescence) were predictors of non-consumptive mortality. Additionally, there was no evidence of the influence of tidal state, either in terms of a flow or ebb tide, neap, spring and intermediate tides or tidal height.

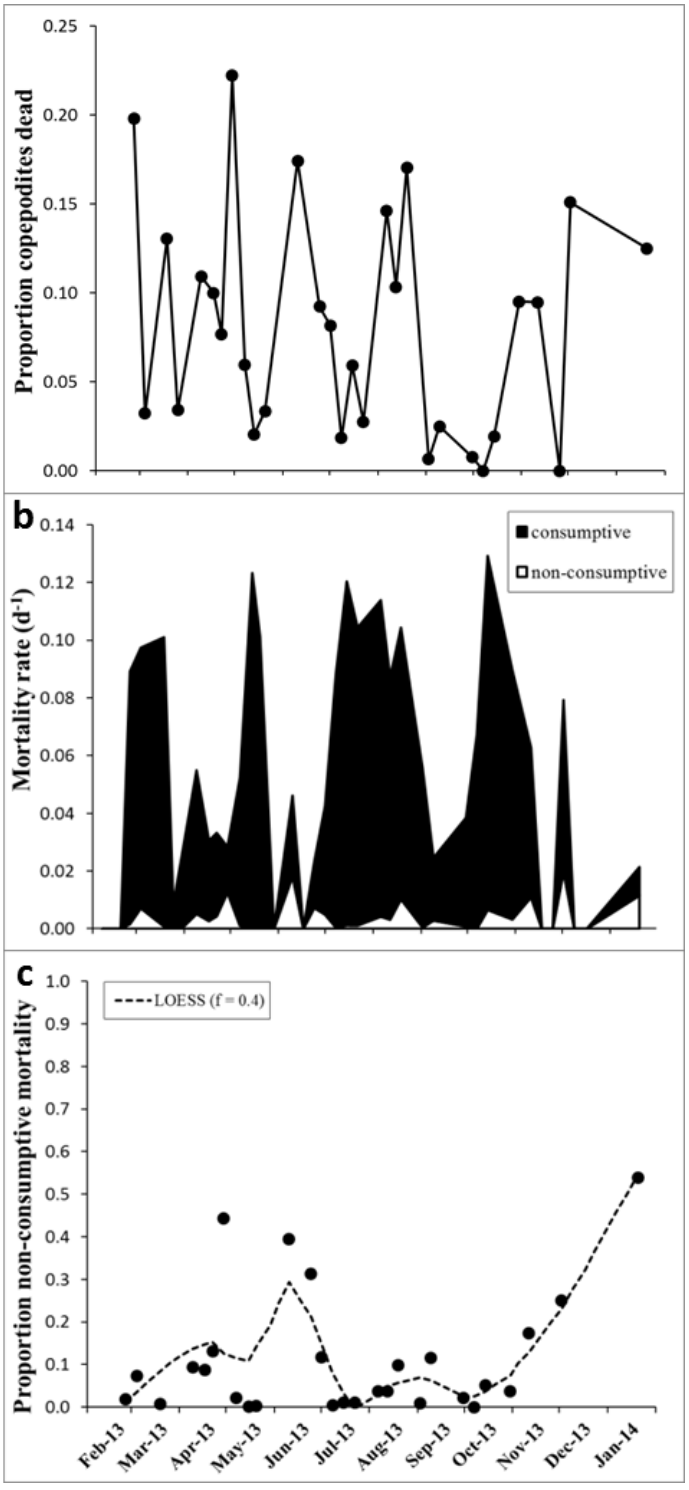

Figure 4.12. *Calanus helgolandicus* copepodite non-consumptive mortality at station L4 (February 2013 – January 2014). (a) proportion of total copepodites (CI-CVI) collected which were classified as dead (not stained by neutral red stain) from weekly sampling; (b) non-consumptive and consumptive mortality rates (CI to CVI); (c) proportion of non-predatory mortality in relation to total mortality (CI-CVI), and LOESS smooth (black dashed line); zero rates represent weeks with missing data or where rate could not be estimated.

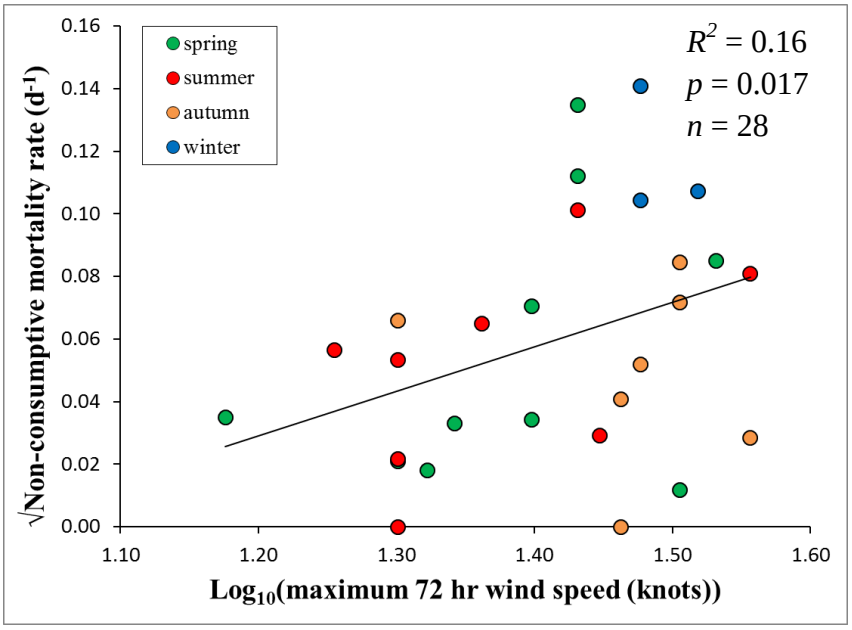

Figure 4.13. Relationship between maximum 72-hour wind speed and non-consumptive mortality rate of *C. helgolandicus* copepods (CI-CVI) (2013); symbol colours indicate seasons to illustrate distribution of rates.

Comparison of the sexes (CV-female and CV-male)

The proportion of adult male carcasses (13.4% of the adult male population) was greater than the respective value for females (5.5%). This was the case at all times of year except autumn, when no male carcasses were collected (Figure 4.14). Annual mean non-consumptive mortality rates were 0.01 day^{-1} and 0.02 day^{-1} for CV-females and CV-males respectively, and annual mean consumptive rates were 0.06 day^{-1} and 0.21 day^{-1}, indicating that CV-males were subject to both higher consumptive (~6 times) and non-consumptive mortality rates (~1.5 times) than CV-females.

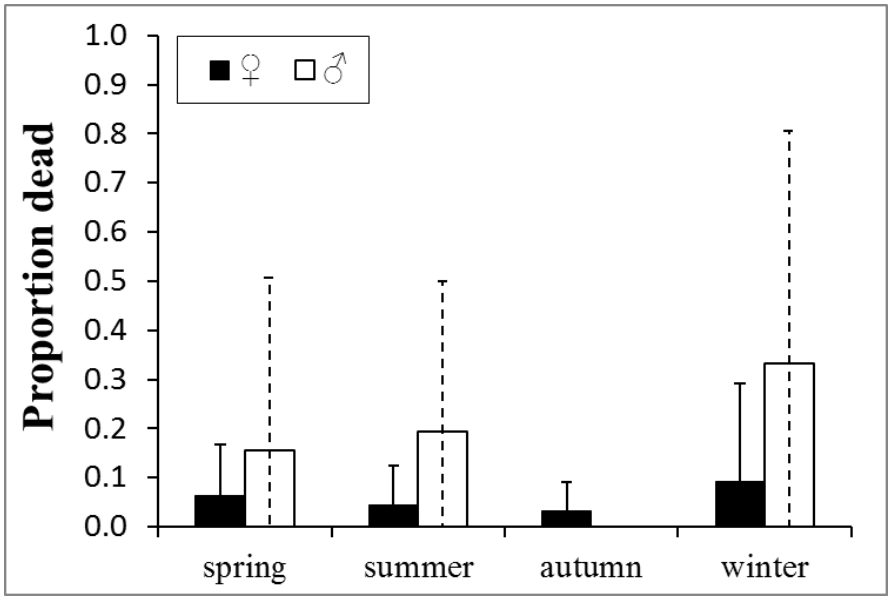

Figure 4.14. Proportion of dead *C. helgolandicus* by sex (filled bar represents ♀; white bar represents ♂) and season (spring = March-May; summer = June-August; autumn = September-November; winter = December-February). Values are seasonal means during 2013, with standard error bars representing standard deviations; numbers above bars indicate number of ♀ and ♂ analysed.

Consumptive rates contributed more to the total mortality than non-consumptive rates for both sexes; accounting for an average of 86% for CV-males and 76% for CV-females (95% CI: -0.091 – 0.005). The greatest consumptive mortality rates occurred in autumn for CV-males, whereas rates were relatively stable across seasons in CV-females (Figure 4.15). Non-consumptive mortality was greatest during the summer and winter months, for both sexes. It should be noted that the number of adults counted, whether alive or dead was very low during some of the winter weeks (i.e. < 20), so I urge caution with these data.

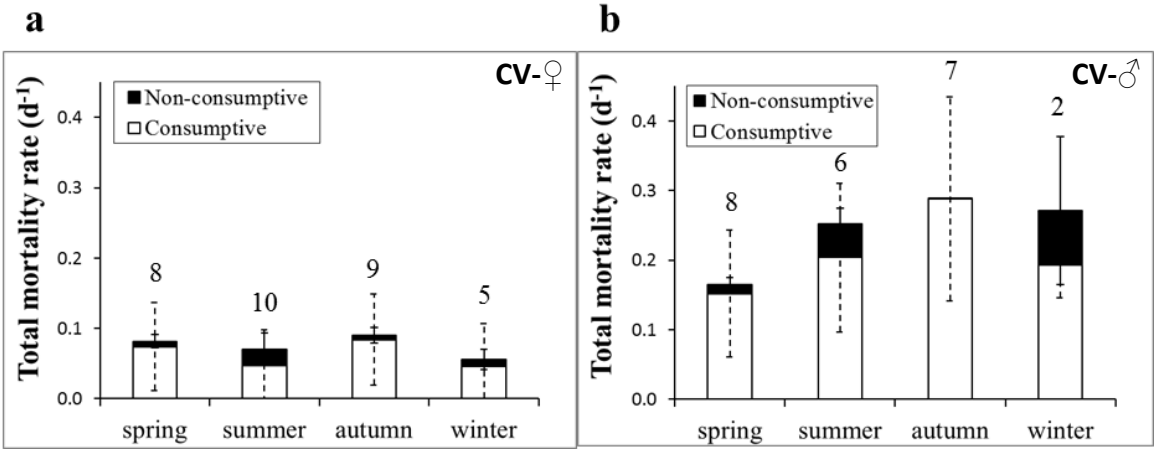

Figure 4.15. *Calanus helgolandicus*. Total mortality rates, divided into the consumptive (white bar) and non-consumptive mortality rates (filled bar). Values are seasonal means during 2013, with error bars representing standard deviations for each of the two mortality components. (a) CV-♀, and (b) CV-♂. Spring = March-May, summer = June-August, autumn = September-November, winter = December-February; numbers above bars indicate number of samples analysed; consumptive mortality error bars represented by dashed line, non-consumptive mortality error bars represented by solid line, plus bars shown only.

The CV-male consumptive mortality rates were related both to chaetognath and siphonophore abundance, and siphonophore + medusae abundance in a multiple gls regression. SST was a significant predictor of CV-male, but not CV-female consumptive mortality. When the relationship between SST and CV-male consumptive mortality was accounted for, medusae abundance was the only significant predator. The CV-female consumptive mortality was related to chaetognath abundance only. Neither CV-male nor CV-female non-consumptive mortality rates were related to any environmental factors, including SST. The results of all significant mortality regression analyses are presented in Table 4.6.

Table 4.6. *Calanus helgolandicus*. Generalised least squares analysis of weekly consumptive mortality rates of CV-♀ and CV-♂ with temperature and predators (2013): coefficients, standard error (SE), *t*-value, *p*-value and AIC value, single and multi-variable GLSs; SST = sea surface temperature; *n* = 21 (CV-♀), *n* = 17 (CV-♂); all abundances were $log_{10}(x+1)$ except CV-♀ chaetognath.

Stage and Sex	Model predictor(s)	Coefficient (slope)	SE	*t*-value	*p*-value	AIC
CV-♀						
	Chaetognath abundance	0.006	0.002	2.667	0.009	-425.6
CV-♂						
	SST	-0.027	0.011	2.450	0.027	-18.2
	Chaetognath abundance	0.092	0.037	2.502	0.020	-15.2
	Siphonophore abundance	0.086	0.014	6.152	0.00001	-27.8
	SST +	0.036	0.010	3.667	0.002	
	medusae abundance	-0.012	0.044	-2.634	0.020	-13.1

4.3.4 Matrix mortality model

The weekly proportional mortality (PM) results integrate multiple weeks of egg laying (Figure 4.16), and span the one to two month *C. helgolandicus* generation timescale, therefore I have limited any analysis of the weekly PM values. Instead, I initially used an April-August time-span (the summer growth period and also time of most robust data) to calculate a mean PM index for each year; followed by the usage of specific months pertinent to the seasonality of each specific predator group to calculate the mean PM. The median predicted *C. helgolandicus* CI-CV copepodite peak abundance of ~10,000 m^{-3} occurred during June-July, with the median observed peaks of ~70 and ~100 m^{-3} occurring in June and August (Figure 4.17a). This indicates that each year, 95 - 99% of all the potential *C. helgolandicus* copepodites (CI-CV) are lost at some point before they reach adulthood, whether this be a failure of eggs to hatch, naupliar abnormalities, predation on eggs/nauplii/copepodites or some other form of non-consumptive type of mortality.

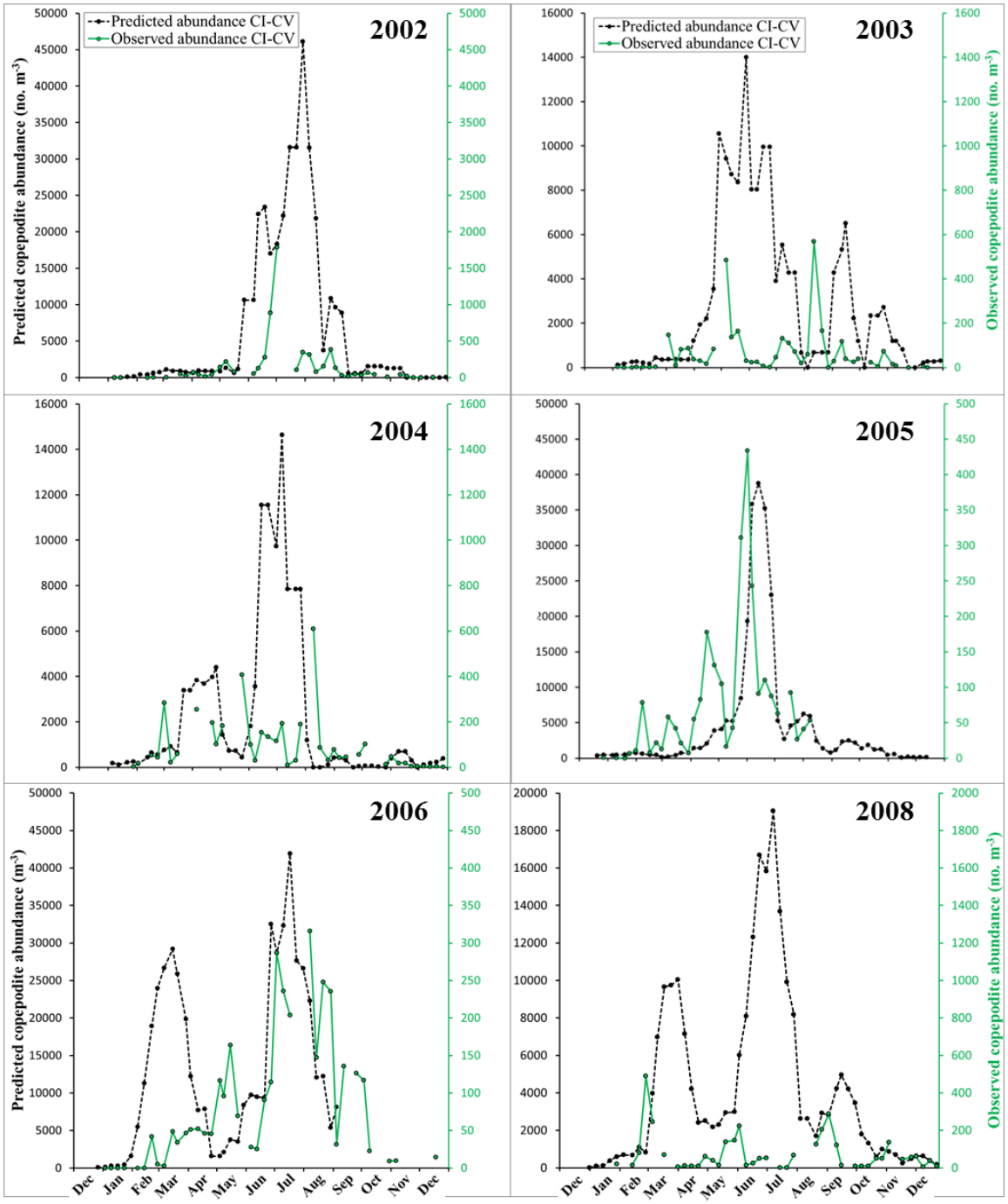

Figure 4.16. Matrix mortality model method presenting predicted *Calanus helgolandicus* copepodite (CI-CV) abundance and observed copepodite abundance (2002-2015, excl. 2007) at station L4.

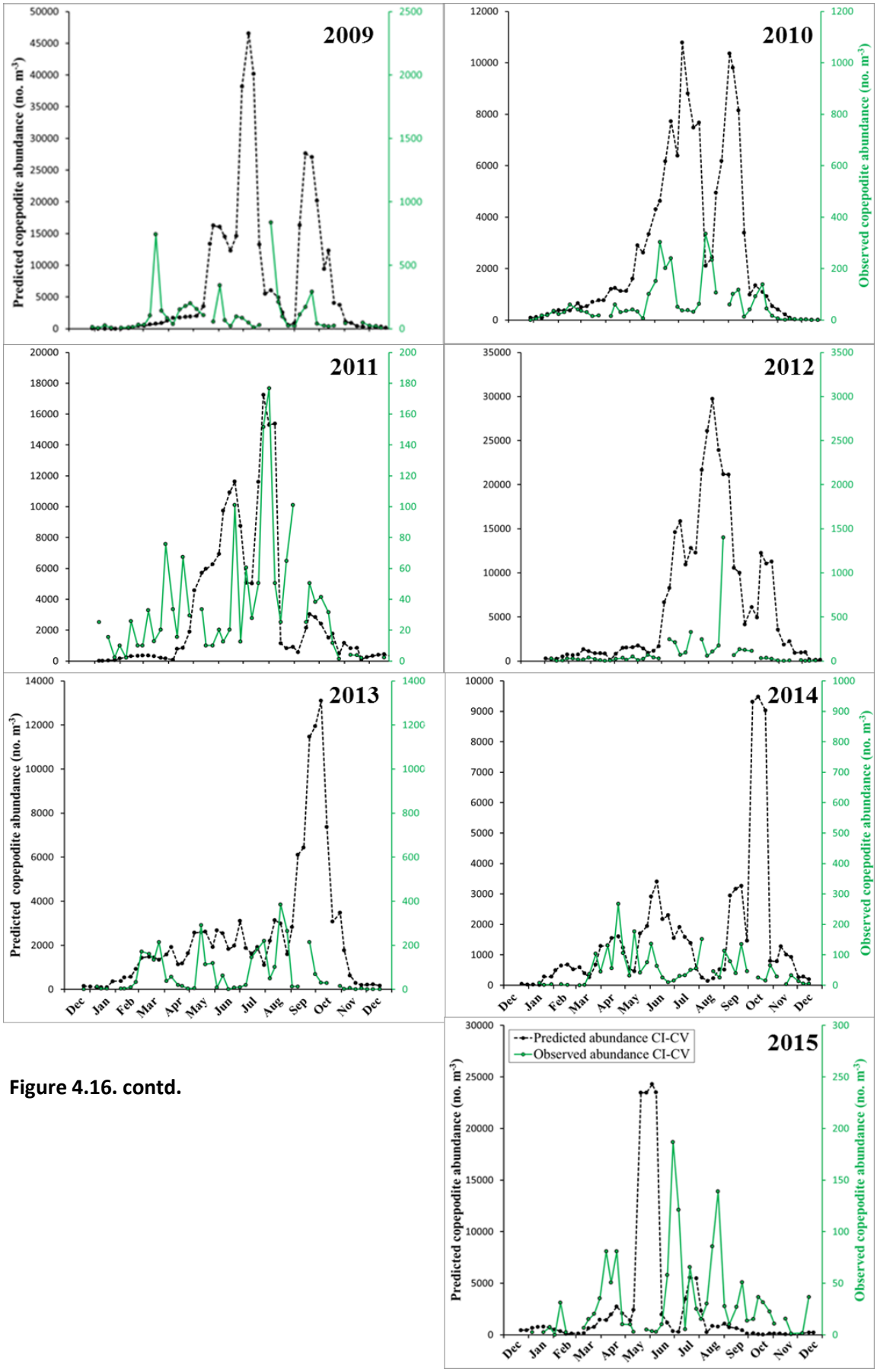

Figure 4.16. contd.

Copepodite PM was highest in the earlier part of the summer (June/July) but decreased thereafter (Figure 4.17b). Mean April-August PM for 2002-2015 (excl. 2007) ranged from 94 to 99%, with a median of 98% (Table 4.7). Table 4.8 details the summary statistics of each of the constituent variables within the matrix mortality and demonstrates that it is the ~7-fold variation of the female abundance, translating to the ~11-fold variation in TRO that was most important in deriving the variability in absolute mortality derived by this matrix model.

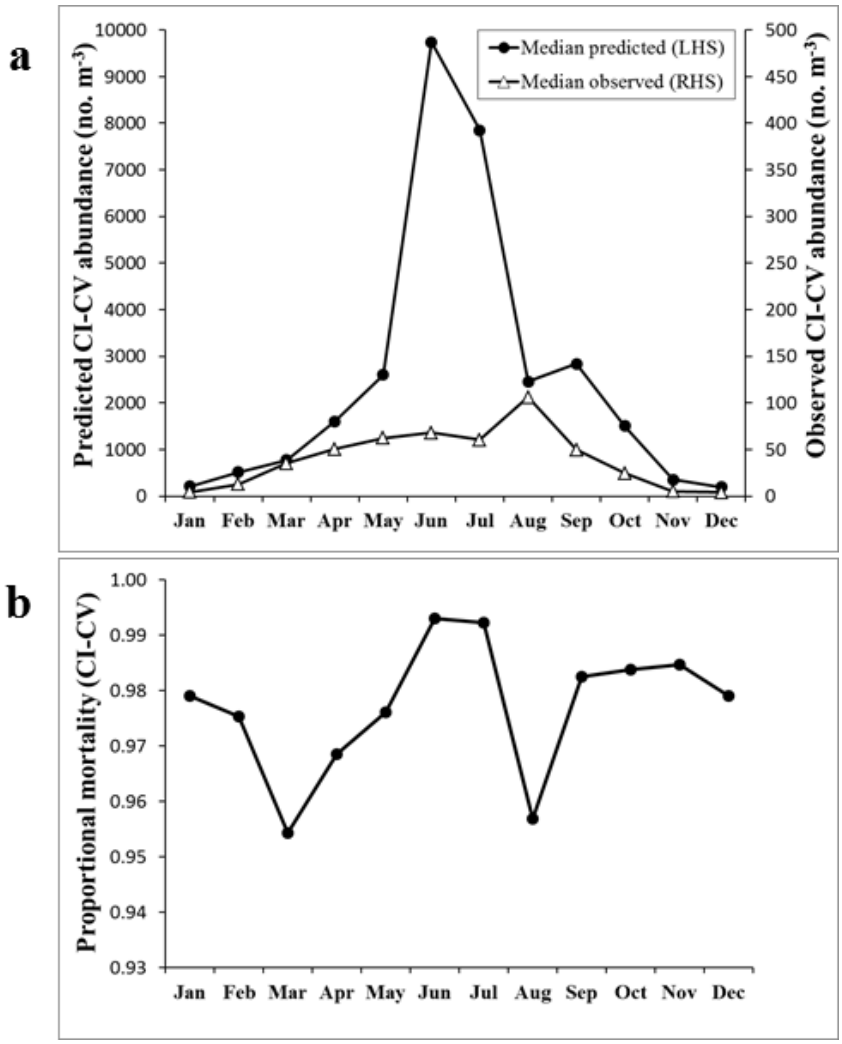

Figure 4.17. Matrix mortality model results (2002-2015, excluding 2007); (a) median monthly abundance of CI-CV copepodites as observed (right-hand axis (LHS)) and predicted (left-hand axis (RHS)), note change in scale; (b) proportional mortality, as derived from the difference between the observed and predicted values.

Table 4.7. *Calanus helgolandicus*. Summer (April – August) proportional mortality rates between egg and CI-CV stage, as determined by the matrix mortality method; mean, standard deviation and *n*; number of sampling time-points (2002-2015, excluding 2007).

Year	Proportional mortality (%)	
	Mean	*n*
2002	98.0	18
2003	98.1	19
2004	96.5	16
2005	98.8	18
2006	99.1	17
2008	99.0	18
2009	98.8	19
2010	97.7	20
2011	99.2	21
2012	98.4	19
2013	95.4	22
2014	94.5	20
2015	99.2	20

Table 4.8. *Calanus helgolandicus*. Minimum, maximum, mean, median and range amplitude of variables used over 13 years within matrix mortality method (April-August), and resulting mortality estimates. Amplitude is maximum/minimum range value.

Variable	Min	Max	Mean	Median	Range amplitude
♀ *C. helgolandicus* abundance (no. m^{-3})	3.70	27.06	12.55	13.13	7.3
C. helgolandicus egg production rate (EPR) (eggs $female^{-1}$ day^{-1})	18.43	28.75	23.63	24.47	1.6
C. helgolandicus total reproductive output (TRO) (eggs m^{-3} day^{-1})	65.50	717.75	337.96	265.03	11.0
C. helgolandicus copepodite abundance (CI-CV) (no. m^{-3})	49.47	284.67	119.57	108.81	5.8
Absolute mortality (no. m^{-3})	1378.39	13927.42	7558.74	6715.45	10.1
Proportional mortality (%)	88.7	98.3	95.0	96.2	-

Mean April-August PM was not related to mean April-August SST. A backwards stepwise multiple gls regression including the main zooplankton predators (chaetognaths, siphonophores, medusae and ctenophores) resulted in a model with only one significant explanatory variable; chaetognaths (Figure 4.18a). There was no relationship between total gelatinous predator abundance and PM, unlike my findings for summer VLT mortality rates. Mean annual gelatinous zooplankton biomass related positively to mean PM, although not significantly (2002-2015, excl. 2005-07) (R^2 = 0.35, p = 0.056, n =10), while no such relationship was found for the non-gelatinous component.

Because the April-August averages integrate seasonal as well as interannual variability I focussed analysis on time periods specific to the peak of each predator. Thus a highly significant relationship was found between mean May-June PM and mean May-June ctenophore abundance (2008-2015) (Figure 4.18b) and June to October mean PM was related to mean medusae abundance (2002-2015, excl. 2005-07) (Figure 4.18c).

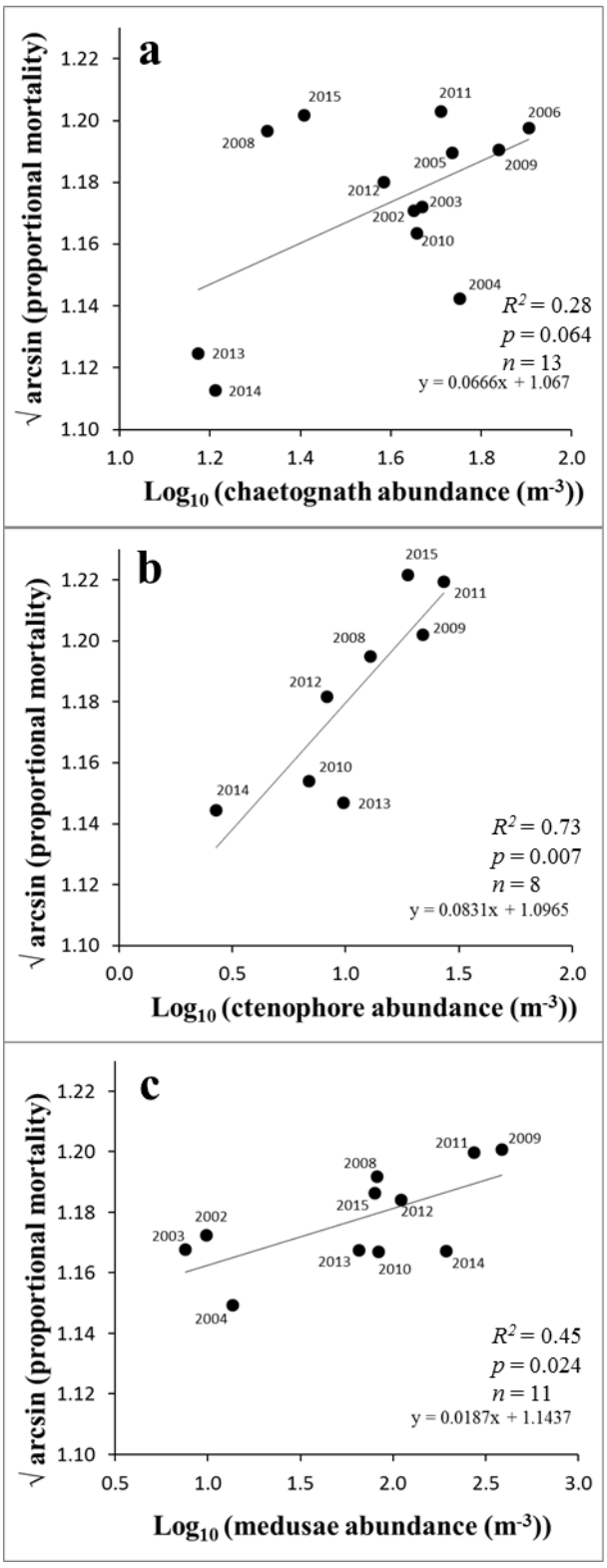

Figure 4.18. ***Calanus helgolandicus*** **matrix mortality mean proportional mortality (PM) versus predator abundance; (a) mean chaetognath abundance (April to August); (b) mean ctenophore abundance (May to June); (c) mean medusae abundance (June to October); station L4, western English Channel, UK.**

4.4 Discussion

The three mortality methods provided complementary insights into the mortality dynamics of *C. helgolandicus*. Mortality was calculated over various temporal resolutions, including over 4 years (CV-adult copepodite VLT), seasonal over 1 year (consumptive vs non-consumptive), and across 13 years (matrix method). I discuss these in combination and focus on agents of total mortality, non-consumptive mortality rates and causes and differences in mortality rates between males and females.

4.4.1 Total mortality in relation to temperature and predators

VLT total mortality rates were highest during summer and autumn, lowest in winter, and rates for both sexes followed similar seasonal patterns. Kvile et al. (2016) proposed a statistical regression approach to take account of the issues related to a traditional VLT method when trends in recruitment were present. A downward trend in recruitment to the stages leads to underestimated mortality rates, whereas the reverse is true for an increasing trend in recruitment. While my data is unsuitable for this approach, the basic finding of high mortality in autumn at a time of reducing recruitment to late stages would be supported even more strongly if mortality was indeed underestimated at this time.

Sea surface temperature was a positive predictor of both CV-male and CV-female VLT mortality rates. There may be several factors behind this. First, predator activity is higher in warmer summer conditions resulting in higher food demands. Second, higher metabolic rates of *C. helgolandicus* would be expected to result in higher natural mortality. These temperature related factors seem to modulate the seasonality of mortality rather than its inter-annual variability, since the matrix model did not invoke temperature variations between years.

Considering the effect of the predator landscape, chaetognaths, siphonophores and total gelatinous zooplankton abundance were all significant predictors of late-stage *C. helgolandicus* VLT mortality rates. Simple calculations of the potential

predatory impact of chaetognaths suggest that they are capable of removing up to 82% of the *C. helgolandicus* copepodites daily from the water column (using a mean clearance rate of 1.2 L ind^{-1} d^{-1} provided by Tönnesson and Tiselius (2005), the maximum chaetognath abundance of 568 m^{-3} and the L4 water column depth of 55 m). An investigation of the content of the gastrozooids of *Muggiaea atlantica* found prey ranging from 0.1 to 0.9 mm in length (Purcell, 1982), which indicates that only the naupliar and CI copepodite stages of *C. helgolandicus* could be ingested. Using mean May-September total naupliar NI-NVI and CI abundances (2002-04 and 2013), I calculated a clearance rate of 11.1 L $siphonophore^{-1}$ d^{-1}, which translated to a population clearance rate of 1377 m^3 (assuming a maximum siphonophore abundance of 1.27 L^{-1}) equivalent to >2500% of the 55 m^3 water column.

Analysis of the separate 2002-04 and 2012-13 datasets suggested that chaetognaths and siphonophores were most influential during the earlier time period, and medusae were more important during the latter, when they were more abundant. This suggests that there are inter-annual fluctuations in the predatory influence of each of the gelatinous zooplankton, and in some years there are blooms of various medusae that may significantly impact the copepod population. The matrix model suggested that chaetognaths, medusae and ctenophores were important at an inter-annual scale, but each at specific times of the year. The role of these groups is also corroborated by the fact that mean annual total gelatinous zooplankton biomass was a predictor of annual PM (proportional mortality), but mean annual non-gelatinous zooplankton biomass was not. Total zooplankton biomass and abundance at L4 is dominated for most of the year by the non-gelatinous zooplankton, which in turn is dominated by the Copepoda. These two sets of results at different scales substantiate that gelatinous predators (including chaetognaths) are important in structuring *C. helgolandicus* populations in the western English Channel.

Parasagitta setosa and *Parasagitta elegans* are the two dominant chaetognaths recorded at L4. *Parasagitta* spp. are major predators of copepods (Rakusa-Suszczewski, 1969) and *Calanus* spp. have been found to predominate in the diet of *Parasagitta elegans* in particular (Grigor et al., 2015). Various studies have

linked *C. helgolandicus* abundance with chaetognath abundance (Southward, 1984; Clark et al., 2003; Bonnet et al., 2010). The fact that the matrix mortality method also found a relationship with chaetognath abundance is noteworthy in that it suggests the predation pressure by chaetognaths is strong enough to be detected at an inter-annual scale. Of the Siphonophora, the Calycophorae *Muggiaea atlantica* and *Muggiaea kochi* are the main species at L4. Blackett et al. (2014) reported that high densities of *Muggiaea* spp. were often associated with high abundance of copepods. *C. helgolandicus* abundance was also negatively correlated with siphonophore abundance in the 1989-2003 L4 study (Bonnet et al., 2010). Medusae were found to influence proportional mortality (PM) during June to October, the time of their peak abundance. There are ~35 species of hydromedusae and scyphomedusae recorded at L4, the majority of which are reported to include copepods in their diet (see Chapter Five). The numerically dominant species include *Aglantha digitale*, *Obelia* spp., *Liriope tetraphyllae* and *Lizzia blondina*, with two of these, *A. digitale* and *Obelia* spp. recorded as feeding specifically on *Calanus* spp. (Lebour, 1922). Ctenophores are dominated by *Pleurobrachia pileus* at L4 and are usually restricted to a month or so of extreme abundances in early summer. Although they are present for only a short period, their total number and biomass can be substantial; hence the highly significant relationship with *C. helgolandicus* mortality during these months.

There has been only limited mention in the literature of the relationship of *C. helgolandicus* abundance with fish larvae, despite this species being a major prey item of many larval fish species (Lebour, 1918; Rice, 1963; Robb and Hislop, 1980; Rowlands et al., 2008; Lynam et al., 2013). My study found no relationships between fish larvae and mortality rates. Although these infrequent predators may target *Calanus* spp., it may be that they are usually too sporadic to have a major impact on copepod abundances.

4.4.2 Non-consumptive vs consumptive mortality rates

My non-consumptive mortality estimates are, to my knowledge, the first for a large copepod, and certainly for this species. Carcasses accounted for an average of 9%

of all *C. helgolandicus* copepodite stages. This was a similar percentage to that found by Elliott and Tang (2011a) who reported a mean of 12-15% *Acartia tonsa* copepodite carcasses in the lower Chesapeake Bay, USA. Non-consumptive mortality of CI-CVI stages contributed from 0-54% of total mortality, with an average of 11%, demonstrating that this type of mortality can be substantial. Consumptive mortality of *C. helgolandicus* accounted for an average of 89% of the total mortality, which is higher than the 75% estimated by (Hirst and Kiørboe, 2002) in their study of global patterns in mortality rates. Non-consumptive mortality rates of total copepodites (CI-CVI) were high during the summer and winter, but dropped during late-summer to autumn, whereas consumptive rates tended to be much higher in the autumn. This indicates that in the autumn, at a time when predator abundance is at its highest, consumptive processes may be most important in controlling the *C. helgolandicus* population, but at other times, non-consumptive factors contribute more strongly. During the derivation of non-consumptive mortality rates, I made the simplifying assumption that carcasses were re-suspended at L4 through turbulent action, with no losses due to sinking. *C. helgolandicus* carcasses are large and likely to sink; therefore actual non-consumptive mortality rates may in fact be greater than I have calculated.

Physico-chemical factors have been investigated as causative agents for non-consumptive mortality. Elliott and Tang (2011b) established a positive relationship between *Acartia tonsa* non-consumptive mortality and SST in Chesapeake Bay. Other studies report no relationships at all with environmental parameters (Tang et al., 2006; Beşiktepe et al., 2015). In this study, of the environmental factors considered, only maximum wind speed in the preceding 72-hours was a predictor of total copepodite (CI-CVI) non-consumptive mortality, suggesting that increased wind and storminess may play a role. South-west England and its coastal areas are subject to storm events and strong winds from the south-west and north-east, particularly in the winter. The January 2014 sampling point occurred after a succession of extreme weather events linked to cyclone conditions throughout December 2013 into January 2014, with gusts of > 70 knots recorded off the coast of Plymouth.

The occurrence of wind is a key factor in the generation of turbulence, as kinetic energy is added to the environment (Kiørboe and Saiz, 1995). It is recognized that copepods adjust their behaviour and swimming effort according to the background flow (Michalec et al., 2015), and that minimal turbulence can cause enhanced heart-beat rate and activity of copepods (Alcaraz et al., 1994). Whilst small-scale turbulent eddies are known to enhance encounter rates and increase grazing rates (Alcaraz, 1997), higher levels of turbulence can decrease the period of contact with food items (Prairie et al., 2012) and inhibit swimming, growth efficiency and development. Therefore increased turbulence is thought to have a dome-shaped effect on the fitness, condition and production of zooplankton (Tóth et al., 2011).

Tank experiments simulating turbulence experienced in Lake Balaton (wind velocity of 11.8 ms^{-1} which equates to ~23 knots) found that the increased turbulence and low water-level caused a decrease in survival rates in the calanoid copepod *Eudiaptomus gracilis* (Tóth et al., 2011). In comparison to Lake Balaton, Station L4 frequently experiences gales and strong winds with a mean wind velocity > 40 knots and gusts exceeding 80 knots (~26 ms^{-1}) and is a relatively shallow shelf site (~50 m), so it would be reasonable to hypothesise that copepod mortality due to extreme weather may be important at certain times of the year.

Global wind speeds have been shown to exhibit an increasing trend, along with wave height (data from 1985-2008) (Young et al., 2011). Mean annual scalar wind speed at L4 has increased significantly from 1960 to 2014 (R^2 = 0.75, p < 0.00001, n= 54) (International Comprehensive Ocean-Atmosphere Data Set (ICOADS); *COPEPOD: the global plankton database.* ONLINE. 2009. http://www.st.nfms.noaa.gov/copepod), which may be an indication of changing weather patterns. Climate change is predicted to manifest in increased rainfall and higher temperature, resulting in the increased incidence of extreme weather events, including strong winds (Fischer and Knutti, 2015); therefore non-consumptive zooplankton mortality may occur at higher rates in the future.

4.4.3 Comparing mortality rates between sexes

One of the main aims of the research was to explore how the *C. helgolandicus* mortality rates (both consumptive and non-consumptive) varied between the sexes. The proportion of adults which were dead at the point of their collection was generally greater for males than for females. This result is akin to that reported by Elliott and Tang (2011a) with 40% of adult males of *A. tonsa* collected being carcasses, with only 9% of adult females being carcasses. Based on VLT mortality methods, CV-male total mortality rates were ~2.5 times greater than CV-female rates; a phenomenon that has been reported with other copepod species. For example, *Oithona similis* male mortality rates were estimated to be ~12 times that of females in polar waters (Hirst and Ward, 2008), and *Calanus pacificus* male rates were 2-3 times higher than female rates in California (Ohman and Hsieh, 2008). An important assumption I have made in this study is the 1:1 sex ratio of CV copepodites. This is based on Fisher's principle (Fisher, 1930), which states that most animal species must produce approximately equal numbers of males and females, and that any skew will adjust back to equal ratios through the process of natural selection. However, Conover (1988) found that the sex ratio of *Calanus* CVs, based on the appearance of the gonad, was strongly skewed towards females, and more recent evidence reported a female-skewed sex ratio at birth in the calanoid copepod *Acartia tonsa* (Burris and Dam, 2015), therefore this assumption may need to be reviewed as new data comes to light. The relative mortality rates of males and females reflect the assumption of a CV sex ratio; for example Figure 4.19 (Appendix B) provides for comparison the male and female mortality rates derived using a female-skewed 5:1 CV sex ratio.

The difference in copepod mortality rates between the sexes has been attributed either to male-skewed predation (MSP), defined as elevated consumptive mortality during more active mate-finding behaviour; or the shorter life-expectancy exhibited by males and subsequent earlier death from natural causes (Hirst et al., 2010). The male skewed predation theory purports that male copepods move more frequently and faster in finding females, and so are more likely to encounter predators (Kiørboe and Bagøien, 2005; Kiørboe, 2008). The shorter physiological longevity of

males is implicated through various different studies, and importantly *Calanus* spp. are believed to adopt a semelparous reproduction strategy (Mayor et al., 2009; Daase et al., 2013), defined as a single reproductive period in their lifetime (Hairston and Bohonak, 1998), after which the adults die. This may contribute to some of the non-consumptive mortality observed, particularly later in the season. Laboratory experiments with *C. helgolandicus* demonstrated that, in general, males moulted and died in culture sooner than the females (Mullin and Brooks, 1967). Female *Temora longicornis* were more sensitive (faster swimming velocities) to the presence of food than males (Moison et al., 2013) and gut fullness ratios were lowest in male *Calanus sinicus* (Chen et al., 2010) indicating that male copepods simply do not forage or ingest as much food as females. *C. finmarchicus* (Ohman et al., 2004) and *Calanus pacificus* (Ohman and Hsieh, 2008) male mortality rates were explained by atrophied mouth-parts and reduced feeding rates of adult males, leading to an exhaustion of lipid reserves. Rodríguez-Graña et al. (2010) reported that males had more oxidative damage than females. Some studies question the male predation theory and suggest that differential longevity of the sexes is more important than previously considered (Gusmão et al., 2013). Others suggest that depending on genera and species, both predation and a shorter life-expectancy are likely to be important (Hirst et al., 2010; Kiørboe et al., 2015).

By separating consumptive and non-consumptive mortality rates for CV-female and CV-male *C. helgolandicus*, I found that consumptive mortality contributed most to the total mortality (at 70% and 85% of the annual mean rate for CV-females and CV-males respectively). CV-male consumptive mortality rates were on average ~6 times greater than that of CV-females; whereas CV-male non-consumptive rates were on average only ~1.5 times that of CV-female rates. As death from senescence would result in carcasses, while predation typically does not, my results suggest consumptive mortality is of greater significance to male loss rates in comparison to that of females. My approach provides a new method to explore differential causes of mortality in the sexes, and in this study provides evidence that predation on males is much greater than on females in *C. helgolandicus*.

My investigation has improved our knowledge of *C. helgolandicus* mortality rates and sources at L4. The estimation of mortality rates and attributing causal factors is necessary to elucidate the full set of parameters that are responsible for initiating and regulating copepod populations. I conclude that total mortality rates are dominated by predation mortality, and gelatinous predators in particular are important in *C. helgolandicus* population regulation. However non-consumptive mortality is not inconsequential and at times of the year, this may contribute more than consumptive sources. Major agents of non-consumptive mortality may include increased turbulence, which could become progressively more important in a future with a more extreme climate. *C. helgolandicus* males experience higher rates of both consumptive and non-consumptive mortality. The six-fold difference between CV-male and CV-female consumptive rates indicates that it is the male's higher susceptibility to predation that explains most of the increased total mortality; however the higher male rates likely reflect both their different behaviour and their shorter natural lifespan.

CHAPTER FIVE

An investigation of the major predators of *Calanus helgolandicus* in the English Channel

Gelatinous zooplankton (including ctenophores, medusae and siphonophores) are purported to be voracious carnivorous predators, particularly of copepods. Various gelatinous species have been implicated as important predators of late-stage Calanus helgolandicus *in the western English Channel. The siphonophore* Muggiaea atlantica *was the most abundant gelatinous species recorded at L4, followed by the ctenophore* Pleurobrachia pileus*. During the spring and summer of 2015 I observed a succession of gelatinous zooplankton in the routine sampling at the station L4, and opportunistically collected specimens of the ctenophore* Pleurobrachia pileus *and the hydromedusa* Leuckartiara octona *for molecular gut-content analysis (*Muggiaea atlantica *were not collected as their stomachs are consistently detached from the nectophores in the net-sampling process). DNA was extracted and amplified from 87* P. pileus *and 36* L. octona *(whole specimens) collected between May and July 2015. I employed primers targeting the V9 region of the 18S nuclear small subunit (nSSU) ribosomal RNA (rRNA) gene and next-generation sequencing (NGS) techniques to sequence the mixed DNA assemblage.* C. helgolandicus *sequences were detected in every pooled sample and contributed a mean of 4% to the* P. pileus *diet (57% of copepod sequences) and a mean of 8% to the* L. octona *diet (79% of copepod sequences). However, DNA sequences from other gelatinous species proved to be dominant in almost all samples of both species. This surprising result suggests that copepods may not be the main prey species of gelatinous predators, and that they may survive mostly on other gelatinous species.*

5.1 Introduction

Many gelatinous zooplankton are reported to be carnivorous zooplanktivores and numerous studies highlight the importance of copepods in their diet (e.g. Lebour, 1922; Greene et al., 1986; Chandy and Greene, 1995). Maud et al. (In review) found that gelatinous predator biomass (including ctenophores, siphonophores and medusae) and chaetognaths were significant predictors of late-stage *C. helgolandicus* mortality rates. However, abundance and mortality rate correlations, and laboratory-based feeding experiments, do not necessarily prove predator ingestion of prey in a natural system, hence, the use of gut-content analysis of wild organisms to substantiate predator-prey interactions is of considerable benefit.

Traditional studies of gut-content have involved time-consuming and painstaking dissection of gelatinous zooplankton and identification of undigested and half-digested prey fragments (e.g. Fraser, 1970; Purcell, 1982; Chandy and Greene, 1995), which may lead to a bias towards prey items that are harder to digest (with a longer gut transit time) and identifiable from indigestible body parts. The development of molecular gut-content analysis techniques and the use of the polymerase chain reaction (PCR) has afforded the identification of prey DNA in the guts of predators, and species-specific primers have provided evidence for the ingestion of a target prey species (e.g. Jarman et al., 2002; Vestheim et al., 2005; Bonnet et al., 2010). Such techniques are becoming widely used in both aquatic [i.e. seahorses (Corse et al., 2015), fish (Leray et al., 2013) and dolphins (Dunshea et al., 2008)] and terrestrial ecological studies [i.e. parasitoid wasps (Rougerie et al., 2011), carabid beetles (Eitzinger and Traugott, 2011), birds (Sutherland, 2000) and bats (Zeale et al., 2011)].

More recently, metabarcoding approaches (where DNA regions, or "barcodes", are sequenced for every organism in a sample), as well as universal primers, the advent of high through-put (HTP) technology and next generation sequencing (NGS) platforms have allowed for the sequencing of DNA from natural mixed assemblages, thus assessing the true biodiversity (Lindeque et al., 2013; de Vargas et al., 2015; Bucklin et al., 2016). Applied to gut-content analyses, metabarcoding and NGS have provided a more accurate and complete characterisation of the diversity of prey items

(e.g. Blankenship and Yayanos, 2005; King et al., 2008; Pompanon et al., 2012; Maloy et al., 2013), revealing previously unknown predator-prey interactions, and providing semi-quantitative assessments of the contribution each prey species to the diet (Albaina et al., 2016).

Whilst many studies have extracted DNA from the dissected gut of the predator, this may be tricky for smaller and less robust zooplankton (e.g. nauplii, gelatinous zooplankton); hence extraction of DNA from the whole organism is necessary. This technique, however, means that the predator, as well as the prey DNA, may be amplified and the predator DNA will likely dominate the PCR product and mask the prey DNA (Piñol et al., 2014). Methods using predator-specific blocking primers have been employed to block the amplification of the predator DNA (e.g. Vestheim and Jarman, 2008; Deagle et al., 2010), however they may also block prey DNA, particularly if it is closely related to the targeted predator, thereby introducing a different bias into the analysis. Piñol et al. (2014) promoted the use of NGS without the use of blocking probes to analyse an invertebrate diet (a terrestrial arachnid), concluding that this technique provided ample sequences for the analysis of the prey diversity.

Here I was able to exploit the sampling of a progression of large blooms of ctenophores and medusae at L4 throughout the 2015 season and undertake molecular gut-content analysis of multiple gelatinous predators to confirm the presence of, and assess the importance of, *C. helgolandicus* as prey. I utilised the Illumina MiSeq NGS platform and 18S rRNA V9 primers following the protocol of the novel study by Albaina et al. (2016). The V9 region is suitable for metabarcoding of gut content as it has a broad amplification range, short amplicon size to maximise the signal returned from partially digested prey and is represented extensively in public databases (Albaina et al., 2016 and references therein). To my knowledge, this is the first study to use NGS to evaluate the prey field of gelatinous zooplankton using whole organisms.

The research aimed to answer the following questions; (1) is there evidence for the predatory impact of gelatinous zooplankton on *C. helgolandicus* in the L4 time

series? (2) which gelatinous zooplankton prey on *C. helgolandicus?* and (3) what contribution do they make to the diet, at L4?

5.2 Materials and methods

5.2.1 Molecular analysis of gut contents

Selection of gelatinous zooplankton for DNA extraction

Between 15/05/15 and 14/07/15 live *Pleurobrachia pileus* ctenophores and *Leuckartiara octona* hydromedusae were selected from the routine weekly vertical WP-2 200 µm net samples (individual ctenophores and medusae were picked as soon as possible on return from L4 whilst in a controlled-temperature laboratory at ambient L4 temperature). Specimens were chosen on their prevalence in the sample and ease of identification so that many specimens could easily be collected. Each specimen was identified using microscopy and triple rinsed in 0.2 µm filtered sea water (FSW) to ensure complete removal of any external contaminating organic material. Excess water was removed, the animal placed in a suitably sized Falcon or Eppendorf tube and frozen in liquid nitrogen. Samples were stored at -80^{o}C.

DNA extraction

The frozen gelatinous specimens were allowed to thaw before pooling the required number of specimens (Table 5.1) in a 15 mL Falcon tube and 300 µL of CTAB solution (2% cetyl trimethyl ammonium bromide (CTAB), 100 mM Tris-HCl pH 8, 20 mM EDTA, 1.4 M NaCl, 0.2% β-mercaptoethanol) was added per ctenophore/medusa. Physical homogenisation was then carried out using a 5 ml syringe and 19 G needle. Molecular grade proteinase K was added (0.37 µg/ml per organism) and the samples physically homogenised for a second time before incubation at 55°C for 24 hours. The homogenate was extracted once with an equal volume of chloroform:isoamyl alcohol (24:1) and gently inverted before centrifugation at 7700 G for 10 min. The aqueous phase was transferred to a fresh Falcon tube and precipitated with 2 volumes of 95% ethanol at -80°C for 1 hour. The samples were centrifuged again at 10,000 G for 30

mins, before pouring off the excess ethanol. The resultant DNA pellet was washed with 70% ethanol and centrifuged for a final time at 7000 G for 15 mins. Excess ethanol was removed and the remaining pellet air dried for 45 mins before resuspension in 200 µL TE and storage at 4°C. The DNA extractions were analysed to assess quality and quantity of DNA present using a NanoDrop 1000 Spectrophotometer (ThermoScientific, Delaware USA).

Table 5.1. Gelatinous species, number of individuals analysed, number of samples, total number of specimens analysed and total number of pooled samples (May to July 2015).

Species	18 Jun	25 May	1 Jun	8 Jun	15 Jun	22 Jun	29 Jun	6 Jul	14 Jul	Total
Pleurobrachia pileus										
No. individuals analysed	18	18	18	15	18					87
No. pooled samples	3	3	3	3	3					15
Leuckartiara octona										
No. individuals analysed	3	9	9	3	3	2	1	3	3	36
No. of pooled samples	1	3	3	1	1	1	1	1	1	13

DNA amplification

Primers (Illumina_Euk_1391f and Illumina_EukBr), designed by the Earth Microbiome Project (EMP), were chosen for amplicon generation. These primers target the V9 region of the 18S nuclear small subunit (nSSU) ribosomal RNA (rRNA) gene and flank a region that is highly divergent (Albaina et al., 2016).

Polymerase chain reaction amplification was performed in triplicate using 1 µL of genomic DNA template (1:10 dilution) in 25 µL reactions containing 2.5 µL of 10x buffer, 2.5 µL 200 µM dNTPs, 2 µL 25 mM $MgCl_2$, 14.8 µL DNA water, 0.5 µL of 10 µM primers, 0.5 µL 5x Q solution and 0.2 µL of 2.5 Unit/reaction Taq DNA Polymerase

(Qiagen). The PCR conditions involved a 2 min denaturation at 95°C followed by 27 cycles of 30 secs at 95°C, 45 secs at 57°C, 45 secs at 72°C and a final extension of 7 min at 72°C. The pooled triplicate PCR products and negative controls was visualised on a 1% agarose gel before the 180 base pair (bp) amplicons were purified using the QIAquick PCR Purification Kit (Qiagen). The cleaned PCR products were sent to MR DNA for sequencing (www.mrdnalab.com, Shallowater, TX, USA) on an Illumina MiSeq following the manufacturer's guidelines. A 5 cycle PCR was undertaken, to add multiplexing labels to the amplicons, using the HotStart Taq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 minutes, followed by 5 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. Following PCR, all amplicon products from different samples were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA).

Sequence data processing

Sequence data were processed using MR DNA's analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, consensus sequences were formed from the forward and reverse reads, barcodes were removed, then sequences <150bp and those with ambiguous base calls were removed. Sequences were de-noised (the removal of errors introduced in DNA library preparation and amplification), operational taxonomic units (OTUs) generated and chimeras removed. OTUs were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified (at the level of 95% homology or above) using BLAST+ version 2.3.0 and the BLASTn database https://blast.ncbi.nlm.nih.gov. The assigned taxonomies were verified and manual BLAST searches were run on non-local species.

The number of sequences for each species was calculated as a percentage of the total number of sequences returned for that sample. As whole organisms were homogenised including their guts, the OTUs containing sequences pertaining to the relevant predator species were removed from the dataset and percentage sequences

recalculated. Where necessary, replicates from the same sampling date were averaged to obtain one set of percentage data for each prey species for each date.

5.2.2 Mesozooplankton abundance and biomass

Sampling, identification and enumeration of mesozooplankton collected during 2007 to 2015 [including adult *Calanus helgolandicus* (male and female) and total copepodites (CI-CV)] were undertaken following the methods described in Chapter Two (Section 2.2.1). Abundance data for *C. helgolandicus*, ctenophores, medusae and siphonophores were extracted from the time series. Total ctenophore data were available from 1988, however I have utilised data spanning from 2007-2015 only, as there are doubts over data accuracy before this time (due to time delay from capture to analysis and possible fragmentation in formaldehyde). Species-specific medusae, ctenophore and siphonophore data were collected from 2009, thus I collated seven years only (2009-2015). Predator biomasses were estimated by measuring lengths of L4 specimens and applying literature length-mass conversions [see Chapter Three (Section 3.2.1)].

5.2.3 Data analysis

Simple linear regressions were undertaken between annual and May-July mean gelatinous zooplankton abundance and estimated biomass values against total *C. helgolandicus* abundance and biomass.

The percentage of *C. helgolandicus* in the diet of *P. pileus* and *L. octona* was analysed over time and analysed in relation to the percentage *C. helgolandicus* biomass of the total zooplankton biomass (mg C m^{-3}).

5.3 Results

5.3.1 Abundance and seasonality of gelatinous zooplankton at L4

The gelatinous zooplankton species recorded at L4 and an assessment of their likelihood to ingest *C. helgolandicus* is presented in Table 5.2. This was based on the published literature and various online registers of marine species [e.g. Marine Species Identification Portal (http://species-identification.org/index.php) and the World Register of Marine Species (WoRMS) (WoRMS Editorial Board, 2017)].

Figure 5.1 presents the total abundance of each species of gelatinous zooplankton recorded over 2009-2015. Only two ctenophores generally appear at L4, *Pleurobrachia pileus* and *Beroe cucumis,* the most abundant being *P. pileus*, a predator of copepods. *Beroe* spp. are predators of other ctenophores and can grow very large (~150mm) and although reported to be rare at L4 (only appeared in the L4 zooplankton dataset in 2013, unpublished data), have appeared more abundant during the summers of 2015 and 2016.

Muggiaea spp. are known to feed on copepods and are the predominant siphonophore at L4, as well as the most abundant of all gelatinous zooplankton. Other species of siphonophore include the physonectae *Agalma elegans* and *Nanomia cara*. The athorybia larvae of *A. elegans* are occasionally seen at L4, along with many individual nectophores from siphonophores that have disintegrated during sampling.

Of the medusan species, five are clearly the most abundant at L4, *Obelia* spp., *Lizzia blondina*, *Liriope tetraphylla*, *Solmaris corona* and *Aglantha digitale*. *Obelia*, *L. tetraphylla* and *A. digitale* are predators of copepods, the diet of *L. blondina* is uncertain and *S. corona* mostly feeds on other gelatinous zooplankton (Larson et al., 1989). It is likely that at least a ⅔ (if not many more) of the 30 species of gelatinous predator found at L4 are potential predators of *C. helgolandicus* (Table 5.2).

Table 5.2. List of gelatinous zooplankton species recorded at L4 (2009-2015); indicating occurrence at L4 (Conway 2012), diet and whether is a potential predator *C. helgolandicus.*

Phylum	Class	Order	Species	Occurrence at L4	Diet	Predator of *Calanus*?
Cnidara	Scyphomedusae		*Aurelia aurita*	Rare	molluscs, crustaceans, tunicate larvae, rotifers, young polychaetes, protozoans, diatoms, eggs, fish eggs, hydromedusae, ctenophores	✓
Cnidara	Hydromedusae		*Liriope tetraphylla*	V. common	herbivorous crustaceans, chaetognaths, and fish eggs and larvae	✓
Cnidara	Hydromedusae		*Aglantha digitale*	V. common	copepods	✓
Cnidara	Hydromedusae		*Obelia* spp.	V. common	crustaceans, copepods, worms, detritus	✓
Cnidara	Hydromedusae		*Clytia hemisphaerica*	Common	?	?
Cnidara	Hydromedusae		*Leuckartiara octona*	Occ. common	copepods, fish larvae, decapods	✓
Cnidara	Hydromedusae		*Solmaris corona*	Occ. common	gelatinous zooplankton	x
Cnidara	Hydromedusae		*Sarsia* spp.	Occ. common	copepods	✓
Cnidara	Hydromedusae		*Coryne prolifera*	Occ. common	?	?
Cnidara	Hydromedusae		*Corymorpha nutans*	Occ. common	crustaceans	✓
Cnidara	Hydromedusae		*Lizzia blondina*	Occ. common	?	?
Cnidara	Hydromedusae		*Amphinema* spp.	Occ. common	copepods, chaetognaths, other hydromedusae	✓
Cnidara	Hydromedusae		*Rathkea octopunctata*	Occasional	crustaceans, fish larvae/eggs & *Parasagitta* spp.	✓
Cnidara	Hydromedusae		*Bougainvillia muscus*	Occasional	microzooplankton	?
Cnidara	Hydromedusae		*Phialella quadrata*	Occasional	copepods	✓
Cnidara	Hydromedusae		*Eutima gracilis*	Occasional	copepods, chaetognaths, other hydromedusae	✓
Cnidara	Hydromedusae		*Ectopleura dumortierii*	Occasional	?	?
Cnidara	Hydromedusae		*Turritopsis nutricula*	Rare	?	?
Cnidara	Hydromedusae		*Hydractinia borealis*	Rare	?	?
Cnidara	Hydromedusae		*Cosmetira pilosella*	Rare	fish larvae, *Pleurobrachia pileus*	✓
Cnidara	Hydromedusae		*Mitrocomella brownei*	Rare	?	?
Cnidara	Hydromedusae		*Lovenella clausa*	Rare	?	?

Table 5.2. Continued

Phylum	Class	Order	Species	Occurrence at L4	Diet	Predator of *Calanus*?
Cnidara	Hydromedusae	Siphonophora	*Muggiaea spp.*	V. common	copepods	✓
Cnidara	Hydromedusae	Siphonophora	*Agalma elegans*	Common	copepods, euphausiids, fish larvae, chaetognaths	✓
Cnidara	Hydromedusae	Siphonophora	*Nanomia cara*	Quite common	copepods, euphausiids, fish larvae, chaetognaths	✓
Cnidara	Hydromedusae	Siphonophora	*Apolemia uvaria*	Rare	copepods, crustaceans, fish, other siphonophores	✓
Ctenophora	Tentaculata		*Pleurobrachia pileus*	Common	copepods, fish larvae, eggs and other crustaceans	✓
Ctenophora	Tentaculata		*Bolinopsis* spp.	Rare	copepods, euphausiids	✓
Ctenophora	Nuda		*Beroe cucumis*	Rare	*Pleurobrachia pileus*, *Bolinopsis* spp. - other ctenophores	x

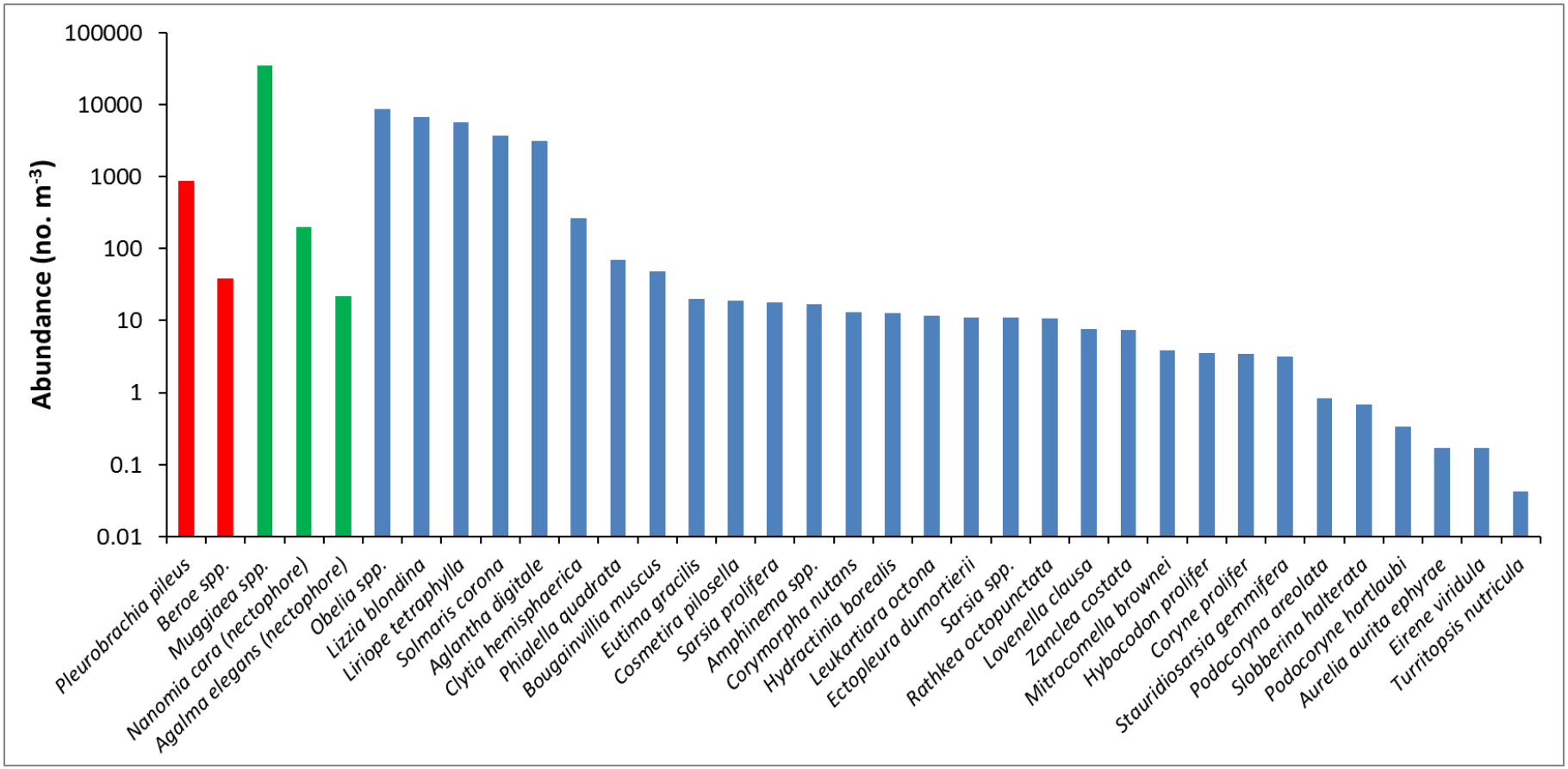

Figure 5.1. Total abundance of gelatinous zooplankton (sum of all samples recorded from 2009-2015) at L4; red columns are ctenophores, green are siphonophores and blue are medusae.

5.3.2 *The abundance of* P. pileus *and* L. octona *during 2015*

Pleurobrachia pileus were present from mid-April to late-June, with a peak at the end of May (Figure 5.2). *Leuckartiara octona*, which temporally overlapped and subsequently succeeded *P. pileus*, were present from late-April, high throughout May and June and disappeared in July. Both species were recorded over periods of ~10 weeks long. Two peaks of *C. helgolandicus* flank the annual occurrence of these two species, indicating that *P. pileus* and *L. octona* in combination may be key predators capable of significantly depressing the population of *C. helgolandicus* at this time of the year.

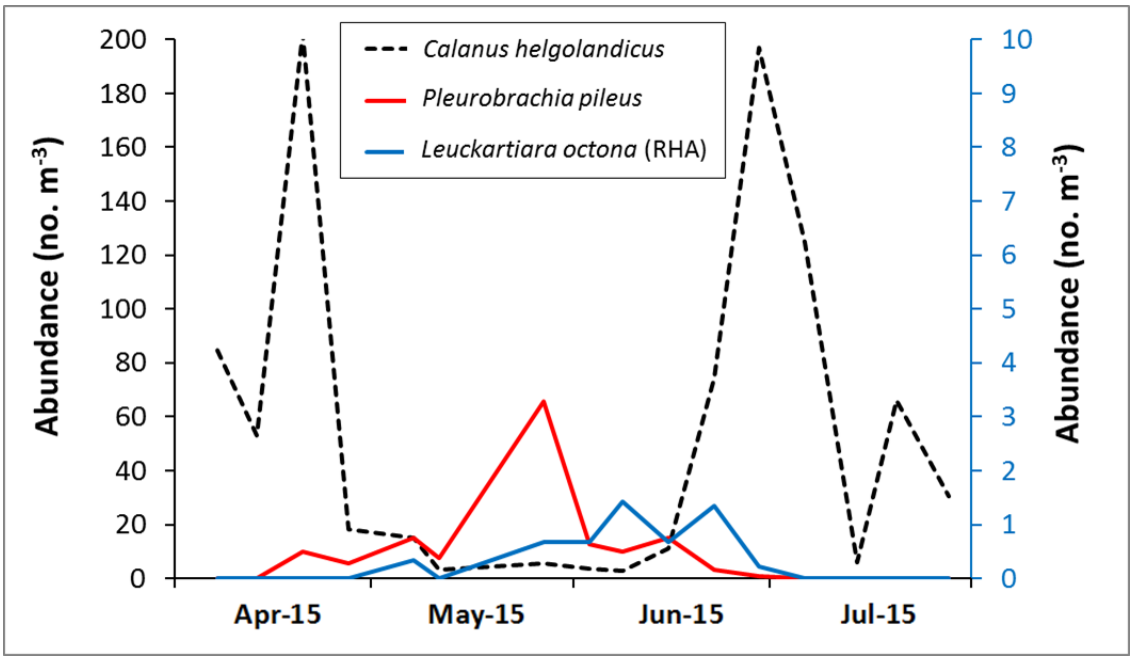

Figure 5.2. Abundance of *P. pileus*, *L. octona* (right-hand axis) and *C. helgolandicus* at L4 (April - July 2015); RHA is right hand axis.

Pleurobrachia pileus tend to appear only once a year, peaking around May to June (Figure 5.3) and have been known to wash up on beaches in their thousands. Although the average abundance at L4 is only ~3 m^{-3}, a maximum of 70 m^{-3} was recorded in June 2011. *Leuckartiara octona* generally follow the same seasonality as *P. pileus*, but at a maximum density of only ~1.5 m^{-3}.

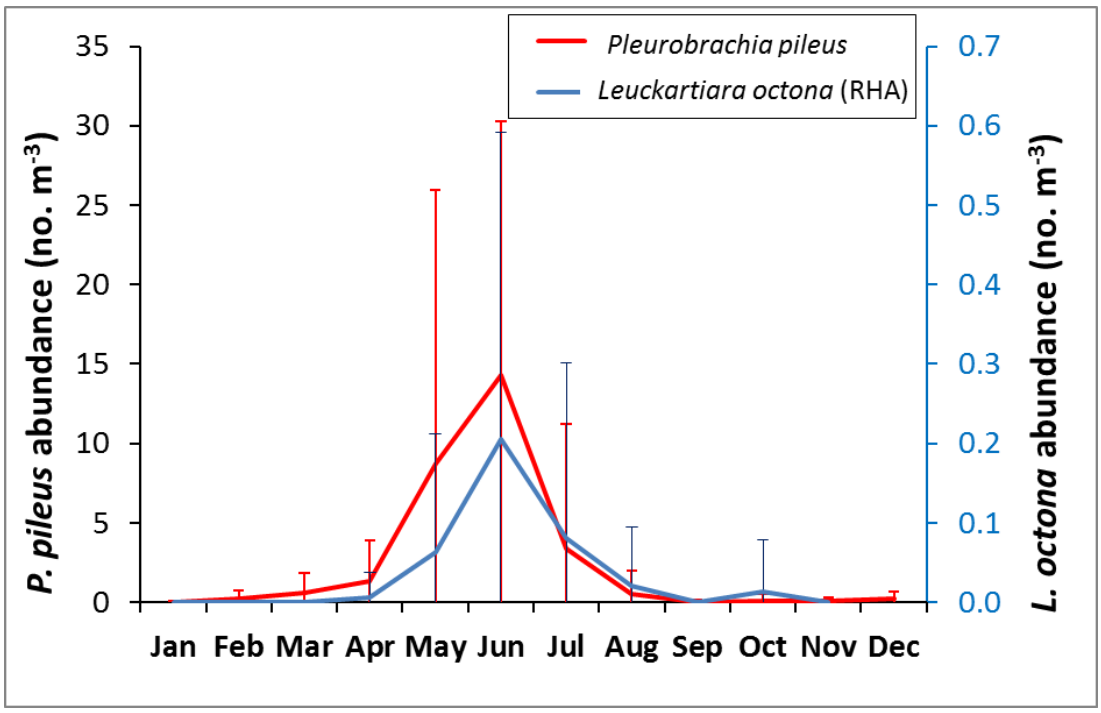

Figure 5.3. Seasonality of *P. pileus* and *L. octona* (right-hand axis) (mean monthly abundance, 2009-2015); RHA is right hand axis.

5.3.3 Relationships between gelatinous predators and C. helgolandicus *abundance*

Annual mean total *C. helgolandicus* abundance was negatively related to total ctenophore abundance, although not at the 5% significance level (Figure 5.4). The relationship with *L. octona* was negative but not significant.

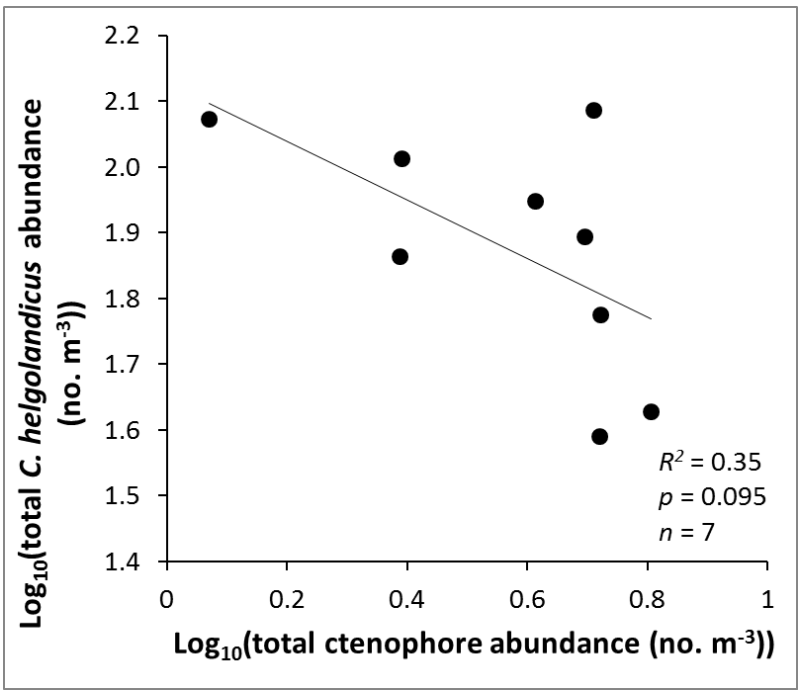

Figure 5.4. Relationship between total *C. helgolandicus* copepodite (CI-CVI) abundance and total ctenophore abundance (annual mean; 2007-2015).

5.3.4 Molecular analysis of gut content

Pleurobrachia pileus

Pleurobrachia pileus DNA sequences constituted a mean of 75% of the total sequences (mean of 31700 sequences per ctenophore). The sequences indicate that the diet consisted mostly of medusae (mean of 38%), with fish and polychaete larvae dominating at times, but on average contributing 14% and 13% respectively (Figure 5.5). Copepods (including *C. helgolandicus*) were detected in every sample, but at low percentages (3-15%). Parasites constituted on average 10% of the sequences. Interestingly, other ctenophores and siphonophores were also identified as prey, and together with the medusae, the gelatinous zooplankton contributed between 24 and 84% of DNA sequences in the *P. pileus* diet.

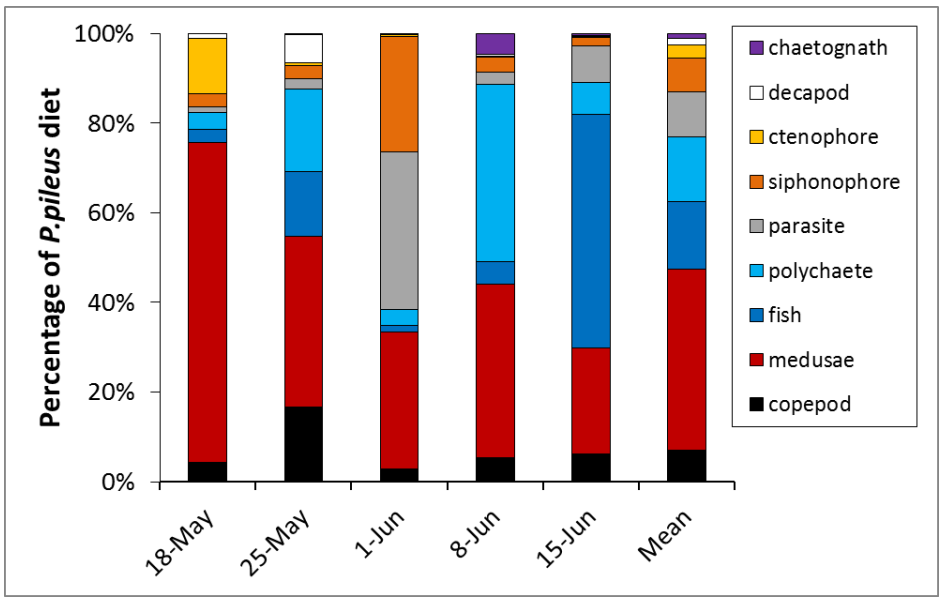

Figure 5.5. Percentage composition of DNA sequences obtained following molecular analysis of whole *P. pileus*, excluding *P. pileus* sequences (thereby indicating species ingested) (May-June 2015 and mean).

Calanus helgolandicus sequences constituted between 1.3 to 7.9% (mean of 3.8%) of the *P. pileus* diet and appeared to demonstrate a decreasing contribution from May to June, although this increased again in mid-June (Figure 5.6).

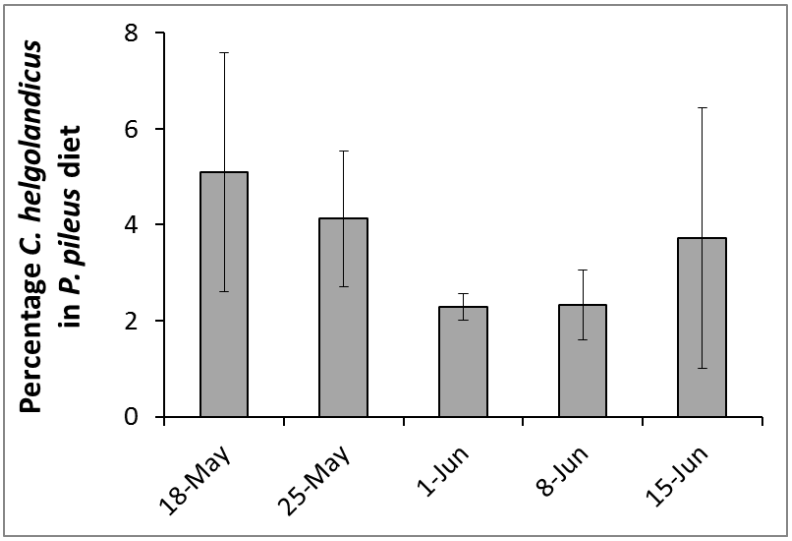

Figure 5.6. Percentage of *C. helgolandicus* DNA sequences obtained following molecular analysis of whole *P. pileus*, and excluding *P. pileus* sequences (thereby indicating species ingested) (May-June 2015, with error bars representing standard deviation).

Looking at the contribution of copepods specifically, Figure 5.7 shows that *C. helgolandicus* was one of the key copepods (mean of 57% of total copepods) in the diet of *P. pileus*. Other calanoids and harpacticoids contributed ~20% each.

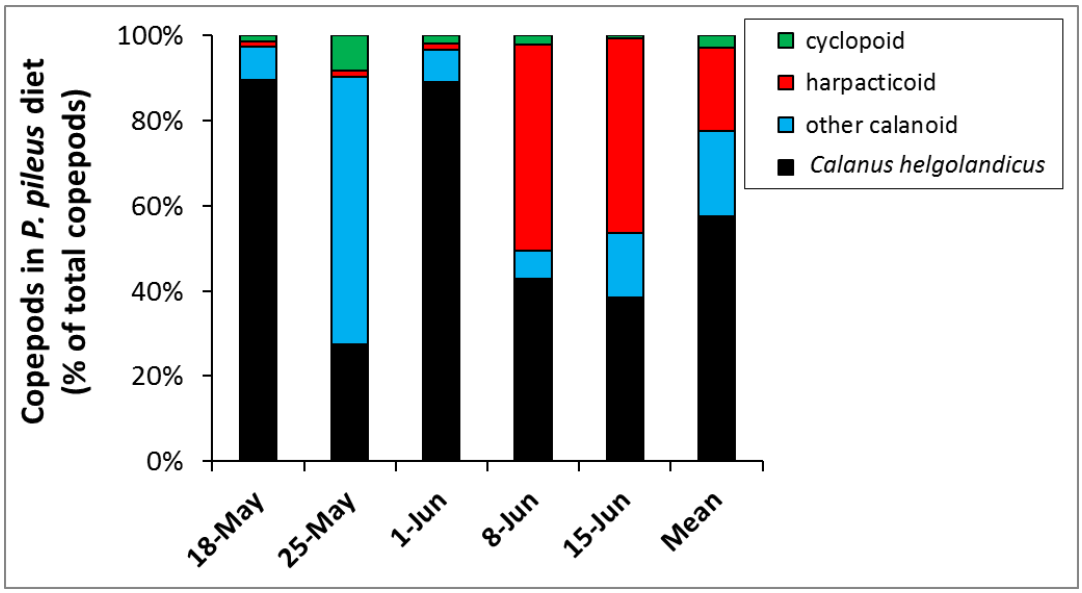

Figure 5.7. Percentage composition of copepod DNA sequences obtained following molecular analysis of whole *P. pileus*, and excluding *P. pileus* sequences (thereby indicating species ingested) (May-July 2015 and mean).

Leuckartiara octona

A mean of 115522 sequences were obtained from each *L. octona* medusae. Predator DNA sequences accounted for a mean of 65% of total sequences. The prey DNA sequences indicate that the diet of *L. octona* was chiefly dominated by ctenophores (mean of 50%), other medusae (18%) and polychaete larvae (10%). Copepods contributed much less, with a mean of 8% (1-15%) (Figure 5.8).

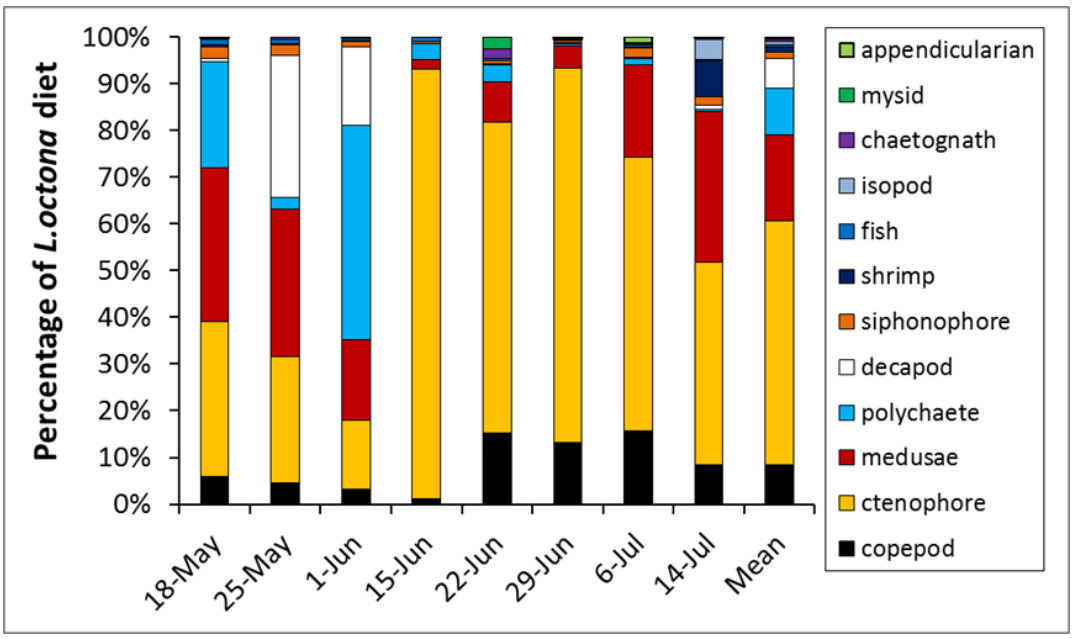

Figure 5.8. Percentage composition of DNA sequences obtained following molecular analysis of whole *L. octona*, excluding *L. octona* sequences (thereby indicating species ingested) (May-July 2015 and mean).

Calanus helgolandicus constituted 0.7 to 14% to the *L. octona* diet, and contributed most in late-June and July (Figure 5.9).

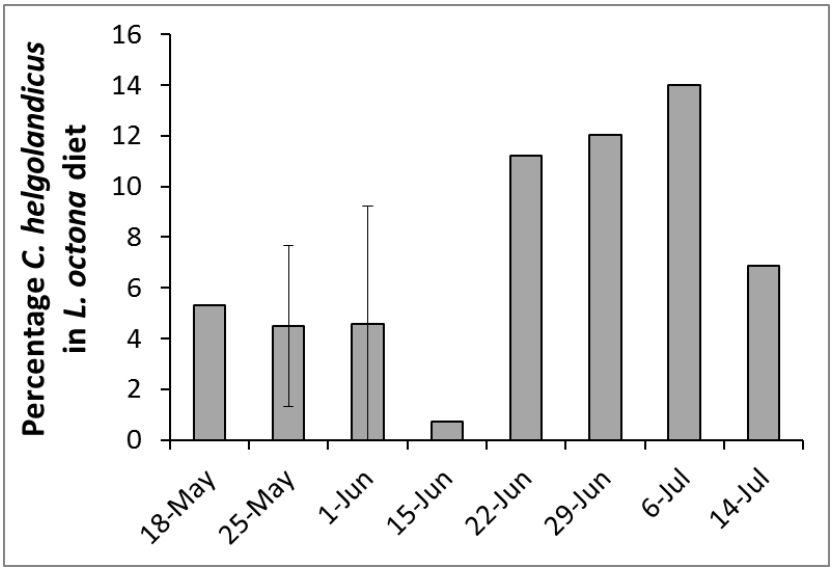

Figure 5.9. Percentage of *C. helgolandicus* DNA sequences obtained following molecular analysis of whole *L. octona*, and excluding *L. octona* sequences (thereby indicating species ingested) (May-July 2015, with error bars representing standard deviation).

Calanus helgolandicus was the predominant copepod in the *L. octona* diet and comprised a mean of 79%. Other calanoids contributed ~17% (Figure 5.10).

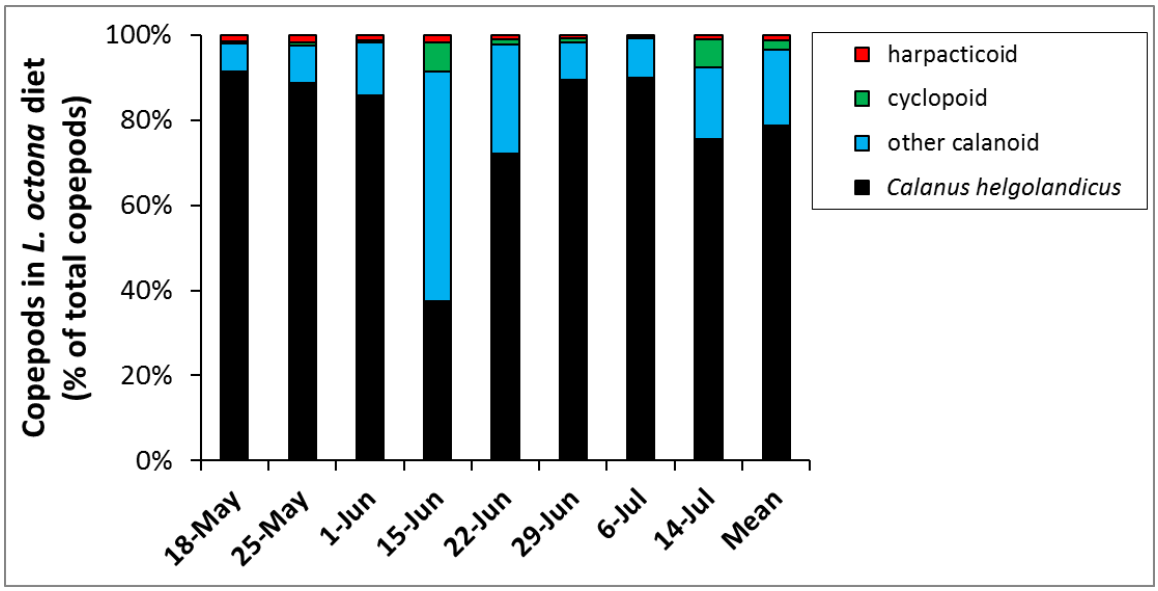

Figure 5.10. Percentage composition of copepod species in the diet of *L. octona* (May-June 2015 and mean).

5.3.5 Relationships between C. helgolandicus *in the diet and biomass at L4*

There was a highly significant logarithmic relationship between the *C. helgolandicus* biomass as a percentage of the total biomass at L4 and the percentage *C. helgolandicus* detected in the *L. octona* diet (Figure 5.11). This suggests that an increasing contribution of *C. helgolandicus* to the zooplankton biomass stimulates greater ingestion rates by *L. octona*, but that at a threshold biomass, ingestion levels off.

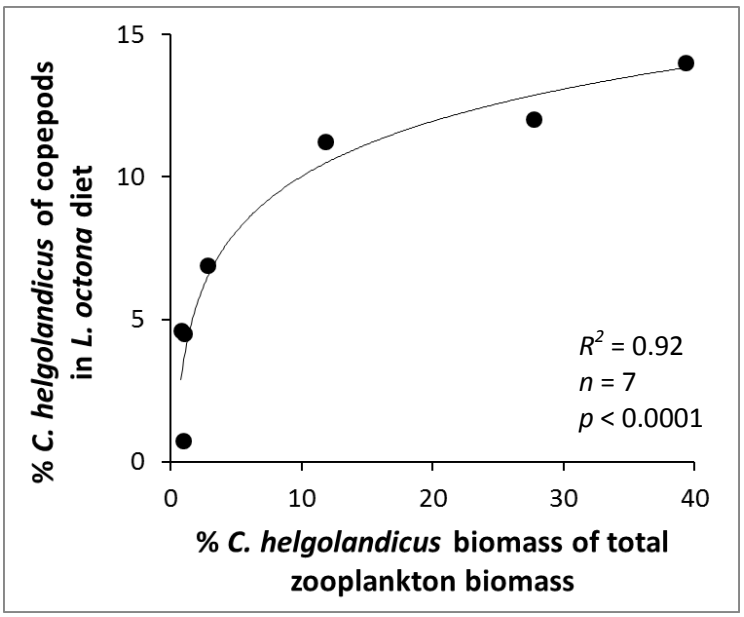

Figure 5.11. Relationship between percentage *C. helgolandicus* biomass of total zooplankton biomass at L4 and percentage contribution made by *C. helgolandicus* to the *L. octona* diet; logarithmic equation: $y = 2.788 \ln(x) = 3.604$.

5.4 Discussion

Large abundances of the ctenophore *P. pileus* and hydromedusae *L. octona* were observed in routine L4 sampling during 2015 and coincided with significantly depressed *C. helgolandicus* populations. Molecular gut content analyses of *P. pileus* and *L. octona* revealed that *C. helgolandicus* had been ingested by specimens in every pooled sample and constituted up to 8% (mean of 4%) and 14% (mean of 8%) of their diet respectively. In addition, I determined a strong positive link between *C. helgolandicus* availability at L4 and the relative contribution to the *L. octona* diet. Here I discuss the significance of these results in terms of what is currently known about the feeding habits of carnivorous gelatinous predators, their ability to modify the zooplankton community and how NGS metabarcoding techniques may alter the perception of jellyfish diets.

5.4.1 Pleurobrachia pileus

The wealth of literature available suggests that *P. pileus* feeds mainly on copepods and other crustaceans (e.g. Fraser, 1970; Frank, 1986; Møller et al., 2010), and is a non-selective carnivore, whose diet is reported to reflect the ambient food environment (Fraser, 1970). Clearance rates of 50-100 L per day were estimated by Gibbons and Painting (1992). Numerous studies have highlighted their predatory impact; for example 70% of the diet of *P. pileus* off south-west Nova Scotia were large crustacean zooplankton and extremely low levels of haddock (*Melanogrammus aeglefinus*) larvae were attributed to the presence of *P. pileus*, due to the removal of their zooplankton prey (Frank, 1986); and in the southern Benguela upwelling system, it was estimated that the *P. pileus* population could remove up to 27% of integrated mesozooplankton standing stocks per day, and in excess of 100% at some depths (Gibbons et al., 2003).

My study suggests that copepods contribute considerably less (a mean of 6.5%) to the *P. pileus* diet at L4 and finds that other gelatinous species form ~50% of the diet. These are surprising results, given that gelatinous zooplankton are not detailed as prey in the published literature. These results are discussed in more detail later in Section 5.4.3.

The capacity of ctenophores to significantly reduce copepod populations has been well documented (Deason and Smayda, 1982; Sullivan and Reeve, 1982; Purcell and Decker, 2005; Tiselius and Møller, 2017). Their tremendous regulatory capacity is the result of individuals being able to ingest up to ten times their body carbon per day and growth responses enabling ctenophore populations to nearly double their biomasses each day (Greene et al., 1986).

The Pleurobrachiidae are frequently discussed in the literature alongside their major predator, the ctenophore *Beroe* spp. Haddock (2007) reported that *Beroe* can engulf *Pleurobrachia* completely and Lebour (1922) observed *Beroe cucumis* that were "packed tight with *Pleurobrachia*". *Beroe*, therefore, are seen as important in the regulation of the *P. pileus* population and thus promoting copepod population recovery (Greve, 1981). I observed large numbers of *Beroe cucumis* at L4 following the *P. pileus* bloom during 2015 (Figure 5.12a, Appendix B), which appeared to

demonstrate a trophic cascade. Here *P. pileus* appeared to hugely reduce the *C. helgolandicus* population, but a subsequent peak of *Beroe* ultimately released *C. helgolandicus* from this predation impact, so that they were able to re-establish peak abundances. Expressed as biomass, Figure 5.12b shows the phenomenal biomass of *Beroe* compared to that of *P. pileus* (~50 times greater), however I include the caveat here that there are considerable uncertainties about the *Beroe* biomass data, due to the fragmentation of specimens in samples, which may have inflated abundance and the resulting biomass conversions. A rough calculation of the predatory impact of *P. pileus* during the months of May to July [mean annual maximum abundance (2007-15) of 39.4 ind. m^{-3}], and using a clearance rate of 61.24 L d^{-1} [calculated assuming the population consisted of large *P. pileus* with a polar diameter of 15mm, using the formula of Gibbons and Painting (1992)], estimated a *P. pileus* population clearance rate of 133 m^3, which equates to 241% of the water column (~55m). This suggests that at times the *P. pileus* population is capable of decimating the *C. helgolandicus* population.

This type of trophic cascade has been documented previously; Greve and Reiners (1980) in particular, discuss a self-regulatory system in ctenophore dynamics where the detrimental effect of *Pleurobrachia* is offset by *Beroe,* which conveys long-lasting protection against the negative impact of *P. pileus* on copepod population.

5.4.2 Leuckartiara octona

The literature provides only a few studies of the feeding habits of *L. octona.* It was reported by Russell (1953) to be a voracious feeder of copepods, decapod larvae, polychaete larvae and fish larvae, and capable of catching and eating organisms larger than itself (Fraser, 1969). *Leuckartiara* spp. were observed to feed on the hydromedusae *Aglantha digitale* and *Sarsia* spp. (Fraser, 1969) and the siphonophore *Nanomia cara* (Alvariño, 1985). More recently Regula et al., (2009) have highlighted that *L. octona* preferentially selects other small hydromedusae over copepods in feeding experiments.

Here, my results suggest that ctenophores and other medusae were the dominant prey of *L. octona,* and copepods contributed only ~8% of DNA sequences. This outcome is discussed further in the following section. Although it is unlikely that *L.*

octona in itself is capable of reducing the *C. helgolandicus* population, it may, in conjunction with other predators, exacerbate the predatory impact of *P. pileus*.

5.4.3 Proportion contribution of C. helgolandicus *to the diet*

Copepods are reported to be the major prey of many gelatinous zooplankton species; for example, in Scottish waters 80-97% of the *P. pileus* diet (analysed using visual examination of gut contents) was found to be copepods (Fraser, 1970); the main prey of *Aglantha digitale* were *Oithona* and *Temora* spp. copepods (González and González, 1996); and Lebour (1922) observed *Sarsia tubulosa* feeding on copepods, but no other species. However, these results were obtained from either feeding experiments and/or gut-content analysis and it is recognised that visual identification often fails to identify gelatinous zooplankton in particular, due to their high digestion rates (Purcell, 1992).

My results, provided by molecular techniques, suggest that although copepods were a constant presence in the diet of two gelatinous species, they were far outweighed by the dominance of other gelatinous zooplankton. Medusae were consistently important in the *P. pileus* diet and ctenophores were consistently important in the *L. octona* diet. A few, limited studies report on the feeding habits of gelatinous carnivores; Purcell (1991) states that the diets of hydromedusae commonly include gelatinous zooplankton, and as mentioned previously, *L. octona* were observed feeding on hydromedusae and siphonophores (Fraser, 1969), and *Pleurobrachia* were observed to prey on other ctenophore species (Alvariño, 1985). Hence, my results may be indicative of the fact that copepods may not be as dominant in the diet of gelatinous species as originally thought and that many gelatinous species may survive mostly on a gelatinous diet. It is only the full characterisation of the gelatinous zooplankton diet through molecular techniques along with clearance rates that can reveal their true predatory impact.

I recognise that these results may reflect a sampling effect, as the gelatinous zooplankton were not picked from the L4 samples until they were back at the laboratory (at times a couple of hours later), and the gelatinous zooplankton may have ingested species they would not in a natural situation. However, a later study of the

diet of L4 gelatinous predators that picked and preserved animals on the research ship, within minutes of their removal from the sea, also indicated high proportions of gelatinous species in the gelatinous predator diet (Parry et al., 2017, manuscript in preparation); thus corroborating the results presented here.

5.4.4 Other gelatinous zooplankton species

Here, I have detailed the diversity of the diet of only two gelatinous predators. Other gelatinous species have been investigated for their predatory impact, and many report the ability of jellyfish to regulate their prey populations. *Aurelia aurita* in particular, has received much attention; for example, it was classed as a keystone species that regulated the zooplankton of a shallow cove (Olesen, 1995); it reduced the zooplankton population to almost nothing in Skive Fjord (Denmark) (Møller and Riisgård, 2007); and removed two-thirds of daily secondary production in years of high abundance in the Kiel Bight/western Baltic (Schneider and Behrends, 1994).

Other medusan species include *Sarsia* sp. and *Rathkea octopunctata,* which were deemed collectively responsible for the predation impact on copepods in Limfjorden, Denmark (Hansson et al., 2005). The total biomass of five hydromedusans (*Sarsia* sp., *A. digitale*, *R. octopunctata*, *Cosmetira* sp. and *Euphysa tentaculata*) and *A. aurita* varied reciprocally with zooplankton biomass, suggesting collective population control by the medusae off Nova Scotia (Matsakis and Conover, 1991). The leptomedusan *Phialidium gregarium* predation pressure on zooplankton population released phytoplankton from grazing pressure in a British Columbian fjord (Huntley and Hobson, 1978). Species-specific feeding by medusae may suggest a strong predatory impact on the prey species; however this is not always the case; the hydromedusan *Nemopsis bachei* in Chesapeake Bay, USA fed primarily on *Acartia tonsa* copepodites, but even at peak abundance was incapable of reducing *A. tonsa* populations (Purcell and Nemazie, 1992).

I mention here the siphonophore *Muggiaea* sp. as they are the most abundant gelatinous predators at L4. I was unable to carry out molecular gut-content analyses due to the feeding and digestive gastrozooids (which form part of the cormidia), being almost always removed from their polygastric stages during the net-sampling process.

Therefore, although the nectophores at times may be very numerous in the L4 samples, analysis was not possible due to their stomachs being likely unattached. This is unfortunate as *Muggiaea* are believed to prey mostly on copepods and Blackett et al. (2014) reported high abundances to be associated with high abundances of copepods. There is also evidence for their invasive ability; where an invasion of *Muggiaea atlantica* in the German Bight in 1989 (~500 m^{-3}) depressed small copepod populations and caused a phytoplankton bloom (Greve, 1994), and a similar invasion of the Adriatic in 1995 created a restructuring of the copepod community (Kršinic and Njire, 2001).

5.4.5 Molecular analyses of gelatinous predator gut-contents

I am aware of only a handful of studies that have employed molecular techniques to establish prey ingestion by gelatinous zooplankton and I believe ours is the first study to use NGS and the 18S rRNA V9 region to characterise the gut contents using whole gelatinous zooplankton specimens. The research by Meredith et al. (2016) used NGS to characterise the diet of the scyphomedusae *Chrysaora quinquecirrha*, however they performed gut lavages and gastric pouch/tentacle picks, rather than using whole organisms.

Two studies have used species-specific primers to verify the presence of prey; for example, picocyanobacteria in the gut of the ctenophore *Mertensia ovum* after feeding experiments (Majaneva et al., 2014), and quantitative polymerase chain reaction methods (qPCR) were used to quantify the consumption of specific prey items of wild doliolids *Dolioletta gegenbauri* (Frischer et al., 2014).

Pyrosequencing (a sequencing technique that is based on the detection of released pyrophosphate (PPi) during DNA synthesis) was used to investigate the bacterial communities of ctenophora (Hao et al., 2015), and although this study focused on the colonisation of bacteria rather than gut contents, the same methodology (extraction of DNA from whole organism) was employed.

5.4.6 Limitations of the metabarcoding approach in this study

The results provided from NGS are only semi-quantitative, and can only provide relative proportions of sequences detected of each prey species. However, the fact that I determined a very strong link between the proportion of *C. helgolandicus* in zooplankton biomass and the proportion of *C. helgolandicus* in the diet of *L .octona* verifies that NGS techniques can provide evidence that prey intake reflects the prey field.

At times DNA sequences from parasites were an important contributor to the *P. pileus* diet. The most frequently-occurring species was *Hysterothylacium aduncum,* a fish parasite, which is a nematode parasite of salmonids that has a copepod first intermediate host and is also capable of surviving free in the sea and susceptible to predation (González, 1998). Whether or not these parasites were ingested along with their copepod hosts or were captured as free-living stages is unknown, but this result may be an example of a prey-parasitism error (a consequence of a predator consuming prey that is parasitised).

5.4.7 Conclusions

I conclude that the ctenophore *P. pileus* is a major predator of *C. helgolandicus*, and capable of temporarily removing most of their biomass and initiating trophic cascades. *L. octona* also ingests *C. helgolandicus*, and may (probably in conjunction with other medusae and siphonophores) intensify the predatory impact of *P. pileus*. In addition, my results, obtained by molecular methods, strongly suggest that the characterisation of the full range of prey species may have been limited by traditional visual gut-content analyses. Molecular gut-content analyses may correct the bias towards the identification and significance of zooplankton prey with exoskeletons and allow for a more correct balance of prey items that include other gelatinous zooplankton.

I acknowledge that I have only characterised the diet of two gelatinous species over a handful of weeks, and that there were multiple co-occurring potential predators of *C. helgolandicus* present in the water column that I have not investigated. However I exploited an abundance of easily identifiable gelatinous species to investigate their

diet. It is clear that a targeted collection and molecular analyses of other species would allow a more complete view of the predatory habits the gelatinous zooplankton at L4. I emphasise that each gelatinous species has a different ecology and whilst many may ingest a diverse range of taxa including copepods (e.g. *P. pileus*), others may not target copepods at all (e.g. the ctenophore *Beroe* sp. and the medusan *Solmaris corona* which is known to prey mostly on other medusae (Larson et al., 1989)). Therefore generalisations about the predatory impact of jellyfish may lead to spurious results.

CHAPTER SIX

General Discussion

This thesis has explored the population dynamics of the copepod *Calanus helgolandicus* in the western English Channel. My research is centred around the 28-year *C. helgolandicus* abundance time series and a 25-year weekly egg production time series from station L4. It has pursued four lines of inquiry; (1) the interannual variability of *C. helgolandicus* through interrogation of the L4 time series and the role of mortality in population regulation; (2) the mortality of early-stage *C. helgolandicus*, through egg and naupliar viability, and the importance of predators; (3) the mortality of copepodites in terms of both consumption by predators and non-consumptive processes, and (4) a specific focus on gelatinous predators (ctenophores and hydromedusae), and direct investigation of their consumption of *C. helgolandicus* through molecular gut-content analysis. Here I summarise my main findings and then contextualise their significance within the wider marine environment. I also discuss potential directions for future work.

The *C. helgolandicus* population density was constrained within a relatively narrow inter-annual envelope, and although the species may grow to adulthood in a matter of weeks, and are capable of producing three to five generations per year (Bonnet et al., 2005 and references therein), there was only a four-fold inter-annual variation in abundance (Chapter Two). This suggests that powerful processes act to regulate the population. A simple population growth model to explore egg to CV population progression (assuming no mortality) and comparison with observed abundance indicated that ~99% of individuals (eggs/nauplii/copepodites) were lost before adulthood (Chapter Two). Therefore, I conclude that multiple mortality sources modify population growth and ultimately regulate the population each year.

Perhaps surprisingly, few decadal-scale trends were detected in the abundance and the timing of key population growth periods; key phenological indices (i.e. the start of the season and centre of gravity) did not shift significantly with time or temperature and an increase in winter *C. helgolandicus* abundance was the only significant trend (Chapter Two). This winter increase was positively correlated both to a temporal increase in spring-summer temperatures and total egg output, but the effects were not

carried over to the next season, as spring and summer abundances were more stable over time.

The rates and sources of mortality losses differed considerably between developmental stages. Egg mortality rates were related both to total copepod biomass and to *C. helgolandicus* copepodite abundance, emphasising the importance of intraguild and cannibalistic predation (Chapter Three). By contrast, late-stage *C. helgolandicus* copepodite mortality rates were positively related to gelatinous zooplankton predators, particularly chaetognaths, medusae and ctenophores (Chapter Four). This was particularly apparent in 2015, when I observed a possible trophic cascade initiated by ctenophores that removed most *C. helgolandicus* copepodites for a six week period in late-spring (Chapter Five). Molecular gut content analysis of carnivorous gelatinous zooplankton using metabarcoding techniques confirmed that *C. helgolandicus* was a constituent of the diets of both the ctenophore *Pleurobrachia pileus* and the hydromedusa *Leuckartiara octona* (Chapter Five).

Separating the constituent consumptive and non-consumptive mortality rates revealed that adult males experienced mortality rates from consumption that were ~6 times higher than females, whilst adult male non-consumptive mortality rates were only ~1.5 that of female rates, indicating that predation was the primary mortality source in male *C. helgolandicus* (Chapter Four). Non-consumptive mortality rates were generally much lower than consumptive rates, but at certain times of the year contributed up to 50% to total mortality, and were positively related to maximum wind speed (used as a proxy for turbulence), suggesting that extreme weather events may be detrimental to copepod health and longevity.

Losses of early-stage *C. helgolandicus* also occurred through the incidence of non-hatching eggs and naupliar abnormalities (NA), both of which were higher in spring (Chapter Three). Together, egg hatch success (EHS) and early stage mortality were responsible for the loss of between 30% and 70% of egg-NI stages before reaching naupliar stage II and were mostly controlled by food availability. EHS was enhanced and NA were reduced when dinoflagellates and ciliates were abundant in the seston, and conversely diatom fatty acid biomarkers in the seston related to low EHS and higher NA. Below I discuss in more depth some of the key strands to emerge from this study.

Dual control of the *C. helgolandicus* population

Understanding the method of regulation of population density is paramount before we can understand ecosystems sufficiently to project their response to future scenarios (Hairston et al., 1960). In addition, population density is ultimately determined by a range of species interactions, coupled with available resources and environmental conditions (Begon et al., 2006). Mechanisms for population control are focused primarily on resource limitation (bottom-up regulation) and predation (top-down regulation). Historically, there was a focus on bottom-up factors as the dominant mechanisms in the structure of marine ecosystems, but it is now acknowledged that some predatory species can exert powerful top-down influence that cascades through pelagic food webs (Verity and Smetacek, 1996).

In situ marine examples of strong top down control often feature gelatinous zooplankton (Tiselius and Møller, 2017); and the ctenophore *Mnemiopsis leidyi* in particular, is a frequently-studied species [e.g. the most recent research found *M. leidyi* was capable of reducing their copepod prey by a factor of five and thus releasing the primary producers from grazing in Gullmar Fjord in Sweden (Tiselius and Møller, 2017)]. However, we often do not understand the relative importance of bottom-up direct physical forcing vs. top-down biological interactions (Mackas et al., 2012b), although more recent literature has attempted to assess the key mechanisms in different regions and ecosystems. For example, bottom-up processes were described as the dominant control mechanism of zooplankton in the Bay of Biscay (Poulet et al., 1996; Stenseth et al., 2006; Lassalle et al., 2011), and in the North Sea, (Munk and Nielsen, 1994; Heath, 2005), whereas top down processes were most important in the Norwegian Sea, Georges Bank (Huse et al., 2012; Ji et al., 2012), and the western North Pacific (Tadokoro et al., 2005), where forage fish were thought to play the dominant role.

More recently, research has highlighted the importance of both bottom-up and top-down processes acting together to determine population size, with various factors affecting the timing, duration and strength of each (Hunt and McKinnell, 2006). I found that the dominant population control processes changed with season and with developmental stage. I determined that bottom-up factors such as food availability and food-related mortality (i.e. toxicity) drive the numbers of healthy eggs and early-stage nauplii produced in spring and early summer, but from mid-summer, predation by

mostly gelatinous predators removes the bulk of the population. This suggests that the dominant processes may switch at some key point during the year. Others have reported similar results; for example, Hunt et al. (2011) reported that the epipelagic food web of the Lazarev Sea in the Antarctic shifted from being bottom-up controlled in summer to top-down controlled in winter; and Kiørboe and Nielsen (1994) found that the seasonal development of copepods in the Kattegat in the Baltic Sea depended on both productivity and mortality, with a shift from bottom-up to top-down after the spring bloom.

It is evident that the effect of predators on zooplankton populations cannot be overlooked and it is likely that both resource limitation and predator pressure play important roles, dependant on ecosystem and seasonality. Indeed Daewel et al. (2014), in their review of zooplankton dynamics, found that predation was important in all six ecosystems considered, but exerted different strengths at different spatial and temporal scales. I also stress the importance of considering other non-fish predators as key drivers of populations; many studies appear to focus on fish species only, which may at times be inadequate, given that I only found an impact of gelatinous zooplankton and not ichthyoplankton.

The role of gelatinous predators

Gelatinous zooplankton have been highlighted in this study as important predators of *C. helgolandicus* (Chapters Four and Five). The literature highlights the tremendous capacity of ctenophores in particular to regulate the abundance of their prey (Greene et al., 1986) and almost a century ago, Bigelow (1926) noted that *Pleurobrachia pileus* ctenophores could "sweep the water clean of zooplankton" and that copepods were exterminated locally. Other gelatinous zooplankton may exert strong predation pressure on copepods, for example the hydromedusa *Aglantha digitale* was reported to have the capacity to considerably reduce copepod abundance (Nicholas and Frid, 1999), and scyphomedusa *Aurelia aurita* density negatively impacted on copepod abundance in the western Baltic Sea (Behrends and Schneider, 1995).

There has been much attention given to jellyfish populations in recent years and newspaper headlines like "Global warming is causing swathes of jellyfish to flock to British beaches" (The Telegraph, 2016) seem to occur every summer. There is still much

scientific debate over the reason for seemingly larger and more frequent jellyfish blooms. On the one hand, it is thought that jellyfish in the Northeast Atlantic show cyclic changes in population sizes (c. 20 year cycle in oceanic waters and 30 year cycle in shelf seas) (Edwards et al., 2013). On the other hand, warming has been related to large populations, particularly in temperate regions, as a consequence of increased metabolism and asexual production (Purcell, 2005). It is also thought that increased jellyfish populations may result from the human harvesting of forage fish (and the removal of direct competitors for zooplankton prey), which releases the jellyfish from competition (Robinson et al., 2014).

It is widely-recognised that medusae, siphonophores and ctenophores are ubiquitous throughout the world's oceans across the environmental spectrum and there are a range of species that can adapt to all niches (Lucas et al., 2014; Mills, 1995). Therefore, whether increasing jellyfish population densities are a consequence of climate, fisheries or natural cycling, their ability to remove large copepod abundances (as I have shown in Chapter Five) may have important knock-on effects for higher trophic levels.

The importance of intraguild predation and cannibalism

Intraguild predation (IGP) is defined as the killing or eating of other species occupying the same guild, and exploiting the same resources (i.e. competitors), and it is a ubiquitous and often powerful interaction central to the structure and functioning of many natural communities (Polis et al., 1989). IGP not only benefits the predator by the consumption of a food resource, but also acts by reducing the competition. I found that both intraguild predation by other copepods and cannibalism by *C. helgolandicus* copepodites (CI-CVI) were important sources of *C. helgolandicus* egg mortality at station L4. Whilst cannibalism of early stages has been investigated and discussed in the literature to some degree (Daan, 1988; Ohman and Hirche, 2001; Bonnet et al., 2004; Basedow and Tande, 2006), intraguild predation has received much less attention.

Numerous studies exist that attempt to discern whether copepods have the ability to feed selectively (and by what metric); for example, prey size is often invoked as a key factor (Mullin, 1963; Hall et al., 1970; Frost, 1972), whilst others report their capacity to select their prey based on nutritional quality (Fileman et al., 2010; Meunier

et al., 2015). Regardless of the mechanisms involved, it is plausible that zooplankton would exploit such plentiful and lipid-rich resources as copepod eggs and nauplii and in fact many studies have observed IGP on eggs [(e.g. *C. finmarchicus* eggs eaten by other calanoids (Plourde et al., 2009a and 2009b); and *Metridia longa* guts coloured orange from ingestion of *Calanus hyperboreus* eggs (Conover and Huntley, 1991)]. Of course some copepod species are primarily carnivorous predators (i.e. *Candacia armata*, *Paraeuchaeta norvegica*, *Metridia lucens*). *Paraeuchaeta norvegica* for instance is reported to feed mainly on *Calanus* species (Lowndes, 1935), so nutritious eggs and nauplii may be a natural target for many copepods.

Studies that feature specific examples of IGP as a key mortality source are scarce. *Acartia clausi*, *Centropages hamatus*, *Centropages typicus*, and *Temora longicornis* adult females were all observed to ingest their own and all other species eggs and nauplii in feeding experiments (Boersma et al., 2014), and Dufour et al. (2016) quantified IGP between *Metridia longa* and the dominant *C. hyperboreus* and reported that *C. hyperboreus* egg concentration was the only variable to have an impact on *M. longa* ingestion rates. Early life stage mortality rates of *C. finmarchicus* were much higher in the north of its geographical range and were correlated with the increased abundance of the congener species *Calanus glacialis* and *Calanus hyperboreus*; thus indicating IGP (Melle et al., 2014). *C. finmarchicus* itself was reported to play a key role in structuring the community in the North Atlantic through IGP on the early stages of *Acartia*, *Oithona*, and *Metridia lucens* (Irigoien and Harris, 2006).

The mechanisms that regulate unconstrained population growth and prevent dominant monocultures are not well understood, and my research contributes insights on this by showing that predation on copepods is a function of a multitude of biophysicochemical factors involving complex trophodynamics. The majority of nutrient-phytoplankton-zooplankton (NPZ) models represent zooplankton losses via mathematical closure functions poorly grounded in empirical measurements (Daewel et al., 2014). Therefore they do not fully explore the mechanisms of IGP or cannibalism. For example the 3D ecosystem model of fish-consumptions in the North Sea includes IGP of mesozooplankton (Maar et al., 2014), and the ERSEM (European Regional Seas Ecosystem Model) marine biogeochemistry and plankton model incorporates various zooplankton groups, which are capable of consuming themselves, hence a general

cannibalism term (S. Sailley, 2017, personal communication, 9th March). According to Mitra (2009), the use of fixed zooplankton closure terms is inappropriate and unnecessary, and there is no justification for their continued use as they may misrepresent the trophic dynamics. Hence, my results would support calls for further research on the occurrence and quantification of zooplankton carnivory and IGP in zooplankton dynamics.

The effects of extreme weather events

An important feature of climate change is the predicted increase in frequency and intensity of extreme weather events, including rainfall, heatwaves, storms and flooding (IPCC, 2013). Changes in the intensity and extent of turbulence in natural aquatic systems, such as those driven by climate change, could have significant repercussions on the biological communities (Zhou et al., 2016). For example Gardner et al. (2005) estimated that Caribbean coral reefs took over eight years to recover from damage incurred by storms, and Sheehan et al. (In prep) found massive decreases in abundance and diversity of seabed organisms at depths of 20-30 m due to storm events in Lyme Bay, following a procession of storms during the winter 2013/14. This was related to the effects of turbulence and scouring from displaced sand moving over the seabed.

I found a relationship between non-consumptive *C. helgolandicus* mortality rates and increasing wind speeds (Chapter Four). This study is one of the first to report a link between zooplankton mortality and high turbulence levels in an open sea environment; other turbulence studies have focused on lakes and mesocosm experiments, although even these are few in number. Turbulence was found to suppress zooplankton growth and biomass compared to calm water in a mesocosm study (Zhou et al., 2016); a large mesocosm experiment with wave-makers resulted in negative copepod growth (Blottiere, 2015) and copepod densities were found to be higher in calm environments (Zhou et al., 2016; Blottière et al., 2017). Increased turbulence was also found to shift community structure and food web interactions in a small lake in the Eastern Adriatic (Ciglenečki et al., 2015), and a shift occurred from a copepod dominated community to a rotifer dominated community in a simulated lake experiment (Zhou et al., 2016).

Physical mechanisms for these effects include decreased feeding ability, decreased visibility, increased predator encounter, and a reduced ability to maintain position (Härkönen, 2014). Feeding behaviour in particular has received attention; *C. finmarchicus* feeding rates appeared to decline with increased turbulence (Irigoien et al., 2000b), and turbulence caused impairment of ingestion through erosion of the feeding current and interfered with the detection of prey (Kiorbøe and Saiz, 2005). Copepods may also expend more energy sorting food from suspended sediments, which may be of lower nutrient quality and may interfere with chemical/biological signals from prey (Blottière et al., 2017).

The effects of turbulence are not limited to zooplankton species, for example, a major ecological change in the phytoplankton community of a large lake ecosystem in Florida was linked to sediment resuspension, elevated biologically available nutrients and increased turbidity, caused by multiple hurricanes (Beaver et al., 2013). Diatom abundance decreased and the number of dead cells increased after exposure to episodic turbulence simulating breaking waves (Garrison and Tang, 2014). Extreme weather events may also affect zooplankton in other ways, including the freshening of coastal waters and increasing pollution via runoff (Sheahan et al., 2013). I conclude that there will be multiple combined impacts of extreme events on zooplankton populations and that understanding these will be necessary to assess the importance of this in future climate scenarios.

Calanus helgolandicus in a changing climate

North Atlantic sea surface temperatures have risen by an average of 0.5^{o} C in the past 50 years (Brun et al., 2016). Most zooplankton and climate change research has so far focused on the direct and indirect effects of warming seas. Indirect climate-induced effects can occur through changes in bottom-up food web forcing from primary production or through top-down effects caused by changes in upper trophic levels and cascading food-web effects (Drinkwater et al., 2010). Warming-related consequences include increased stratification leading to reduced zooplankton population size (Roemmich and McGowan, 1995), and decreasing zooplankton biomass (Chust et al., 2014). The decreasing size of ectotherms within a community has been described as the 'third universal ecological response to global warming' (Daufresne et al., 2009). Smaller

plankton may accumulate lower lipid levels (Wilson et al., 2016b), which may in turn change diapause duration, which declines at higher temperatures because of increased metabolic rates, and lower lipid levels (Wilson et al., 2015).

At the beginning of this PhD research (2012), it was reported by many that *C. helgolandicus* populations were expanding northward and that *C. finmarchicus* was likely to decline as its ecological niche was squeezed (Beaugrand, 2003; Helaouët and Beaugrand, 2007). It was also recognised that the penetration of warmer water masses and their fauna (the so-called "Atlantification" of the Arctic Ocean) (Schiermeier, 2007) may create a new niche for *C. finmarchicus* (Hirche and Kosobokova, 2007), as a species that is largely confined to ice-free waters (Conover, 1988). These phenomena were reported to be part of a large-scale structural reorganisation in calanoid copepod diversity triggered by an increase in SST in the west European basin (Beaugrand, 2003). Now, four years later, has anything changed? Does current scientific thinking still lead to the same conclusions, or has research progressed to revise the hypotheses and projections?

A search for ["*C. helgolandicus"* and "climate change"] as a topic in Web of Science currently yields 64 studies, and we now have evidence to suggest that, for example, the NE Atlantic population is expanding in all directions and not just northwards (Chust et al., 2013). A habitat suitability model predicts an extinction of *C. helgolandicus* from the Mediterranean north, through to the Bay of Biscay, in the second half of this century (Villarino et al., 2015). Maar et al. (2013) predicted the relative occurrence of North Sea *C. helgolandicus/finmarchicus* is unlikely to change with warming seas, but that the seasonal patterns and phenology may shift. A stage-structured spatial model of both *C. finmarchicus* and *C. helgolandicus* developed by Wilson et al. (2016a) suggested that large parts of the northern N. Atlantic are currently sufficient to support only one generation of *C. helgolandicus* per year, but under a high carbon emissions scenario, conditions would encourage faster development; also that *C. helgolandicus* may become more of an oceanic species as a consequence of warming deep-water coming to the surface. Finally, Reygondeau et al. (2015) report a climate driven ecosystem shift in the western English Channel, with modification of the dominance of key planktonic groups, although a change for *C. helgolandicus* was not

detected. Therefore I suggest that the most recent studies both substantiate and diverge from the predictions of only a few years ago.

This research has taken a reductionist approach by focussing on each of the various factors that affect birth rates and death rates of *C. helgolandicus* at one station. Whilst I found little evidence for the effect of increased temperatures on *C. helgolandicus* at L4 so far, the potential effect of more frequent extreme weather events is of concern. This is compounded by the findings that copepods and gelatinous predators play a key role in regulating population size, and the prediction of increasing jellyfish blooms may have significant knock-on effects, both for *C. helgolandicus* and the copepod predators of *C. helgolandicus*. A key conclusion is that an understanding of multi-species biological interactions is needed to inform climate modelling efforts and that population dynamics are unlikely to be predicted from temperature and/or chlorophyll-*a* alone. In addition to the effects of warming, climate change is predicted to manifest via other mechanisms such as acidification and deoxygenation, creating a "deadly trio" of combined impacts on marine hydrodynamics, biochemistry and ecosystems (Bijma et al., 2013). Therefore to be able to better predict the effect of climate change on populations and communities, we need to take a whole ecosystem approach, based on biological, chemical, physical, ecological, behavioural and genetic factors, in relation to multiple climate-related stressors.

6.1 Directions for future study

I have established the importance of viable egg production and both predator and non-predator related mortality at a site thought to be within its population gravity centre (Chust et al., 2013), hence an optimal habitat. These findings can be taken in two different directions along the broad themes of: 1) what else can we learn from a species in its optimal habitat? And 2) how do the key population control processes vary throughout and at the edges of the *C. helgolandicus* distribution? i.e. what changes so that *C. helgolandicus* does not survive?

C. helgolandicus at L4

Given the importance of gelatinous zooplankton in the modification of *C. helgolandicus* populations, and that I have identified a handful of gelatinous species that prey on them [(ctenophores and medusae in this study; chaetognaths in a study by Bonnet et al. (2010)], it would repay to determine the range of gelatinous species that prey on *C. helgolandicus* and investigate the importance of this species in their diet. A succession of gelatinous species was recorded throughout the spring and summer of 2015 and 2016 at L4 and I could predict that some of the other dips in *C. helgolandicus* abundance would be linked to these blooms.

The correlation between non-consumptive mortality and wind speed over one season is perhaps the first *in situ* hint that large-scale turbulence is harmful to copepods. Extended monitoring of non-consumptive mortality over more seasons is needed to explore any relationship further and reveal other important factors. Further research is necessary to elucidate the mechanisms and thresholds, with focus on both the lethal and sub-lethal effects (reproduction, egg hatch success, clearance rates, growth, etc.) of turbulence within mesocosms or wave-tanks. Leading on from this, knowing how the effects of multiple stressors of extreme weather events (i.e. turbulence, decreased salinity, suspended sediments causing increased turbidity, allochthonous pollution and nutrient enrichment) affect copepod populations would improve marine climate change models.

The metabarcoding of whole gelatinous ctenophores and medusae in this PhD research provided evidence of their role as hosts (or predators) of parasites and their colonisation by bacteria; a fact recently highlighted by Lindeque et al. (2013). These molecular techniques could assist with understanding the prevalence and importance of other sources of non-consumptive mortality, namely pathogens and parasites. *Ellobiopsis* alveolate ectoparasites of *C. helgolandicus* (Albaina and Irigoien, 2006) were observed periodically through routine L4 zooplankton sample processing and picking of females for egg production experiments, but little attempt has been made to identify, quantify or even understand the effect of these parasites.

C. helgolandicus throughout its distribution

There are 62 marine monitoring sites in the North Atlantic Basin distributed from the Mediterranean to the Barents Sea (O'Brien et al., 2013). Investigation of the *C. helgolandicus* data in these time series would improve our knowledge if comparison were made between the L4 population and other sites to ascertain, for example, how reproduction and mortality are affected at the northern and southern edges of their geographical range. A comparison of the interannual variability of *C. helgolandicus* at multiple sites could also be made, including an investigation of the predominant population control processes, i.e. are gelatinous zooplankton always key predators? One of the major expected changes in respect to climate change relates to the phenology of key life-stage events, and although I found no evidence of a shift in the initiation of the *C. helgolandicus* growth season at L4, investigations at other sites may elucidate how *C. helgolandicus* responds to warmer conditions and also how this change impacts on higher trophic levels.

Many models have been developed to investigate copepod population dynamics, including stage-structured models (SSM), weight-structured models (WSM), cohort models (CM) and individual-based models (IBMs) (Carlotti et al., 2000). The use of IBMs in ecology in particular has increased rapidly in the last 20 years (DeAngelis and Grimm, 2014) and they account for the development, reproduction and mortality of individuals and attempt to bridge the gap between the level at which environmental impacts occur (individuals) and the level at which observations are made (populations) (Neuheimer et al., 2010). I am currently unaware of any attempts to build a population model specifically for *C. helgolandicus* [although IBMs have been constructed for *C. finmarchicus* (Carlotti and Wolf, 1998; Hjøllo et al., 2012)]; therefore the development of a population model for *C. helgolandicus* would be a natural future research objective. A *C. helgolandicus* model, coupled with a NPZ and/or hydrodynamic model could allow us to test specific hypotheses relating to the mortality of *C. helgolandicus*; for example, how does the population vary in relation to changing temperatures and/or a changing predator landscape (i.e. more gelatinous carnivores)? What are the modelled effects of density-dependent population self-regulation? How does the population respond to extreme weather (increasing turbulence)?

Bonnet et al., (2005) in their review of *C. helgolandicus* in European waters called for the development of a population model and provided a list of the key parameters required (e.g. feeding rates as a function of temperature and mortality rates due to starvation). Whilst it is recognised that one of the major limiting factors in ecological modelling is data parameterisation, empirical research using time series of key marine species such as *C. helgolandicus* can provide valuable data. Overall, as time series lengthen and techniques such as metabarcoding become cheaper and we have better data to parameterise these models, the scientific community will be in a more-informed position to predict the effects of and respond to climate change.

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APPENDIX A:

Published paper:

J. L. Maud et al. (2015). How does *Calanus helgolandicus* maintain its population in a variable environment? Analysis of a 25-year time series from the English Channel. *Process in Oceanography* 137 (Part B): 513-523.

APPENDIX B

Table 3.10. Summary of *in situ* studies reporting on effects of toxic phytoplankton species or related variables on copepod egg hatch success (EHS) and/or naupliar abnormalities (NA); NVO – non-volatile oxylipin; PUA – polyunsaturated aldehyde; ↔ indicates no effect/relationship; ↓ indicates negative effect

Phytoplankton	Copepod	EHS	NA	Effects	Reference
Chaetoceros spp. biomass	*Calanus* spp.	↔		No relationship	Jónasdóttirr et al. 2005
chorophyll-a	*Calanus helgolandicus*	↔		EHS unaffected	Wichard et al. 2008
chorophyll-a	*Calanus helgolandicus*	↔		No relationship with chlorophyll-a	Laabir et al. 1995b
diatom abundance	*Calanus helgolandicus*	↔		EHS unaffected	Wichard et al. 2008
diatom biomass	*Calanus helgolandicus*	↔		No significant relationship with EHS	Irigoien et al. 2002
diatom biomass	*Pseudocalanus newmanii*	↔	↔	No relationship with EHS or NA	Ban et al. 2000
diatom NVO	*Acartia clausii*	↓		EHS decreased	Ianora et al. 2015
diatom NVO	*Calanus helgolandicus*	↓		EHS decreased	Ianora et al. 2015
natural phytoplankton mixture	*Calanus finmarchicus*	↔		EHS unaffected	Campbell and Head, 2000
Pseudo-nitzschia delicatissima	*Acartia clausii*	↓		During blooms low EHS	Miralto et al. 1999
Pseudo-nitzschia delicatissima	*Calanus helgolandicus*	↓		During blooms low EHS	Miralto et al. 1999
Skeletonema costatum	*Acartia clausii*	↓		During blooms low EHS	Miralto et al. 1999
Skeletonema costatum	*Calanoides carinatus*	↔		EHS unaffected	Irigoien et al. 2005
Skeletonema costatum	*Calanus helgolandicus*	↓		During blooms low EHS	Miralto et al. 1999
Skeletonema costatum	*Rhincalanus nasutus*	↔		EHS unaffected	Irigoien et al .2005
Skeletonema costatum	*Acartia tonsa*	↔		EHS ≥ 90%	Tiselius et al., 2008
Skeletonema costatum and *Pseudo-nitzschia delicatissima*	*Acartia clausii*	↓		V low EHS post bloom	Miralto et al. 2003
Skeletonema costatum and *Pseudo-nitzschia delicatissima*	*Calanus helgolandicus*	↓		V low EHS post bloom	Miralto et al. 2003
Skeletonema costatum biomass	*Calanus* spp.	↔		No relationship	Jónasdóttir et al. 2005
Thalassiosira spp.	*Calanus pacificus*	↓		Lower EHS associated with *Thalassiosira* blooms	Pierson et al. 2005
Thalassiosira spp.	*Pseudocalanus newmanii*	↓		Lower EHS associated with *Thalassiosira* blooms	Halsband-Lenk et al. 2005
Thalassiosira spp. biomass	*Calanus* spp.	↔		No relationship	Jónasdóttir et al. 2005
total PUAs	*Calanus helgolandicus*	↔	↔	No effect on EHS or NA	Wichard et al. 2008

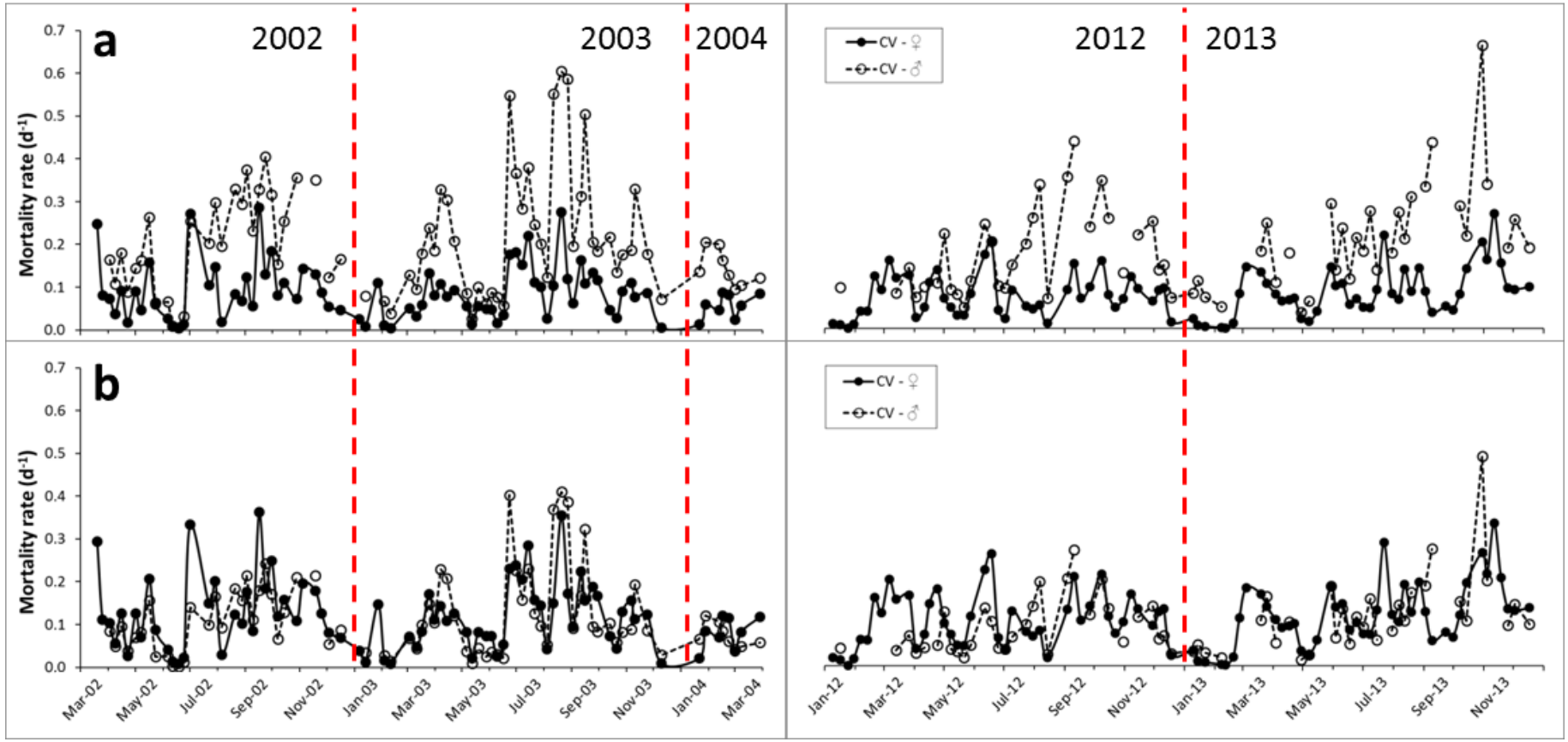

Figure 4.19. *Calanus helgolandicus* mortality rates CV-♀ and CV-♂ stage pairs derived using vertical life table (VLT) stage-pair method (2002-04 and 2012-13) (a) using a 1:1 CV sex ratio, and (b) using a 5:1 ♀ to ♂ CV sex ratio.

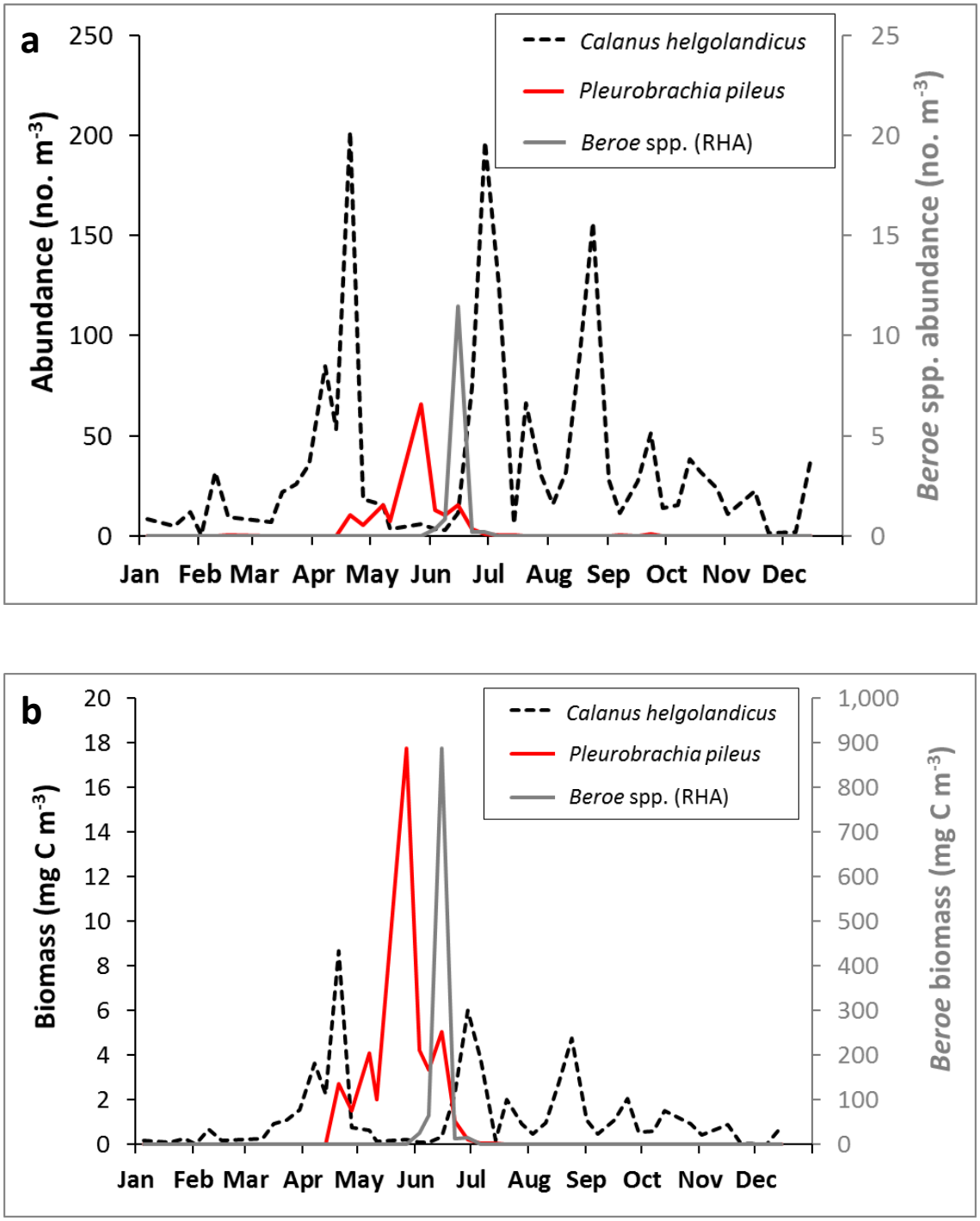

Figure 5.12. ***P. pileus*****,** ***Beroe*** **spp. and total** ***C. helgolandicus*** **at L4 (January -December 2015); a) abundance and b) biomass; RHA is right hand axis.**