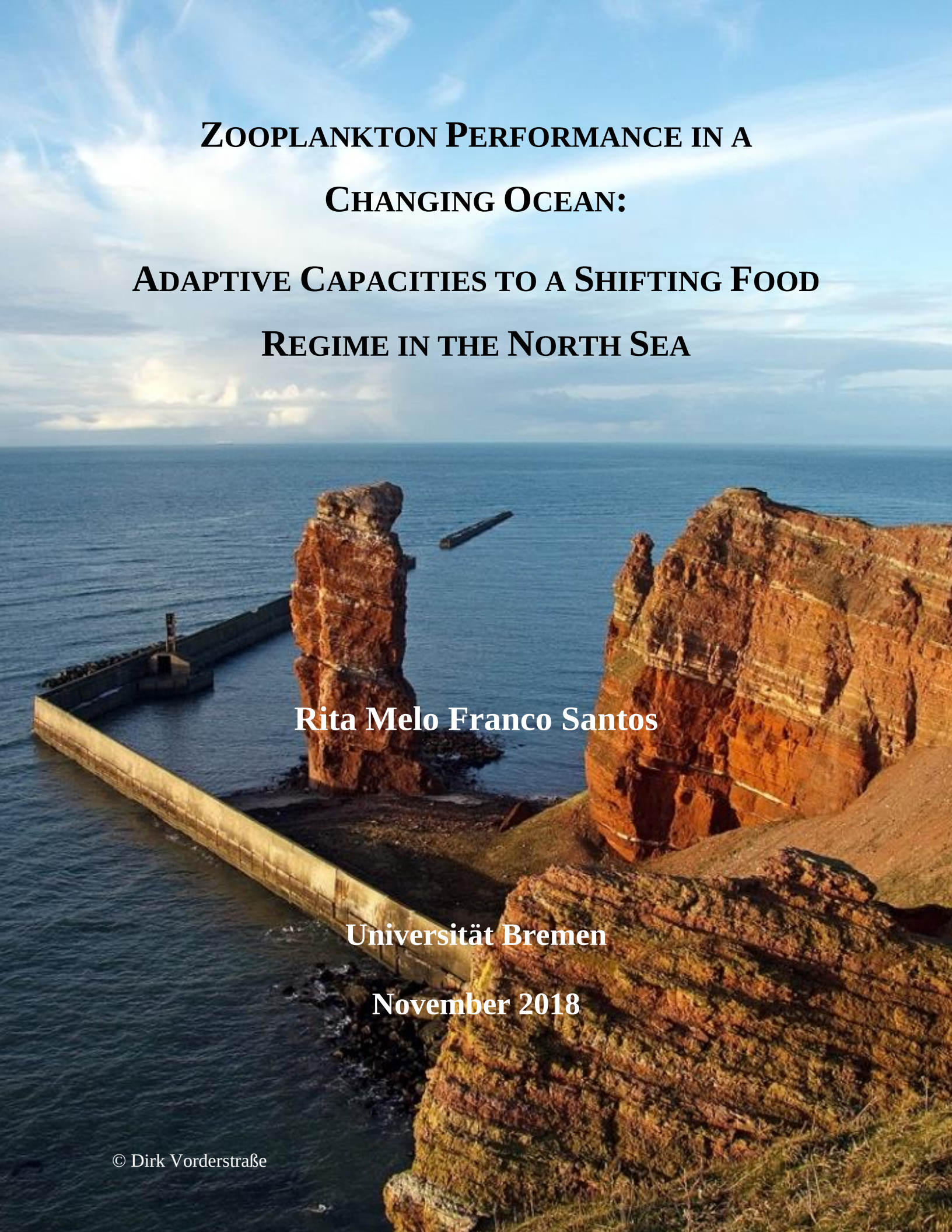
Zooplankton Performance in a Changing Ocean: Adaptive Capacities to a Shifting Food Regime in the North Sea



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ZOOPLANKTON PERFORMANCE IN A CHANGING OCEAN:

ADAPTIVE CAPACITIES TO A SHIFTING

FOOD REGIME IN THE NORTH SEA

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MAR PORTUGUÊS

Ó mar salgado, quanto do teu sal
São lágrimas de Portugal!
Por te cruzarmos, quantas mães choraram,
Quantos filhos em vão rezaram!
Quantas noivas ficaram por casar
Para que fosses nosso, ó mar!

Valeu a pena? Tudo vale a pena
Se a alma não é pequena.
Quem quer passar além do Bojador
Tem que passar além da dor.
Deus ao mar o perigo e o abismo deu,
Mas nele é que espelhou o céu.

Fernando Pessoa

CONTENTS

LIST OF ABBREVIATIONS

AA	Amino acid
AE_C	Absorption efficiency for carbon
AE_N	Absorption efficiency for nitrogen
ANOSIM	Analysis of similarity
ANOVA	Analysis of variance
ASW	Artificial sea water
atom%	Atom percent
APE	Atom percent excess
AWI	Alfred Wegener Institute
BAH	Biologische Anstalt Helgoland
BSIA	Bulk stable isotope analysis
C	Carbon
CPR	Continuous Plankton Recorder
CSIA	Compound-specific stable isotope analysis
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
EPR	Egg production rate
ES	Ecological stoichiometry
FA	Fatty acid

LIST OF ABBREVIATIONS

FAME	Fatty acid methyl ester
FATM	Fatty acid trophic markers
FP	Faecal pellets
FPR	Faecal pellet production rate
GC	Gas chromatograph
GC-c-IRMS	Gas chromatograph/combustion/isotope ratio mass spectrometer
GGE	Gross growth efficiency
LC-PUFA	Long-chain polyunsaturated fatty acids
MUFA	Monounsaturated fatty acids
N	Nitrogen
$NEPE_C$	Net egg production efficiency for carbon
$NEPE_N$	Net egg production efficiency for nitrogen
nMDS	Non-metric multidimensional scaling
NPOC	Non-purgeable organic carbon
$NSGE_C$	Net somatic growth efficiency for carbon
$NSGE_N$	Net somatic growth efficiency for nitrogen
P	Phosphorus
PERMANOVA	Permutational multivariate analysis of variance
PUFA	Polyunsaturated fatty acids
RQ	Respiratory quotient
SFA	Saturated fatty acids

SIA	Stable isotope analysis
SIMPER	Similarity percentages
SST	Sea surface temperature
TDN	Total dissolved nitrogen
TAG	Triacylglycerol
UGhent	University of Ghent

ABSTRACT

The zooplankton is a key group in marine ecosystems – it has high biomass and is the link between primary producers and higher trophic levels. Zooplankton organisms are often vulnerable to fluctuations in food supply, and their population dynamics is directly influenced by changes in phytoplankton availability and nutritional quality. Changes in parameters such as water temperature and nutrient loading can affect the nutritional quality of algae as food for herbivores, as well as phytoplankton community composition and dominance. Alterations in the base of the food web will, consequently, affect all trophic levels. The adaptive capacity of the zooplankton to shifting food regimes will likely be determined by the effects of food quality (a.o.) on their physiology. Understanding such effects is a pressing issue given the gap in the literature regarding how species cope with inadequate (low quality) food supply. In the sourthern North Sea the zooplankton community is dominated by calanoid copepods, with important contributions from polychaete larvae. This PhD project investigated how food quality influenced the performance, the elemental and biochemical compositions, and the assimilation and turnover of carbon (C) in females of the calanoid copepod *Temora longicornis* and in the meroplanktonic larvae of the polychaete *Lanice conchilega*.

Zooplankton samples were collected between May and June 2016 off the German island of Helgoland, in the southern North Sea, and copepods and polychaetes were sorted for five-day laboratory feeding experiments. Two experiments were conducted with *T. longicornis* and one with *L. conchilega*, during which the individuals were fed with diets of different quality - diatoms (*Conticribra weissflogii*) and dinoflagellates (*Oxyrrhis marina*) cultured in nutrient-replete and in nitrogen (N)-depleted conditions. In order to be able to follow dietary lipid C assimilation, prey items were enriched with ^{13}C before being offered as food to copepods and polychaetes. Grazing, respiration, excretion, and egg and faecal pellet production rates were also recorded in incubations conducted during the experiments with copepods. These rates were used to construct C and N budgets in order to evaluate their intake and usage of energy. Performance was also discussed in terms of the efficiency with which these processes occurred.

Copepods feeding on prey with a molar C:N ratio that resembled their own showed the highest investment in somatic and reproductive growth. However, they also showed the highest expenditures with egestion and catabolism, which were expected for copepods feeding on prey

with the highest molar C:N ratio. The major result regarding copepod performance in relation to food quality is that dinoflagellates are a food source of superior or similar quality to diatoms under nutrient-replete or N-depleted conditions, respectively. This result is, however, limited to the temperature tested during the laboratory experiments, as different temperatures will influence copepod metabolism and C requirements. Furthermore, the experiments with *T. longicornis* revealed that egestion is a major pathway for eliminating excess C, and that low food quality can influence respiration and the intensity and speed of DOC leakage from faecal pellets.

The experiments investigating C assimilation and turnover in *T. longicornis* revealed a higher absolute lipid C assimilation in copepods fed with prey whose molar C:N ratio was the most different from their own. The relative lipid C assimilation (as % food ingested), however, was similar between copepods fed with different diets. This result suggests that prey biochemical composition does not have an effect over lipid C assimilation by *T. longicornis*, only over ingestion rates. The high C turnover rates measured indicate an opportunistic feeding strategy for *T. longicornis*, which would accumulate dietary C when it is readily available with a speed that decreases as saturation is approached and its lipid storage is replenished. This strategy is likely employed to maximize reproductive output. In the case of *L. conchilega*, the experiment revealed that larvae are able to regulate their lipid C content (homeostasis) regardless of the fatty acid (FA) availability in their diet. Although lipid C assimilation was low in the larvae (meroplanktonic organisms are usually lipid-poor), the individuals selectively accumulated certain FAs and biosynthesized others (from dietary FA) which were not available in the diet.

Concomitant changes in several ecosystem parameters make it difficult to precisely evaluate how the zooplankton will react to changing food regimes in marine environments and to comprehend the full scale of the effects and feedback mechanisms of ecosystem changes. Nevertheless this thesis presents a robust contribution towards a better understanding of how zooplanktonic organisms might be affected by changes in the quality of their prey. Although the work for this thesis refers to organisms from a very specific location, the ubiquitousness of the species and of the forcing (of environmental change) studied enables the comparison of the results with those from several marine ecosystems.

ZUSAMMENFASSUNG

In marinen Ökosystemen hat das Zooplankton eine Schlüsselrolle – in vielen Regionen ist die Biomasse der Zooplankter hoch und sie sind ein Bindeglied zwischen Primärproduzenten und höheren trophischen Ebenen. Viele Zooplanktonorganismen reagieren sensitiv auf Fluktuationen in ihrer Nahrungsversorgung, und daher wird ihre Populationsdynamik direkt von Veränderungen der Verfügbarkeit und der Qualität der Nahrung, z. B. Phytoplankton, beeinflusst. Die Qualität der Algen als Nahrung für herbivore Organismen wiederum wird von Umweltparametern wie z.B. Wassertemperatur und Nährstoffkonzentrationen beeinflusstb. Änderungen an der Basis des Nahrungsnetzes betreffen folglich alle trophischen Ebenen. Wie gut sich Zooplanktonorganismen an ein sich veränderndes Nahrungsregime anpassen können, wird u.a. dadurch bestimmt, wie sich die Nahrungsqualität auf die Physiologie der Tiere auswirkt. Zur Zeit liegen allerdings nur wenige Informationen dazu vor, wie die Zooplankter auf Nahrung von geringer Qualität reagieren.

In der südlichen Nordsee wird die Zooplanktongemeinschaft generell von calanoiden Copepoden dominiert, aber auch meroplanktische Larven, wie z.B. Polychaetenlarven, können saisonal hohe Abundanzen erreichen. Im Rahmen dieser Dissertation wurde untersucht, welchen Einfluss die Nahrungsqualität auf die Leistungsfähigkeit und die biochemische Zusammensetzung der Weibchen des calanoiden Copepoden Temora longicornis und der meroplanktonischen Larven des Polychaeten Lanice conchilega hat. Außerdem wurde untersucht, wie viel und wie schnell Kohlenstoff (C) unter unterschiedlichen Nahrungsbedingungen assimiliert und umgesetzt wird.

Die Zooplanktonproben wurden in Mai und Juni 2016 in der südlichen Nordsee nahe der deutschen Insel Helgoland gesammelt. Direkt nach dem Fang wurden die Copepoden und die Polychaetenlarven lebend aus den Fängen sortiert und dann für fünf Tage im Labor inkubiert. Mit T. longicornis wurden zwei Versuche und mit Larven von L. conchilega wurde ein Versuch durchgeführt. Dabei wurden die Tiere mit Diatomeen (Conticribra weissflogii) und Dinoflagellaten (Oxyrrhis marina) gefüttert. Die Algen wurden dabei entweder unter hohen Nährstoffkonzentrationen oder unter limitiertem Angebot an Stickstoff (N) kultiviert. Um der Aufnahmen von C in Form von Lipiden zu folgen, wurden die Beuteorganismen mit 13C angereichert, bevor sie den Copepoden und Polychaetenlarven als Futter angeboten wurden. In zusätzlichen Experimenten mit T. longicornis wurden täglich die Ingestions-, Respirations- und

Exkretionsraten der Tiere gemessen. Außerdem wurden die Eier und die Anzahl Kotballen, die Weibchen innerhalb von 24 h produzierten gezählt. Diese Daten wurden dazu verwendet, Kohlen- und Stickstoffbudgets zu berechnen und die Aufnahme und Nutzung von Energie aus der Nahrung in Abhängigkeit von der Nahrungsqualität zu bewerten.

Die Ergebnisse zeigen, dass Copepoden, deren Nahrung ein molares C:N-Verhältnis hatte, welches ihrem eigenen ähnelte, den meisten Kohlenstoff und Stickstoff in somatisches Wachstum und Reproduktion investierten. Diese Tiere gaben jedoch gleichzeitig am meisten C und N über ihre Ausscheidungsprodukte und den Katablolismus ab. Diese Ergebnisse waren eigentlich bei den Copepoden zu erwarten, denen Beuteorganismen mit dem höchsten molaren C: N-Verhältnis angeboten wurden. Ein wesentliches Ergebnis dieser Untersuchungen ist, dass die Dinoflagellaten eine Nahrungsquelle von höherer Qualität waren als die Diatomeen, die unter nährstoffreichen bzw. N-limitierenden Bedingungen kultiviert wurden. Hier ist jedoch zu bedenken, dass dies zunächst nur für die Temperatur gelten kann, die während der Laborexperimente herrschte, da generell die Temperatur ein erheblichen Einfluss auf den Metabolismus der Copepoden und ihren Bedarf an Kohlenstoff hat. Darüber hinaus zeigten die Experimente mit T. longicornis, dass überschüssiger Kohlenstoff vor allem über die Egestion abgegeben wird und dass eine geringe Nahrungsqualität sowohl die Atmung als auch die Intensität und Geschwindigkeit, mit der DOC aus den Kotballen austritt, beeinflussen kann.

Die Experimente, die die Assimilation und den Umsatz von Kohlenstoff bei T. longicornis untersuchten, zeigten, dass Tiere, deren Nahrung ein molares C:N Verhältnis aufwies, welches sich am stärksten von ihrem eigenen unterschied, mehr Kohlenstoff über Lipide assimilierten. Die relative Assimilation von Lipidkohlenstoff (in % des über die Nahrung aufgenommenen Kohlenstoffs) war jedoch bei allen Copepoden trotz Fütterung mit unterschiedlicher Nahrung ähnlich. Dieses Ergebnis legt nahe, dass bei T. longicornis die biochemische Zusammensetzung der Beuteorganismen keine Auswirkungen auf die Assimilation von Lipid-C hatte. Die hohen C-Turnover-Raten deuten auf eine opportunistische Strategie hin. Es ist zu vermuten, dass die Weibchen, Kohlenstoff aus der Nahrung zunächst schnell akkumulieren, und die Geschwindigkeit abnimmt, wenn die Lipidspeicher aufgefüllt sind. Diese Strategie könnte dazu dienen, die Fortpflanzungsleistung zu maximieren.

Im Fall von L. conchilega zeigte das Experiment, dass die Larven dazu in der Lage sind, ihren Lipid C-Gehalt unabhängig von Verfügbarkeiten von Fettsäuren (FA) in ihrer Nahrung zu regulieren (Homöostase). Obwohl die Assimilation von Kohlenstoff in den Lipiden der Larven niedrig war (meroplanktonische Organismen sind gewöhnlich lipidarm), akkumulierten die Individuen selektiv bestimmte FAs und biosynthetisierten andere, die nicht in der Nahrung verfügbar waren.

Gleichzeitige Veränderungen mehrerer Ökosystemparametern machen es schwierig, genau vorherzusagen, wie das Zooplankton auf Veränderungen im Nahrungsregime reagieren wird. Damit ist es schwierig, Auswirkungen und Rückkopplungsmechanismen von ökosystemaren Veränderungen vollständig zu erfassen. Nichtsdestotrotz liefert diese Arbeit einen Beitrag zu einem besseren Verständnis darüber, wie Veränderungen der Qualität der Nahrungsgrundlage Zooplanktonorganismen beeinflussen können.

SAMENVATTING

Zooplankton is een onmisbare schakel in mariene ecosystemen vanwege hun hoge biomassa en de connectie tussen primaire producenten. Zooplankton zijn echter kwetsbaar voor schommelingen in hun voedselvoorzieningen. De populatiedynamiek van zooplankton wordt rechtstreeks beïnvloed door veranderingen in de beschikbaarheid en de voedingskwaliteit van fytoplankton. Veranderingen in parameters zoals watertemperatuur en nutriënten concentraties beinvloeden de voedingskwaliteit, de samenstelling van de gemeenschap en de dominantie van fytoplankton, die een voedselbron zijn voor herbivore zooplankton. Veranderingen aan de basis van het voedselweb zullen bijgevolg alle trofische niveaus beïnvloeden. Het aanpassingsvermogen van zooplankton aan veranderende voedselregimes zal worden bepaald door de effecten van de voedselkwaliteit op hun fysiologie. Het begrijpen van dergelijke effecten is een dringende kwestie, gezien de kloof in de literatuur over hoe soorten omgaan met ontoereikende (lage kwaliteit) voedselvoorzieningen. In de zuidelijke Noordzee wordt de gemeenschap van zooplankton gedomineerd door calanoïde copepoden, met een belangrijke bijdragen van polychaete larven. Dit doctoraatsproject onderzocht hoe voedselkwaliteit de prestaties, de elementaire en biochemische samenstellingen en de assimilatie en turnover van koolstof (C) beïnvloedde bij de vrouwelijke calanoïde copepoden *Temora longicornis* en in de meroplanktonische larven van de polychaete *Lanice conchilega*.

Zooplankton stalen werden verzameld tussen mei en juni 2016 nabij het Duitse eiland Helgoland, in de zuidelijke Noordzee. Copepoden en polychaetes werden geselecteerd uit deze stalen voor vijfdaagse laboratorium voedingsexperimenten. Twee experimenten werden uitgevoerd met *T. longicornis* en één met *L. conchilega*, waarbij de individuen werden gevoed met diëten van verschillende kwaliteit; diatomeeën (*Conticribra weissflogii*) en dinoflagellaten (*Oxyrrhis marina*) werden gekweekt in nutriënten-verzadigde en in stikstof (N) arme voedings media. Om supplementaire lipide C-assimilatie te kunnen volgen, werden prooi-items verrijkt met ^{13}C voordat ze als voedsel aan copepoden en polychaetes werden aangeboden. Productiesnelheden voor graas, ademhaling, excretie, egestie en faecale pellet productie werden gemeten in de incubatie tanken tijdens de experimenten met de copepoden. Deze snelheden werden gebruikt om C- en N-budgetten te construeren en om de inname en gebruik van energie in de copepoden

te evalueren. De algehele prestaties van de copedpoden, in termen van de efficiëntie waarmee deze processen plaatsvonden, worden ook in deze thesis besproken.

Copepoden die zich voedden met prooien met een molaire C:N verhouding die leek op hun eigen samenstelling vertoonden de hoogste investering in somatische en reproductieve groei. Ze vertoonden echter ook de hoogste energetische uitgaven aan eier productie en katabolisme, deze resultaten werden echter verwacht voor copepoden die zich voeden met prooien met de hoogste molaire C:N verhouding. De belangrijkste uitkomst van de copepod prestaties, in betrekking tot de voedselkwaliteit, was dat de dinoflagellaten een voedingsbron zijn van superieure of vergelijkbare kwaliteit wanneer deze werden vergeleken bij diatomeeën onder voedings verzadigde of onder N arme omstandigheden, respectievelijk. Dit resultaat is echter beperkt tot de geteste temperatuur tijdens de laboratoriumexperimenten, omdat verschillende temperaturen uiteraard het copepod metabolisme en de bijgaande C-vereisten zullen beïnvloeden. De de experimenten met *T. longicornis* onthulden tevens dat egestie een belangrijk pad is voor het elimineren van een overmaat aan C, en dat een lage voedselkwaliteit de ademhaling en de intensiteit en snelheid van DOC-lekkage uit fecale pellets kan beïnvloeden.

De experimenten die de C turnover en assimilatie in *T. longicornis* onderzochten, lieten een hogere absolute lipide C-assimilatie zien in copepoden die gevoed werden met prooien waarvan de molaire C: N-verhouding het meest verschillend was van die van henzelf. De relatieve lipide C-assimilatie (als % ingenomen voedsel) was echter vergelijkbaar tussen copepoden die werden gevoed met verschillende diëten. Dit resultaat suggereert dat de biochemische samenstelling van de prooi geen effect heeft op lipide C-assimilatie voor *T. longicornis*, alleen voor ingestiecijfers. De hoge C turnover snelheden duiden op een opportunistische voedingsstrategie voor *T. longicornis*, waarbij C wordt geaccumuleerd wanneer het gemakkelijk beschikbaar is met een snelheid die afneemt naarmate de verzadiging wordt benaderd en de opslag van lipiden wordt aangevuld. Deze strategie wordt waarschijnlijk gebruikt om het reproductievermogen te maximaliseren. In het geval van *L. conchilega* onthulde mijn experiment dat larven hun lipide C-gehalte (homeostase) kunnen reguleren ongeacht de beschikbaarheid van vetzuren (FA) in hun dieet. Hoewel lipide C-assimilatie laag was in de larven (meroplanktonische organismen zijn meestal lipidearm), accumuleerden de individuen selectief bepaalde FA's en biosynthetisereerde ze andere (uit FA-dieet) die niet beschikbaar waren in het dieet.

Gelijktijdige veranderingen in verschillende ecosysteemparameters maken het moeilijk om nauwkeurig te evalueren hoe zooplankton zal reageren op veranderende voedselregimes in mariene omgevingen. Dit maakt het niet triviaal om de volledige schaal van effecten en feedbackmechanismen van ecosysteemveranderingen te vattten. Niettemin presenteert dit proefschrift een krachtige bijdrage, en een beter begrip aan de manier waarop zooplanktonische organismen kunnen worden beïnvloed door veranderingen in de voedingskwaliteit van hun prooi. Hoewel het werk voor dit proefschrift verwijst naar organismen van een zeer specifieke locatie, maakt de alomtegenwoordigheid van deze soorten en de focus op verandering in nutrienten concentraties de resultaten van deze thesis vergelijkbaar met die van verschillende mariene ecosystemen.

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1 SCIENTIFIC BACKGROUND AND OBJECTIVES

1.1 ZOOPLANKTON COMMUNITIES

The zooplankton is a key group in biological and abiotic processes in marine ecosystems, especially since its estimated biomass is larger than that from all other consumers (Conover, 1978). They are the most important link in the energy transfer between the lower and higher trophic levels, and contribute to the cycling of organic matter and to the CO_2 (carbon dioxide) export from the surface to deeper water via the carbon (C) pump (Castellani and Altunbas, 2014; Jónasdóttir et al., 1998; Juul-Pedersen et al., 2006). Zooplankton are not often limited by food quantity in coastal regions (Huntley and Boyd, 1984), but some food sources will be of lower nutritional value (Sterner and Schulz, 1998). Elemental composition, digestion resistance, and biochemical composition all are important factors determining the nutritional value / quality of food particles (Sterner and Schulz, 1998).

The experiments performed during this PhD used zooplanktonic species sampled around the German island of Helgoland, located in the southern North Sea. In this area the zooplankton community is dominated by calanoid copepods, both in terms of density and abundance, but polychaete larvae, harpacticoid copepods, and appendicularians have also been found in great numbers between June and September (Fransz et al., 1991; Hickel, 1975).

1.1.1 Zooplankton species investigated

Temora longicornis

The small (approximately 1 mm prosome length) calanoid copepod *Temora longicornis* (Müller, 1785) (Fig. 1) is one of the dominant species in the coastal zooplankton community in the North Atlantic and North Sea, reaching peak densities (~ 3 µg C L^{-1}) during the spring, early summer, and early fall (Castellani and Altunbas, 2014; Hickel, 1975). Off Helgoland, this abundant species (up to 600 individuals m^3) reproduces all year round (Wesche et al., 2006), and its grazing may have a substantial impact on the phytoplankton standing stock (Gentsch et al., 2009; Maar et al., 2004). They are, thus, an important link in the energy transfer between primary producers and higher trophic levels. Copepods have adapted to different environmental conditions by developing several life history traits, such as high reproduction rates, opportunistic

feeding modes, and efficient energy utilization (Kiørboe et al., 1985, Niehoff et al., 2015). *T. longicornis* is omnivorous (Marshall, 1973), though with weak prey selectivity (DeMott, 1988), and has high metabolic turnover rates (Helland et al., 2003; Kreibich et al., 2008). It is, however, unable to accumulate significant amounts of energy reserves (Båmstedt, 1986; Evjemo and Olsen, 1997; Kreibich et al., 2008), being thus dependent on a constant availability of prey and vulnerable to fluctuations in food supply (Helland et al., 2003; Kiørboe et al., 1985, Kreibich et al., 2008). This can be particularly problematic in dynamic systems such as the North Sea, where plankton community composition can change rapidly (Kiørboe and Nielsen, 1994). The availability of prey for this copepod, both in qualitative and quantitative terms, can be further aggravated by the decrease in nutrient loading around Helgoland (Boersma et al., 2015). Efficient food utilization is, thus, of paramount importance for the survival of *T. longicornis*.

Figure 1: *Temora longicornis*. Confocal image (10x) showing both auto fluorescence and Congo Red Fluorescence (© Jan Michels, Christian-Albrechts-Universität zu Kiel, Germany).

Lanice conchilega

The tube-dwelling polychaete *Lanice conchilega* (Pallas, 1766) has a worldwide distribution (Holthe, 1986). It is commonly found in European waters, especially in the North Sea (Godet et al., 2008; Ropert and Dauvin, 2000, Van Hoey et *al.*, 2008), where it can amount to 15% of the zooplankton biomass between July and September when water temperatures are above 13^{o}C (Hickel, 1975). It is a non-selective suspension-deposit feeder (Braeckman et al., 2012; Bühr,

1976; Bühr and Winter, 1977) which derives its diet from the organic matter available in the water column and in the sediment. Common food items include diatoms, bacteria and microphytobenthos, and the species is able to switch between food sources (Braeckman et al., 2012; Bühr and Winter, 1977; Dubois et *al.*, 2007). *L. conchilega* forms reefs (Fig. 2a) capable of structuring the surrounding habitat and creating a complex and heterogeneous environment (Zühlke et al., 1998; Zühlke, 2001). This, in turn, provides refuge against predation and physical and chemical stresses, but also nursery and feeding grounds to several other species, fish and birds included (Alves et al., 2017; Braber and De Groot, 1973; De Smet et al., 2013; Godet et *al.*, 2008, and references therein; Petersen and Exo, 1999; Rabaut et al., 2007). By modifying its habitat into a more favorable one and with better living conditions, *L. conchilega* positively affects the benthic community living in it, favoring species richness and composition and faunal abundance (Callaway, 2006; Rabaut et al., 2007; Zühlke et al., 1998; Zühlke, 2001). Its reproductive peak in the southern North Sea occurs in spring, and is followed by smaller peaks during the summer and autumn (Van Hoey, 2006), though larval supply can vary between years (Strasser and Pieloth, 2001). *L. conchilega* larvae evolve from a short planktonic to a benthic phase and, after a few days, to a second planktonic stage, the aulophore larvae (Bhaud, 1988) (Fig. 2b). At this stage larvae already present a (transparent) tube, and display morphological features of a juvenile (Rabaut, 2009). They are able to feed in the water column, where they can remain up to 60 days before succeeding to an intermediate benthic stage (Bhaud and Cazaux, 1990). In comparison with adult organisms, few studies have been conducted on the basic ecology of *L. conchilega* larvae, addressing topics such as juvenile settlement and buoyancy (Bhaud and Cazaux, 1990; Callaway, 2003; Ropert and Dauvin, 2000; Strasser and Pieloth, 2001). There is a considerable gap in the literature regarding the feeding ecology of the larvae.

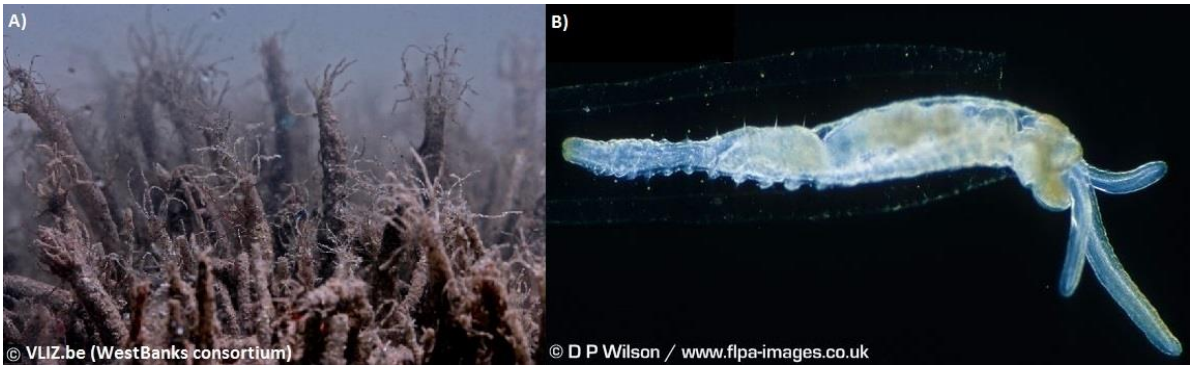

Figure 2: *Lanice conchilega* aggregations, or reefs (a), and aulophore larva stage (b).

1.1.2 Common prey items for the studied zooplankton species

Diatoms and dinoflagellates are common prey for *T. longicornis* and for *L. conchilega* larvae (Bakker and van Rijswijk, 1987; Evans, 1981; Larink and Westheide, 2011). It has been claimed that diatoms, although a stable food source, are of low quality for calanoid copepods and only make up a minor part of their diet, whereas dinoflagellates are consumed in higher proportions and have a higher nutritional value (Gentsch et al., 2009; Jónasdóttir et al., 1998, 2009; Jones and Flynn, 2005; Löder et al., 2011; Saiz and Calbet, 2011).

Diatoms

Diatoms are silicified members of the phytoplankton. They can be responsible for up to 40% of the carbon fixed in the ocean, which characterizes them as key players in the biological pump (Smetacek, 1999). *Conticribra weissflogii* (Grunow) (Stachura-Suchoples and Williams, 2009) (Fig. 3a) is a planktonic, centric diatom that can be found as single cells or in groups in marine environments in many parts of the world. It varies in size from 4 to 32 µm diameter (larger in winter, 15 µm, and smaller in summer, 5 µm) (TW1200 Reed Mariculture Inc. Retrieved 2011-11-18). Great controversy involves the topic of diatoms being good or bad quality diet items for copepods, given that their defense mechanisms against predation (production of compounds toxic to zooplankton, for example) and nutritional quality vary interspecifically (Jónasdóttir et al., 1998; Paffenhöfer et al., 2005). Some studies have further pointed out the suitability of diatoms as food source for the production of eggs in copepods but its reduced success for egg

hatching (Miralto et al., 1999; Ban et al., 1997). Nevertheless they have been successfully used in laboratory feeding experiments with *T. longicornis* (Arendt et al., 2005; Boersma et al., 2016; Niehoff et al., 2015; van Someren Gréve et al., 2017).

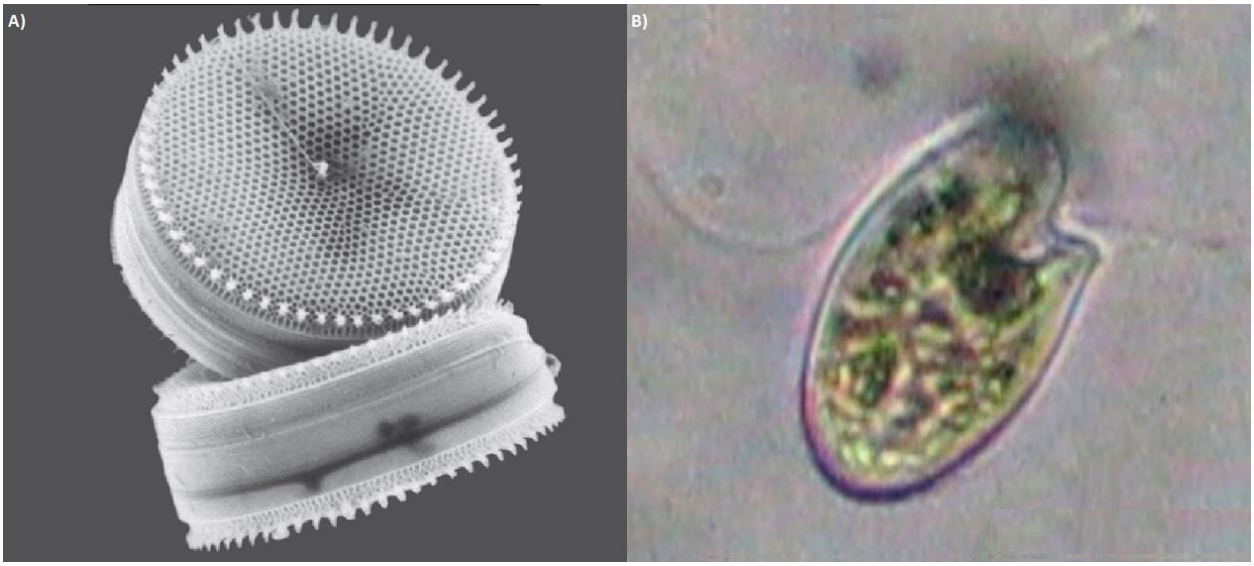

Figure 3: Prey species used during feeding experiments with copepods and polychaete: (a) *Conticribra weissflogii* (© Friedel Hinz, AWI, Germany); (b) *Oxyrrhis marina* (© Liliana Gomez Luna, CNEA, Cuba).

Dinoflagellates

Marine dinoflagellates often display mixotrophy and are able to feed on both phytoplankton and heterotrophically. Some species are toxic and can produce harmful algal blooms (HABs), which can impact water quality (Smayda, 1997). *Oxyrrhis marina* (Dujardin, 1841) (Fig. 3b) is a common heterotrophic dinoflagellate said to be representative of many marine planktonic protists (Roberts at al., 2011). It is considered a cosmopolitan species, though the lack of studies on its geographic may overlook genetic differences between locations (Watts et al., 2011 and references therein). This dinoflagellate is omnivorous and feeds by engulfing its prey, which range from dissolved matter to bacteria, small algae and organisms as large as itself (Hantzsche and Boersma, 2010). It has been shown that, although food quality does not influence dietary uptake in *O. marina*, it can negatively affect growth in cases of phosphorus (P) limitation (Hantzsche and Boersma, 2010). *Oxyrrhis marina* has been routinely used in laboratory experiments for over a century (Montagnes et al., 2011), including for feeding experiments with copepods (Boersma et al., 2016).

1.2 NUTRITIONAL ECOLOGY

The transfer of energy from one trophic level to the next is the most basic process in food web dynamics (Lindeman, 1942). At first, studies on the interactions between the environment and the nutrition of an organism were conducted based on single currencies, such as energy and its transfer through trophic levels in ecosystems (Lindeman, 1942). With the development of the fields of trophic and nutritional ecology, it became clear that individual fitness and ecosystem dynamics, a. o., can be better explained by considering the transfer of multiple elements and nutrients between prey and predator (Sperfeld et al., 2017).

Stoichiometry is the branch of science concerned with the application of the Law of definite proportions and conservation of mass, i.e., with the patterns that can be observed in the proportions of elements or molecules in the reactants and products of reactions. Chemical stoichiometry defines and limits quantitatively how reactants will combine and how the combinations interact with one another. Ecological stoichiometry (ES) sets similar restrictions to interacting biological systems, which are further combined with limitations imposed by the conservation of matter (Sterner and Elser, 2002). In doing so, it provides a framework under which ecologists can establish links between food webs, ecosystem metabolism, and biogeochemistry. There are several other fields for which ES can have practical applications, such as biotechnology (quantifying biomass production and yield), waste removal systems, and livestock production (determining diet for optimum growth) (Sterner and Elser, 2002).

The availability of nutrients, together with light and seawater temperature, can dictate processes within the phytoplankton on the individual (in terms of elemental and biochemical composition) and community (composition, abundance, and dynamics) levels (Harrison et al., 1990; Klein Breteler et al., 2005; Reitan et al., 1994; Wiltshire and Manly, 2004; Wiltshire et al., 2008). The quality and abundance of the phytoplankton will, in turn, influence the developmental time and adult size of several animal species (Evans, 1981). It will also affect mesozooplankton production (Klein Breteler et al., 2005; Malzahn et al., 2010; Nejstgaard et al., 2001; Thor et al., 2007), which itself can determine the recruitment success of organisms in higher trophic levels (Beaugrand et al., 2003; Black et al., 2016; Malzahn et al., 2007).

1.2.1 Effects of nutrient limitation on prey quality

The nutritional value of food particles can be determined by several factors, such as its elemental and biochemical compositions and digestion resistance (Sterner and Schulz, 1998). The availability of limiting nutrients to algal growth can influence the transition between exponential (non-nutrient limited) and stationary (nutrient limited) growth phases. Under nutrient-limiting conditions, green algae will show an increase in cell wall thickness, a defensive mechanism against herbivore grazing when algal growth rates are low and which renders them a poor quality food source for predators (Van Donk and Hessen, 1993, 1995).

When algae have all the resources necessary for photosynthesis, C is allocated to lipid synthesis, growth and cell division (Kattner et al., 1983; Parrish and Wangersky, 1990). Lipids have the highest energetic content (39 kJ g^{-1}) among the major organic compounds in organisms (~ 24 and 17 kJ g^{-1} for carbohydrates and proteins, respectively) (Gentsch et al., 2009). They have several functions, such as energy storage (triacylglycerols (TAG), wax esters, phospholipids, and diacylglycerol ethers), components of biomembranes (cholesterol, phospholipids), as regulatory hormones (ecdysone), antioxidants (tocopherol, pigments), and buoyancy (neutral lipids) (Lee et al., 2006). Under exponential growth, over 50% of the total lipid content may be (n-3) polyunsaturated fatty acids (PUFA) (Falk-Petersen et al., 1998). Under stationary growth, however, algae may become deficient in FAs (Weers and Gulati, 1997), but will most likely accumulate C as TAG (Bromke et al., 2015; Henderson et al., 1998; Kattner et al., 1983; Parrish and Wangersky, 1990), which are richer in saturated (SFA) and monounsaturated (MUFA) fatty acids (Reitan et al., 1994; Roessler, 1990). TAGs, which are formed by a glycerol backbone esterified with 3 FAs, are the main type of storage lipids in animals, and provide the energy necessary for locomotion and vertical migration, feeding-related processes (or survival through starvation), and reproduction (Lee et al., 2006).

1.2.2 Effects of prey quality on predator performance

Ecological stoichiometry attempts to explain the balance of energy and materials between prey and predator in terms of their elemental composition, which can also be considered as a proxy for biochemical composition (Sterner and Elser, 2002). As heterotrophs cannot synthesize or transform elements, they must obtain them from their diet in the necessary quantity in order to

meet their own metabolic requirements (Anderson et al., 2004). Biomass formation and energy supply are usually demands met by C, whereas nutrient elements primarily fulfill a structural role (Anderson et al., 2004). ES often relates the content of an element to that of C, and predicts that, if present in limiting quantities, this element will be used with the highest efficiency (Kuijper et al., 2004; Anderson et al., 2005). It also characterizes consumers in terms of their ability (or lack thereof) to handle nutrient limitation in their prey (homeostasis) and to buffer such effects for higher trophic levels (Sterner and Elser, 2002). In general, autotrophs display a wider range of C:nutrient ratios, whereas marine zooplankton have a narrower range and must cope with excess elements in their diet (Sterner and Elser, 2002). Homeostatic regulation requires the use of extra energy, and stoichiometric imbalances between prey and predator will greatly affect the efficiency with which the latter grows (Sterner and Elser, 2002). In summary, food quantity and quality can influence the physiology of copepods, their efficiency in nutrient uptake, and their ability to produce/metabolize energy (Ambler, 1986; Guisande et al., 2000; Hessen and Anderson, 2008; Jónasdóttir et al., 1995, 2009; Møller, 2007; Peterson and Kimmerer, 1994; Thor and Wendt, 2010).

Temora longicornis has been reported to selectively graze according to its physiological requirements, choosing its prey by their biochemical composition and nutritional value (Cotonnec et al., 2001). Although knowledge on the feeding ecology of calanoids is broad, information on how they react to different food regimes in nature, i.e., before, during, and after blooms, is scarce (Gentsch et al., 2009). It has been pointed out that *T. longicornis* prefers to feed on *Conticriba* spp. in the field (Bakker and van Rijswijk, 1987; Evans, 1981) and that, when algae are not abundantly available, it may prey on heterotrophic species to satisfy its metabolic needs (Gentsch et al, 2009). It has been shown, however, that dinoflagellates are a food source of higher nutritional value to copepods than diatoms, even under N-limitation scenarios, a common situation during the late spring bloom (Jones and Flynn, 2005). Furthermore, certain diatom species can inhibit copepod growth and reproduction (Paffenhöfer et al., 2005), further lowering the quality of such food source.

Although certain studies have addressed the effects of food limitation (in terms of quantity) on polychaete larvae (Almeda et al., 2009; Hansen, 1999; Pedersen et al., 2010), a gap exists in the literature concerning how these meroplanktonic organisms are affected by food quality. No

information is available on the effect of either food quantity or quality for the larvae of *Lanice conchilega*.

1.2.3 Measuring predator performance

Estimation of vital rates

The measurement of vital rates can provide information on the efficiency with which food is utilized and the costs of maintenance of an individual (Almeda et al., 2011). According to the First Law of Thermodynamics, all the energy that is ingested by an organism must equal the sum of energy that is absorbed or lost to the environment. The biological processes which define how energy is absorbed or lost are species-specific and also vary with sex and life stage of an organism. In the case of adult females of copepod species, it would be possible to devise an energy budget by measuring ingestion, respiration, excretion, faecal pellet (FP) production, somatic growth, and egg production rates (Fig. 4). Although performance entails the amount of energy ingested and used by an individual for all these vital activities, the development and continuity of a population is initially constrained by its ability to transform dietary intake into viable offspring (Wendt and Thor, 2015). Performance is, thus, ultimately defined by what is invested in reproduction and how successful this process is. Each of these processes has been extensively documented in copepod species (e.g., Castellani and Altunbas, 2014; Hirst and Bunker, 2003; Ikeda et al., 2001; Jónasdóttir et al., 1995, 1998, 2009; Mayzaud, 1976; Miller and Glibert, 1998; Møller et al., 2003; Thor, 2003), but on few occasions have ingestion, respiration, excretion, egestion, and reproduction been estimated altogether (Debs, 1984; Kiørboe et al., 1985; Lee and Yan, 1994) or in consideration of nutrient unbalances of their food source (Malzahn and Boersma, 2012; Nobili et al., 2013; Siuda and Dam, 2010). Far few studies on the topic have been conducted with polychaetes (Bühr, 1976; De Smet et a., 2016; McHugh, 1993; Ropert and Goulletquer, 2000), and to date no information is available for the larval stage of *L. conchilega*.

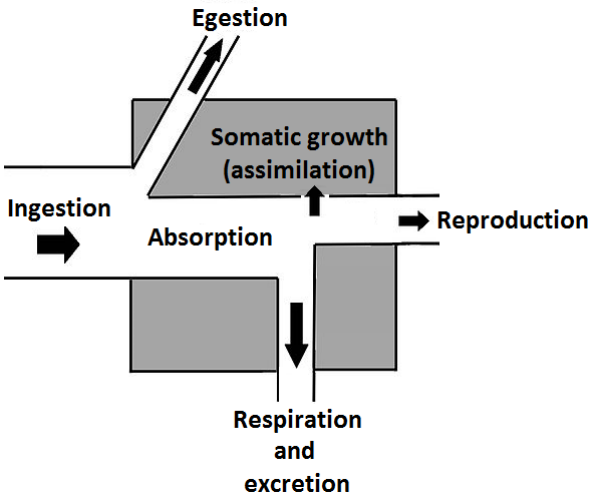

Figure 4: A simplified diagram with the source (food ingestion) and sinks (egestion, respiration, excretion, reproduction and somatic growth) of energy in an individual.

Estimation of carbon assimilation

Feeding experiments and gut content analyses, although widely used to study trophic ecology in marine ecosystems, can only reveal information on short-term feeding patterns of organisms (Dalsgaard et al., 2003; Gentsch et al., 2009 and references therein). A long-term record of feeding can, for example, be accessed by investigating the patterns in lipid deposition within consumer tissues (Dalsgaard et al., 2003). Fatty acid trophic markers (FATM) are compounds that would ideally (a) have a distinctive origin, which can be easily traced; (b) be inert and harmless to organisms; (c) not be selectively taken up and incorporated; and (d) be metabolically stable, thus being qualitatively and quantitatively transferred to the next trophic level (Dalsgaard et al., 2003). FATM are usually assimilated by consumers in a conservative manner (Graeve et al., 1994; Lee et al., 1971, 2006), though this assumption has only been investigated in holoplanktonic organisms with intermediate to high lipid contents. FATM can also provide information on the feeding ecology of organisms on short and long time scales (Dalsgaard et al., 2003). Although no FA can be uniquely found in any single species, some phytoplankton taxonomic classes can be identified based on common features and combinations (presence and/or absence) of certain FA groups (Ackman et al., 1968). 16:1(n-7), 16:2(n-4), 16:3(n-4), and 20:5(n-3) (EPA), for example, are considered to be diatom FATM, whereas 18:4(n-3) and DHA

are considered dinoflagellate FATM (Boissonnot et al., 2016; Braeckman et al., 2012; Dalsgaard et al., 2003; Falk-Petersen et al., 1998; Graeve et al., 2005).

Essential qualitative and quantitative information on pathways of energy flow between prey and predator can be obtained via feeding experiments which combine the use of trophic markers and stable isotopes. Whereas bulk stable isotope analysis (BSIA) can only determine the isotopic composition of a complex mixture, compound-specific stable isotope analysis (CSIA) is a methodology that is able to measure the isotopic composition of individual compounds within a mixture, such as fatty acids (FA) and amino acids (AA) (for a review on CSIA see Meier-Augenstein, 2002). CSIA is a powerful tool for tracing organic matter origin and fate and to investigate biochemical processes and patterns in individuals and ecosystems (e.g., Guilini et al., 2010; Rix et al., 2018; Wegener et al., 2012), and has been applied in research on a variety of fields such as archeology, ecology, and pollution (Evershed et al., 2007). It first became available with the commercial production of the gas chromatograph/combustion/isotope ratio mass spectrometer (GC-c-IRMS) in the early 90's, and has since been recognized by its potential applications (Evershed et al., 2007; Lichtfouse, 2000; Newton, 2010). FAs have been used over the past 40 years as an ecological tool to study topics that range from individual nutrition and metabolism to ecosystem structure and trophic ecology scale (Budge et al., 2006). When FA determination is coupled to measurements of isotopic composition, quantitative estimates can be obtained for the transfer of dietary C from prey to predator and for FA-specific assimilation and potential biosynthesis and bioconversion (Boissonnot et al., 2016; De Troch et al., 2012; Graeve et al., 2005; Kluijver et al., 2013; Middelburg et al., 2000). Unfortunately, FA dynamics in marine invertebrates have been mostly characterized for larger, often herbivorous, calanoid copepods, whereas few studies are available on the lipid biochemistry of smaller copepods (Boissonnot et al., 2016; Dalsgaard et al., 2003; Fraser et al., 1989) or of polychaetes (Braeckman et al., 2012).

1.3 FOOD REGIME SHIFTS IN THE NORTH SEA AND AROUND HELGOLAND

Climate change has the potential to directly and indirectly impact entire food webs. Seawater temperature, together with light and nutrient availability, dictate the phytoplankton composition

and dynamics, and phytoplankton production can affect the recruitment success of zooplankton. The planktonic system has been well studied in the North Sea over the past 60 years (Edwards et al., 2002; Reid et al., 2003; Beaugrand, 2004), and several plankton regime shifts have been observed in relation to temperature (Alvarez-Fernandez et al. 2012). Three water temperature shifts have been recorded in the North Sea in the past 50 years. In the late 70's a cold event was related to air-sea exchange processes, which are dependent on the atmospheric circulation in the North Atlantic (Becker & Pauly, 1996). As a result, diatom blooms were smaller and delayed in time, and individuals from species that were previously common in early blooms and/or dominant in that system, such as dinoflagellates and meroplankton, were present in lower numbers or absent altogether (Becker & Pauly, 1996; Edwards et al., 2002). Two warmer periods were then in the late 80's and early 2000s (Payne et al., 2009). The episode in the 80's caused a shift in the pelagic ecosystem of the Northeast Atlantic towards a warmer dynamic regime, which was characterized by higher phytoplankton abundance in the central North Sea, especially during winter and summer months, an extended phytoplankton season, and a change in the structure of the zooplankton community, with the takeover of warm-water copepod and fish species (Reid et al., 2001, 2003). Recent analyses of continuous plankton recorder data from the entire North Sea have also detected a decrease in dinoflagellate abundance over the last decade (Alvarez-Fernandez et al., 2012). These alterations in climate regimes have the potential to reorganize trophodynamic relationships and to induce changes in community composition and dominance (Alheit and Niquen, 2004; Alheit et al., 2005). It would seem, thus, that zooplankton organisms feeding primarily on these prey items have to cope with severe changes in timing of occurrence (match/mismatch), and in quantity and quality of their food items.

Considerable change in the physical and biological components of ecosystems in the southern North Sea have also been recorded in the past decades (e.g., Alheit et al., 2005; Beaugrand et al., 2003; Edwards et al., 2002; Reid et al., 2003; Wiltshire et al., 2008). The long-term monitoring data from Helgoland has shown (a) increases in water temperature (~ 1.2^{o}C between 1979 and 2011), light penetration, and wind speeds; (b) decreases in P and nitrogen (N) loading (from 25.32 to 13.06 mol N L^{-1} and from 0.83 to 0.56 mol P L^{-1} between 1979 and 2011) and in dinoflagellate abundances (3.82 to 2.14 $*10^{9}$ μm^{3} L^{-1} from 1979 until 2011); and (c) a temperature-related change in copepod phenology (Boersma et al., 2015; Schlüter et al., 2010; Wiltshire and Boersma, 2016). Nutrient availability in the North Sea will become even more

dependent of influx from the North Atlantic in the future (Boersma et al., 2015), and will thus have an even greater impact on the system dynamics than it currently does. Changes in all of these variables make it difficult to evaluate how the zooplankton will react to future prey composition changes in Helgoland. If the availability (or lack thereof) of nutrients for algae leads to changing phytoplankton elemental composition and community structure, copepod populations and production could also be affected, and major consequences could be expected for food webs, ranging from nutrient regeneration to fisheries production (Beaugrand et al.., 2003; Boersma et al., 2015; Jones and Flynn, 2005; Malzahn and Boersma, 2012; Nobili et al., 2013). The scale of the effects and feedback mechanisms of climate change in the southern North Sea are yet to be clarified and merit further investigation. The mechanisms which regulate zooplankton community diversity and secondary production can only be understood in light of zooplankton physiology, and how it is directly and indirectly affected by environmental conditions and changes therein (Castellani and Altunbas, 2014).

1.4 OBJECTIVES AND THESIS OUTLINE

Climate change has become a major topic of discussion in present society given its impact on ecosystems and economies. Changes have already been recorded for several biotic and abiotic parameters of marine ecosystems, such as water temperature, nutrient loading, light penetration, wind patterns, and phenologies of phytoplankton and zooplankton. It would be difficult enough to predict the overall effect to an ecosystem of a change in one of these parameters, let alone understanding the synergetic reactions caused by changes in a suite of parameters. This multiple change scenario is the case in the area monitored by the Helgoland Roads time-series, and is most likely also the case elsewhere. Nevertheless local studies can help to understand these reactions by separately investigating components of an ecosystem and testing possible change scenarios. These, in turn, can be used to understand, predict, and possibly remedy, overall ecosystem response in the face of climate change.

The zooplankton is responsible for the transfer of energy originated from the photosynthesis of unicellular algae to higher trophic levels. They are the foremost consumers of phytoplankton, and serve as food for invertebrates, fish, and baleen whales, which makes them an important

trophic level on the food web. They also play a role in the biogeochemical cycles of C, N, and P, though their part in it has yet to be better understood. Within the zooplankton, copepods are one of the most significant secondary producers, given their worldwide distribution, abundance, and dominance. Due to their rapid biomass turnover and low capacity to accumulate lipid reserves, most copepods rely on continuous food supply (Helland et al., 2003; Kreibich et al., 2008, 2011). Their growth and reproductive activity is, therefore, closely linked to bloom events (Ianora, 1998; Halsband and Hirche, 2001; Halsband-Lenk et al., 2004). Meroplanktonic larvae may occur in high abundances on a seasonal basis (Fransz et al., 1991), and they also have restricted capacity to sustain food limitation. Changes in phytoplankton availability and nutritional quality thus directly influence zooplankton metabolism and lipid dynamics.

The rate with which physiological processes such as ingestion and respiration occur in zooplankton organisms will vary according to parameters such as temperature, but also in relation to how their nutritional requirements are met by the food items ingested. Furthermore, the various members of the zooplankton have different lipid requirements, and will display varying ability to store lipids as energy reserves. A change in the food available to zooplankters could, thus, hinder their ability to fulfill their nutritional requirements, and the adaptive capacity of these organisms to a shifting food regime in the North Sea will likely be determined by the effects of food quality (a.o.) on their physiology. Understanding such effects is a pressing issue given the gap in the literature regarding how species cope with inadequate food supply, especially during reproductive periods (Lee et al., 2006). In an attempt to fill this gaps, this thesis consists of studies on the effects of prey quality on the performance of two members of the zooplankton community at Helgoland, adult females of the calanoid copepod *Temora longicornis* and meroplanktonic larvae from the polychaete *Lanice conchilega*. The former represents holoplanktonic organisms which have intermediate levels of total lipid contents and the latter is a proxy for lipid-poor meroplanktonic organisms, which likely have different lipid requirements and, thus, assimilation strategies. The decrease in nutrient loading in the area surrounding Helgoland was reproduced in the experiments as a limitation in the availability of N for the prey species with which the study species were fed. Although N is a limiting nutrient for primary production in the marine environment (Anderson et al., 2017; Litchman et al., 2007) it does not receive much attention as a limiting nutrient to zooplankton (Sterner and Schulz, 2002). In the context of this PhD, the effects of N-limitation were considered worthy of investigation in the

experiments due to the high N content (and thus demand for) in adult copepods (Hessen, 1992). Prey choice for feeding the copepod and polychaete larvae took into account species which they feed on in their natural habitat which can also be easily cultured.

The major working hypothesis investigated in this thesis is that predators (copepods or polychaetes) will exhibit the best possible physiological performance when feeding on a diet whose elemental and biochemical compositions more closely resemble their own. Chapter-specific hypotheses and how they were addressed in this PhD are described below.

Chapter 1: copepods feeding on a diet with a molar C:N ratio close to their own body composition will have the highest possible growth and reproduction rates and the lowest possible egestion, respiration and excretion rates.

The Ecological Stoichiometry framework proposes that the efficiency with which a consumer utilizes the food it ingests is higher when it feeds on prey with an elemental composition similar to its own. A vast body of literature is available on the functional responses of calanoid copepods to diet quality, but few address all important physiological rates at once. A comprehensive approach should concomitantly estimate metabolism, feeding, growth and reproduction and, thus, assess the energy allocation to different processes in relation to food quality. Chapter 1 presents rates of ingestion, respiration, excretion, egestion, growth, and reproduction for adult females of the copepod *Temora longicornis* fed with different diets, and an analysis of its overall performance in relation to food quality. Quality was herein defined by the elemental composition of prey species, i.e., their molar C:N ratio, and by prey type (diatoms and dinoflagellates).

Chapter 2: how does prey nutritional quality affect the biochemical composition, the dynamics of lipid utilization and assimilation, and the reproductive output of copepods?

A gap in knowledge still exists on the role and importance of lipids in marine zooplankton, and on how species cope with inadequate food supply, especially during reproductive periods (Lee, Hagen, and Kattner, 2006). FA dynamics in marine invertebrates have been mostly characterized for larger calanoid copepods from polar regions, whereas few studies are available on the lipid

biochemistry of smaller copepod species (Boissonnot et al., 2016; Dalsgaard et al., 2003; Fraser et al., 1989). Chapter II aimed at describing qualitatively and quantitatively how the nutritional quality (biochemical and elemental composition) of prey grown under different nutrient regimes affects the biochemical composition, the dynamics of lipid utilization and assimilation, and the reproductive output by *T. longicornis* females. In order to obtain data on lipid dynamics and on reproductive output, copepods were fed with prey cultures previously labelled with ^{13}C, which enabled the calculation of egg production rates, the monitoring of lipid C transfer between prey and predator, and the calculation of FA-specific C assimilation and turnover rates.

Chapter 3: how is lipid C assimilated in the larvae of polychaetes? Can the concept of FATM be applied to the study of lipid dynamics in these lipid-poor meroplanktonic larvae?

The first goal of Chapter 3 was to obtain basic knowledge on the. Experiments investigating FA-related C assimilation have been conducted mostly for holoplanktonic organisms with intermediate to high lipid contents, whose FA profile tends to reflect that of the food items it ingests. Little is known, however, about how meroplanktonic organisms, which are usually lipid-poor, incorporate dietary FAs. It is possible that the lipid content and, thus, requirement, of an organism can dictate whether it will assimilate dietary FAs in an unmodified manner or not. One of the objectives of Chapter 3 was thus to test the hypothesis that the concept of FATM can also be applied in a feeding study with lipid-poor meroplanktonic larvae. Basic knowledge on the feeding ecology of *L. conchilega* larvae is scarcely available, so the other objective of Chapter 3 was to provide information on lipid C assimilation in these organisms.

2 MATERIALS AND METHODS

2.1 SAMPLING AREA – THE NORTH SEA AND THE ISLAND OF HELGOLAND

The North Sea (Fig. 5) is bordered by England, France, Belgium, Holland, Germany, Denmark, Sweden, and Norway. It receives low-salinity discharges from rivers and from the Kattegat sea, and opens broadly to the North Atlantic (Ducrotoy et al., 2000). This large (750 000 km^2), semi-enclosed body of water supports one of the world's most active fisheries (IMM, 1997). The North Sea can be divided into four key areas. The northern North Sea (0-500 m depth) is strongly influenced by oceanic inflow and is mostly stratified in the summer. The southern North Sea (0-50 m depth) receives a strong riverine discharge and has a well-mixed depth layer (ICES, 2016). The English Channel is adjacent to the southern North Sea and opens to the Atlantic Ocean. The Skagerrak and Kattegat region links the North Sea to the Baltic Sea (ICES, 2016).

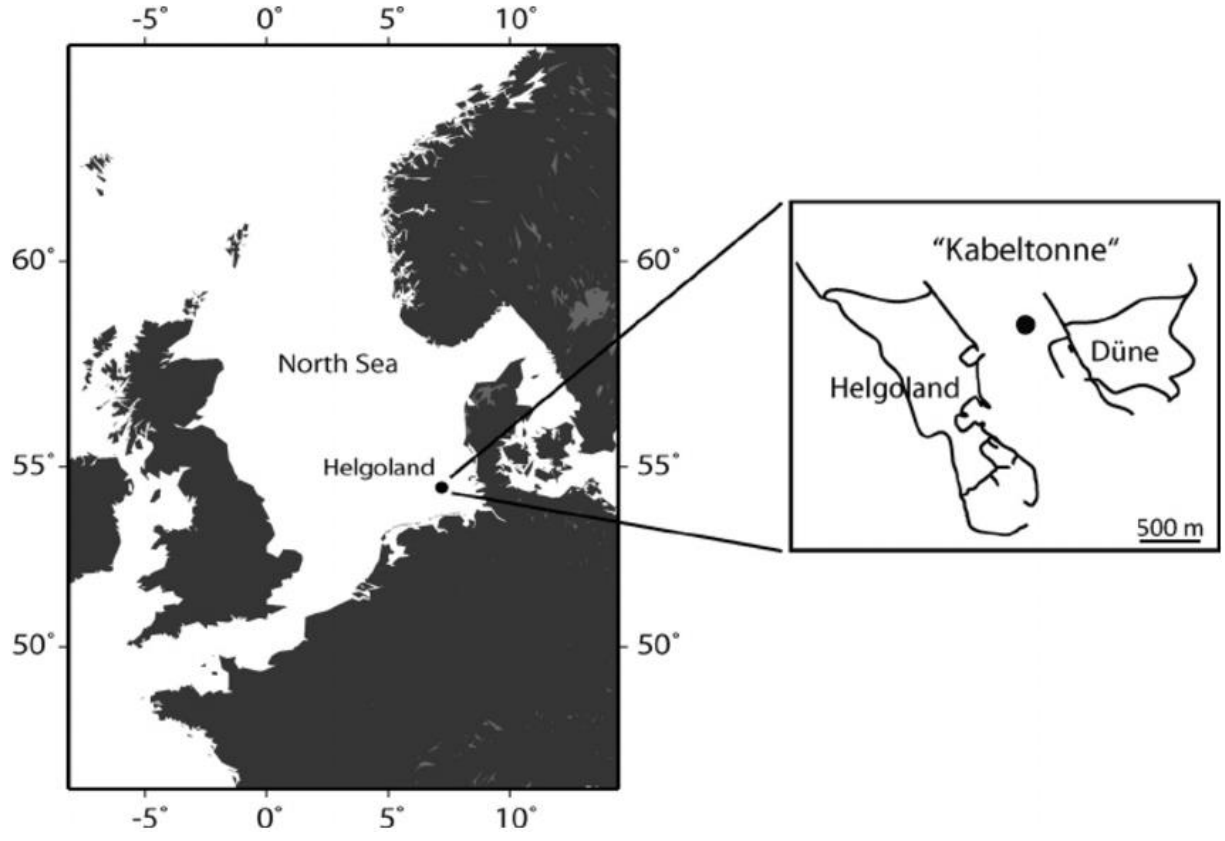

Figure 5: Location of Helgoland, a German island in the southern part of the North Sea. The dot between Helgoland and Düne, named "Kabeltonne", indicates the area where sampling for the Helgoland Roads time-series programme takes place. Source: Pizzetti et al., 2011.

The North Sea is an area with intensive human activities, such as fishing (Fig. 6), shipping, wind farms, aggregate (sand and gravel) extraction and oil and gas production (ICES, 2016). Its food web is characterized by high primary production, which in turn is consumed by the zooplankton and benthos, and passed on to higher trophic levels such as birds, fish, and mammals (ICES, 2016; Fig. 6). It can be currently considered as perturbed, as the larger fish species which used to be the major predators have been depleted by the fishing industry and are now absent or present in reduced numbers in the North Sea (ICES, 2016).

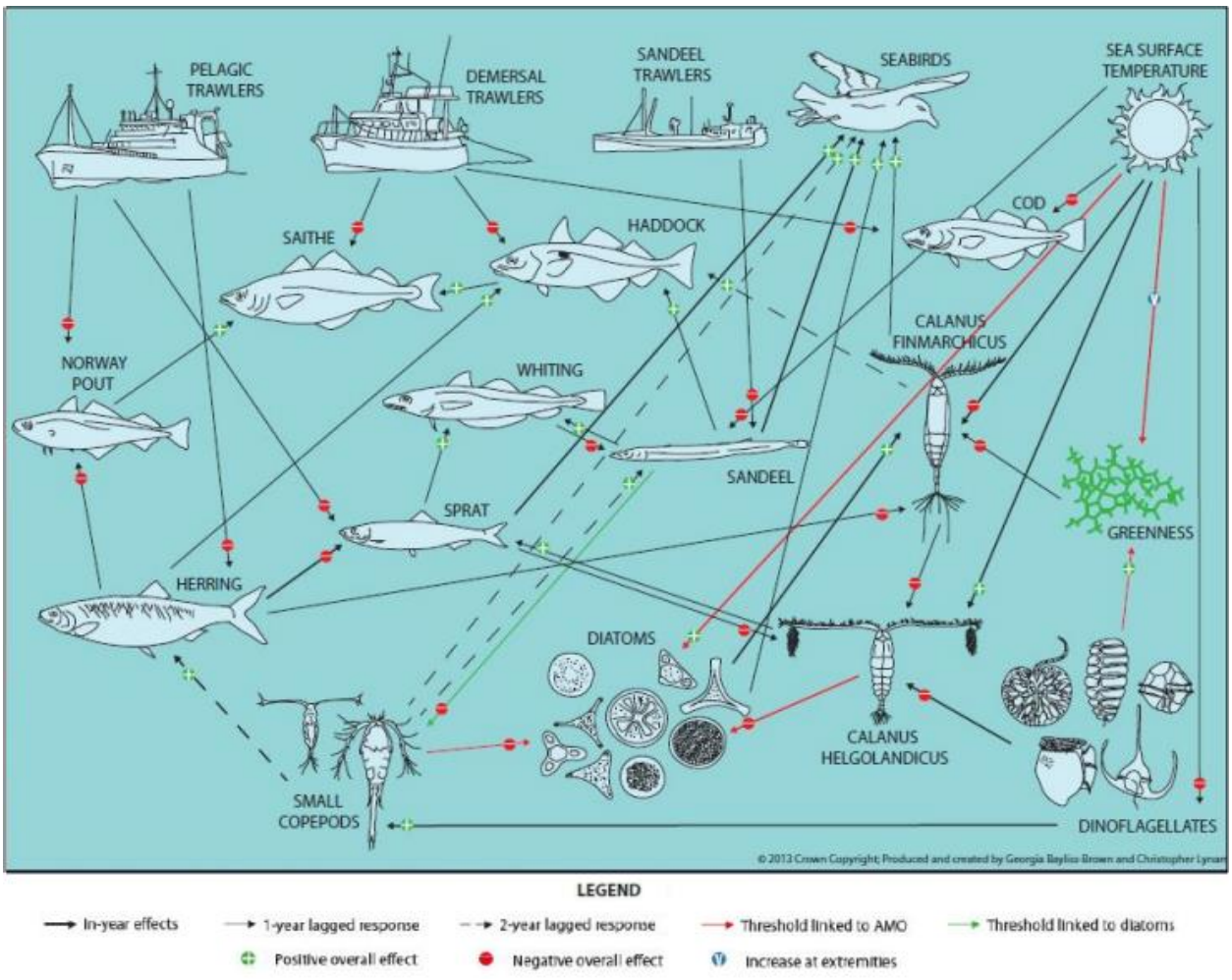

Figure 6: Major components of the North Sea food web and significant interactions with fishing fleets, as modelled with statistical tGAMs. Source: modified from ICES, 2016.

The island of Helgoland is located in the German Bight, in the southern North Sea (Fig. 5). It lies approximately 60km from the mainland and is at the mouth of the River Elbe. Being home to a

rich fauna and flora, Helgoland caught the attention of German biologists in the mid-19th century. The area surrounding Helgoland has been monitored since 1873 (Wiltshire and Manly, 2004), and in 1892 a marine laboratory (formerly Biologische Anstalt Helgoland (BAH), currently a part of the Alfred-Wegener-Institute für Polar- und Meeresforschung (AWI)) was established in the island (Fischer, 2013). It was only in 1962 that a long term monitoring programme was established to record pelagic data on phytoplankton species, salinity, nutrients, and water temperature and transparency (Hickel et al., 1993). The Helgoland Roads time-series, as it is known, is one of the longest datasets in the North Sea, and the one with the shortest time intervals, and is kept up by the BAH (Hickel et al., 1993; Wiltshire et al., 2015). Zooplankton, rocky shore macroalgae, macrozoobenthos and pelagic bacteria are also included in the current sampling scheme (Wiltshire et al., 2010 and references therein). Samples are collected at the site denominated Kabeltonne (54^{o}11' N, 07^{o}54' E), in the narrow channel between the main island of Helgoland and the small, sandy island of Düne nearby (Fig.5).

2.2 ZOOPLANKTON SAMPLING

Zooplankton samples were collected for experiments with *T. longicornis* females and with *L. conchilega* larvae by horizontal hauls with a 500-µm mesh-size CalCOFI net. Tows were conducted for 15 minutes at 5m depth at the Kabeltonne site off the German island of Helgoland (54°11′N, 07°54′E). The samples were then taken to the laboratory, where intact and active individuals were immediately sorted under an Olympus SZX16 stereoscopic microscope.

The quantity of *T. longicornis* females needed for the full experimental design required that the experiment be split into two parts. Samplings were performed as close in time as environmental conditions allowed on 17 (Experiment I) and 30 (Experiment II) May 2016. A total of 1300 females were sorted at each date (t_{0h}), 1080 for the experiments, 30 for analysis of *in situ* body C and N contents, 150 for determination of *in situ* lipid composition and FA-specific ^{13}C isotopic enrichment, and 40 were fixed in 4% formalin buffered with hexamethylenetetramine for measurement of prosome length (PL) under a Leica M205C. Adult male copepods were also sorted and added to the experiments for continuous fertilization of females.

Lanice conchilega larvae were sorted after the sampling on 6 June 2016. A total of 390 individuals were sorted, 30 for determination of *in situ* body C and N contents, 150 for determination of *in situ* lipid composition and FA-specific ^{13}C isotopic enrichment, and 210 for the feeding experiment.

2.3 PREY CULTURE

Three species were cultured in the laboratory, the diatom *C. weissflogii*, the heterotrophic dinoflagellate *O. marina*, and the cryptophycean *Rhodomonas salina* (Wislouch) (Hill and Wetherbee, 1989). The latter was used solely as food for *O. marina*, whereas the diatoms and dinoflagellates were used to feed copepods and polychaetes. Detailed descriptions on how these species were cultured and sampled are available in Chapter II.

A stock solution was maintained for each of the three species. New batch cultures were created daily for five consecutive days by diluting part of the stock solution with fresh nutrient-replete f/2 medium (after Guillard, 1975) or fresh N-depleted medium (f/2 without the addition of nitrate). Silicate was only added to the medium used for diatom culture. Diatom and cryptophycean cultures were directly labelled with ^{13}C by adding ^{13}C-enriched sodium bicarbonate ($NaH^{13}CO_3$) to the medium. *Oxyrrhis marina* was indirectly labelled with ^{13}C via daily feeding on labelled *R. salina*. Cultures were kept in a temperature-controlled room at 18^{o}C. *R. salina* and *C. weissflogii* were provided constant light, while *O. marina* was kept in the dark. Cultures were grown for 5 days and then used as food suspension for copepods during experiments. Cell densities were determined with a BD Accuri C6 Flow Cytometer. *C. weissflogii* and *O. marina* cultures were sampled (filtered) daily for determination of cell C, N, and lipid contents and of FA-specific ^{13}C isotopic enrichment. Filters for determination of prey C and N content were dried at 60^{o}C for 48 h, folded inside aluminum foil, and stored in a desiccator until analysis. Samples for FA analyses were placed into pre-combusted lipid vials and stored at -80^{o}C. The remaining volume of the cultures was then used in the feeding experiments at concentrations of $8*10^3$ and $2*10^3$ cells mL^{-1} for diatom and dinoflagellate diets, respectively.

2.4 EXPERIMENTAL DESIGNS

2.4.1 Feeding experiments with *T. longicornis* females

Experiments were initiated right after field sampling. Figure 7 depicts the distribution of different diet treatments, replication and the sampling scheme. Copepods from Experiment I were fed with diatoms cultured in nutrient-replete (Diat+) and N-depleted (Diat-) medium. In Experiment II the copepods were fed with dinoflagellates cultured with nutrient-replete (Dino+) and N-depleted (Dino-) food (*R. salina*, as described above). Prey were offered to copepods ad libitum (>350 µg C L^{-1}, $8*10^3$ diatom and $2*10^3$ dinoflagellate cells mL^{-1}) during the entire experiment. The only exceptions were the respiration and excretion incubations (described below), which were performed without any food.

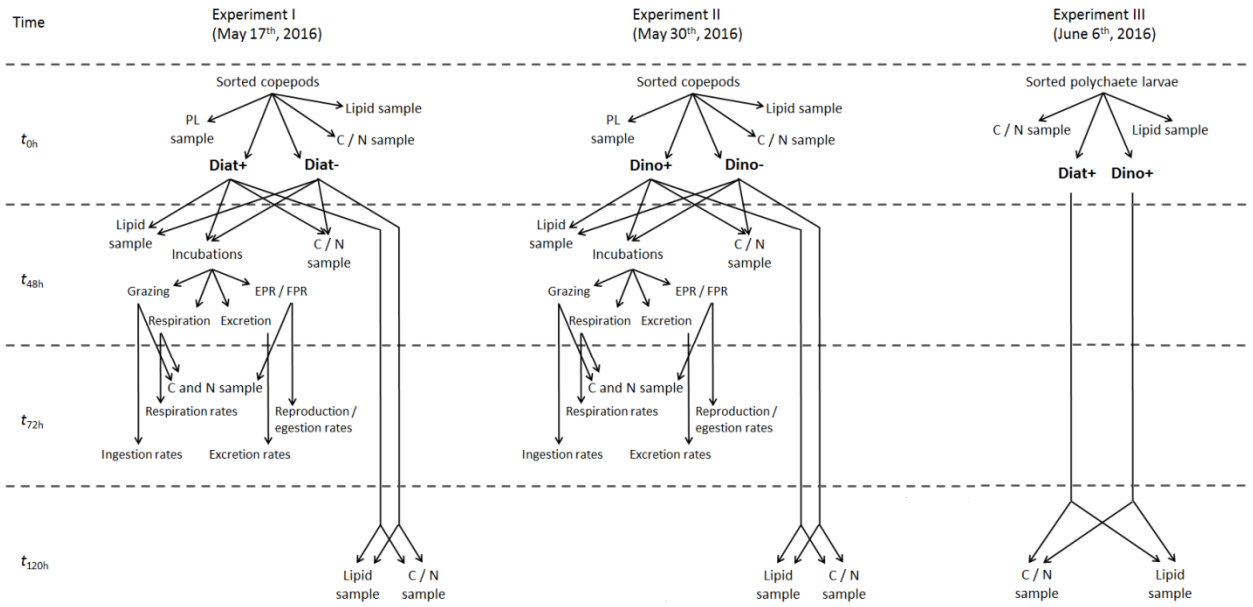

Figure 7: Experimental design used in the feeding experiments with females of the copepod *Temora longicornis* (Experiments I and II) and with larvae of the polychaete *Lanice conchilega* (Experiment III). All experiments took place immediately after zooplankton sampling. The experimental design was almost equal between Experiments I and II, except that the former was conducted with diet treatments (names in bold) consisting of diatoms cultured in nutrient-rich (Diat+) and N-limited (Diat-) conditions, whereas the latter was conducted with dinoflagellates fed with nutrient-rich (Dino+) and N-limited (Dino-) prey. Experiment III was conducted only with nutrient-rich diatom (Diat+) and dinoflagellate (Dino+) cultures. The timing of experimental phases in Experiments I and II (acclimation period, between t_{0h} and t_{48h}; incubations, between t_{48h} and t_{72h}; and remaining feeding time, between t_{48h} and t_{120h}) is shown in the left column. Experiment III consisted only of feeding time (between t_{0h} and t_{120h}). Copepods and

polychaete larvae were kept in triplicates. Sampling of copepods and polychaete larvae for carbon (C) and nitrogen (N) contents and for lipid composition, and of copepods for prosome length (PL) measurement, are included in the diagram, as are the estimations of vital rates for copepods. Source: modified from Franco-Santos et al., 2018 (Chapter I).

Copepods were incubated in groups of 180 females in partially filled 3 L plastic beakers (75 females L^{-1}) fitted with a 300 μm meshed-bottom cylinder to keep the copepods from feeding on eggs and fecal pellets (FP). Although the in situ temperature was different between Experiments I and II (10 and 12°C, respectively), all experiments were conducted in the same temperature, and copepods were kept in a dark temperature-controlled room at 10 ± 0.3°C. The water was gently stirred three times a day for food resuspension. Partial water exchanges (66%) were performed daily in order to remove eggs and FP and to renew copepod food, which was done immediately after exchanges. The copepods were acclimated under these conditions for 48 h. Subsequently, 60 females were sampled from each replicate, 10 individuals to determine body C and N contents and 50 for lipid composition and FA-specific ^{13}C isotopic enrichment (samples referred to as t_{48h}). Some of the remaining individuals were distributed among four experimental incubations in order to separately measure grazing, respiration, excretion and egg and FP production rates (methodologies described in more detail in Chapter I). These were then used to construct C and N budgets and to calculate C and N turnover and efficiencies for copepods. Experimental units were kept in the same temperature-controlled room as the beakers. The remaining copepods were kept in their respective beakers, under the same conditions as during the acclimation, and fed another three days before being sampled (referred to as t_{120h}) to determine body C and N contents, lipid composition, and FA-specific ^{13}C isotopic enrichment. Copepods were gently washed in distilled water, placed into either pre-weighed tin cartridges for body C and N content determination or pre-combusted lipid vials for FA analyses, and stored at -80°C until further analysis. Tin capsules with copepods were dried and stored in a desiccator until analysis.

Grazing

Ingestion rates were estimated based on changes in prey cell concentrations in the absence and presence of copepods (Frost, 1972). The prey cell concentrations at the beginning and end (after 24 h) of the grazing incubation were measured from bottles containing prey or prey+copepods.

At the end of the incubation copepods were sieved and sampled to measure body C and N contents; the food suspension was fixed immediately with 4% buffered formalin for cell densities determination with a BD Accuri C6 Flow Cytometer. Cell densities were used to calculate grazing rates as number of prey cells ingested per female and per day.

Respiration

The sealed chamber method was applied to measure oxygen consumption rates (Harris et al., 2000). The O_2 saturation of water was measured at the beginning and end (after 24 h) of the respiration incubation from bottles which contained aerated artificial seawater (ASW) or ASW+copepods. After the end of the incubation the copepods were sampled to measure body C and N contents. O_2 consumption rates were obtained per female and per day.

Excretion

The sealed chamber method was also used to calculate daily, individual excretion rates. Bottles with ASW and ASW+copepods were incubated for 24 h, after which their contents were filtered and immediately frozen at −20°C. The copepods were trapped inside the syringe filters and could not be sampled for C and N content analysis. Since both the excretion and the respiration incubations were conducted under similar conditions, the C and N contents of the copepods from the latter were also used for calculating the excretion rates. The equation from Miller and Glibert (1998) was used to calculate the total dissolved nitrogen (TDN) and the non-purgeable organic carbon (NPOC) content of filtrate samples.

Egg and faecal pellet production

For each diet treatment, females were placed individually in 12-well cell culture plates filled with filtered seawater and food suspension. The production of eggs and FP was checked under a binocular 1, 6, 12, 18 and 24 h after the beginning of the experiment. After eggs and feces were counted, they were carefully pipetted out of the well, at times being collected for determination of their C and N contents. Food was immediately replenished until the next count, and plates were left undisturbed. Copepods were sampled after 24 h for determination of body C and N contents. Individual egg production rates (EPR) were calculated as a daily estimate based on the production observed over 24 h. FP production rates (FPR) were also observed during 24 h, but

due to problems during the last 6 h period, only the data from the first 18 h of incubation were used to estimate daily rates. Filters with eggs and FPs were dried at 60°C for 48 h, folded inside aluminum foil, and stored in a desiccator until analysis.

Carbon and nitrogen budgets, turnover rates, and efficiencies

The amount of energy ingested by an organism should equal the sum of the amounts of energy egested and used for growth and metabolism. In that sense, the vital rates of an individual can also be expressed as a balanced equation, such that $I = G + R + U + E + F$, where I is ingestion, G is somatic growth, R is respiration, U is excretion, E is egg production, and F is FP production. In order to do so, the vital rates were converted to C and N units (µg C or N female^{-1} day^{-1}), as described in detail in Chapter I.

The budget data were further standardized to daily C and N turnover rates (% body C or N day^{-1}), as described in Chapter I. An arcsine square root transformation was then applied to the calculated percentage values. Given that there were negative values in the N growth term, and that the transformation is not possible for negative numbers, a fixed, minimum value was added to all treatments in that category to ensure values above zero.

Different physiological efficiencies were calculated to evaluate copepod performance in relation to food quality: absorption efficiency (AE_C and AE_N), the percentage of ingested C or N (respectively) absorbed in the gut of copepods; and net somatic growth efficiency ($NSGE_C$ and $NSGE_N$) and net egg production efficiency ($NEPE_C$ and $NEPE_N$), the percentage of absorbed C and N (respectively) that was converted into copepod somatic growth and into reproduction, respectively. The equations and a more detailed description of how efficiencies were calculated are given in Chapter I.

2.4.2 Feeding experiment with *L. conchilega* larvae

Experiments were initiated right after field sampling (Fig. 7). Polychaete larvae were kept for five days in triplicate 500 mL glass beakers fitted with a 300 µm meshed-bottom cylinder (140 ind L^{-1}). Only nutrient-replete cultures of *C. weissflogii* and of *O. marina* were used for this feeding experiment. The diatom and dinoflagellate food suspensions were provided on a daily

basis at a concentration of $8*10^3$ and $2*10^3$ cells mL^{-1}, respectively. Individuals were kept in a dark temperature-controlled room at the temperature recorded when they were sampled in the field, 13.5 ± 0.3°C. The beakers were gently stirred three times a day for food resuspension in the water. Partial water exchanges (66%) were performed daily and followed by the addition of new food suspension. At the end of the experiment the larvae were sampled from each replicate for body C and N content determination and for analysis of FA composition and FA-specific ^{13}C isotopic enrichment (10 and 50 individuals per sample, respectively). Individuals were gently washed in distilled water, placed into either pre-weighed tin cartridges (body C and N content determination) or pre-combusted lipid vials (FA analyses), and stored at -80°C until further analysis. Tin cartridges with larvae were dried at 60°C for 48 h and stored in a desiccator until analysis.

2.5 ANALYTICAL WORK

2.5.1 Carbon, nitrogen, and FA content analyses

The C and N contents of all copepod, prey culture, egg, and FP samples were measured with an elemental analyzer (detection limit: 2 µg C / 0.5 µg N; maximum error: ± 3%, Euro EA 3000, EuroVector S.P.A., Milan, Italy) using acetanilide as a standard.

The extraction and determination of fatty acid methyl esters (FAMEs) in the samples (according to Boissonnot et al., 2016) is described in detail in Chapter III. The chromatograms generated for the FAMEs were evaluated with the ChemStation software from Agilent. The A:B(n-X) shorthand notation was used to refer to FAs, where A is the number of carbon atoms, B is the number of double bonds, and (n-X) gives the position of the double bond closest to the terminal methyl group. Total lipid contents of zooplankton and prey cultures were calculated by adding the mass of all FAs in a sample.

2.5.2 Compound-specific stable isotope analysis (CSIA)

The FA-specific stable isotope composition of carbon in FAMEs extracted was obtained according to Boissonnot et al. (2016) with a Thermo gas chromatography combustion-isotope ratio mass spectrometry (GC-c-IRMS) system, equipped with a Trace GC Ultra gas

chromatograph, a GC Isolink, and a Delta V Plus isotope ratio mass spectrometer connected via a Conflo IV interface (Thermo Scientific Corporation, Bremen, Germany). The chromatograms containing peak areas and C isotope ratios were obtained with the (instrument-specific) software Isodat 3.0. The 14:0 and 18:0 FAME reference standards (Iowa University) with known δ-values were used for further calculations.

The equations used to calculate carbon assimilation (following Boissonnot et al., 2016) are described in detail in Chapter III.

2.5.3 Statistical analyses

Statistical analyses applied to results from the feeding experiments with *T. longicornis* females are described in Chapters I and II, and Chapter III contains the analyses regarding results from the feeding experiment with *L. conchilega* larvae.

Problems with the isotopic enrichment of nutrient-replete *O. marina* cultures in all the feeding experiments hindered the use of the data collected from these treatments for statistical analyses. In the case of *T. longicornis*, data for females fed with the Dino+ diet are presented, but are not included in some analyses, as described in Chapter II. For *L. conchilega*, analyses were only performed on data from the diatom cultures and from larvae fed with them, as described in Chapter III.

3 PUBLICATIONS

The following overview encompasses a publication as first author and another as co-author, and two additional chapters, to be published as first author. These constitute the main part of my PhD thesis. The minor part of the thesis consisted of similar experiments with crab zoeae in Helgoland and with copepods in the Arctic, and of isotopic labelling experiments with the three algae species. The project under which this PhD was developed was first idealized by PD Dr. Barbara Niehoff, Dr. Marleen De Troch, and PD. Dr. Holger Auel for the Doctoral Programme on Marine Ecosystem Health and Conservation - MARES. I conducted the entire laboratory work for chapters I, II, and III, and one of the experiments for chapter IV, at the BAH, in Helgoland. I processed all of my samples at the BAH and at the AWI in Bremerhaven (at the Polar Biological Oceanography and Ecological Chemistry sections). I conducted the data analyses for chapters I, II, and III at the AWI in Bremerhaven and at UGhent in Ghent, Belgium. My contribution (% total workload) for the elaboration of each chapter is also stated below.

CHAPTER I

Franco-Santos RM, Auel H, Boersma M, De Troch M, Meunier CL, Niehoff B

Bioenergetics of the copepod *Temora longicornis* under different nutrient regimes

Experimental concept and design: 70% - the experiments were mainly designed by me and BN, with valuable input from the co-authors.

Acquisition of experimental data: 100% - I gathered all the experimental data during laboratory experiments I performed at the BAH during April-June 2016.

Data analysis and interpretation: 75% - I processed and analyzed the experimental at the BAH, AWI, and UGhent, and BN and HA helped with interpretation.

Preparation of figures and tables: 90% - I prepared all figures and tables for the paper and all co-authors provided feedback and valuable input and suggestions.

Drafting of the manuscript: 75% - I wrote the manuscript and received scientific and editorial advice from all co-authors.

Journal of Plankton Research, volume 40(4), doi:10.1093/plankt/fby016, published in May 2018.

CHAPTER II

Franco-Santos RM, Auel H, Boersma M, De Troch M, Graeve M, Meunier CL, Niehoff B

Life history strategies of the copepod *Temora longicornis* as inferred from lipid carbon assimilation experiments

Experimental concept and design: 70% - the experiments were mainly designed by me and BN, with valuable input from the co-authors.

Acquisition of experimental data: 100% - I gathered all the experimental data during laboratory experiments I performed at the BAH during April-June 2016.

Data analysis and interpretation: 70% - I processed and analyzed the experimental at the BAH, AWI, and UGhent. All co-authors helped with interpretation.

Preparation of figures and tables: 90% - I prepared all figures and tables for the paper and all co-authors provided feedback and valuable input and suggestions.

Drafting of the manuscript: 75% - I wrote the manuscript and received scientific and editorial advice from all co-authors.

The manuscript is under preparation for submission to the journal Functional Ecology.

CHAPTER III

Franco-Santos RM, Auel H, Boersma M, De Troch M, Graeve M, Meunier CL, Niehoff B

Fatty acid bioconversion and homeostasis in the larvae of the sand mason worm *Lanice conchilega*

Experimental concept and design: 90% - the experiments were designed by me and BN.

Acquisition of experimental data: 100% - I gathered all the experimental data during laboratory experiments I performed at the BAH during April-June 2016.

Data analysis and interpretation: 80% - I processed and analyzed the experimental at the BAH, AWI, and UGhent, and HA helped with interpretation.

Preparation of figures and tables: 90% - I prepared all figures and tables for the paper and all co-authors provided feedback and valuable input and suggestions.

Drafting of the manuscript: 85% - I wrote the manuscript and received scientific and editorial advice from all co-authors.

The manuscript was submitted to PLoS ONE on October 31st 2018.

CHAPTER IV

Boersma M, Mathew KA, Niehoff B, Schoo KL, Franco-Santos RM, Meunier CL

Temperature driven changes in the diet preference of omnivorous copepods: no more meat when it's hot?

Experimental concept and design: 0% - the experiments were conceived by MB, BN, and KLS.

Acquisition of experimental data: 10% - I carried out one of the experiments of the study.

Preparation of figures and tables: 10% - I provided feedback on the tables and figures that were prepared for the paper.

Drafting of the manuscript: 10% - I provided scientific and editorial feedback to the drafts.

Ecology Letters, volume 19, doi: 10.1111/ele.12541, published in November 2015.

CHAPTER I

Bioenergetics of the copepod *Temora longicornis* under different nutrient regimes

Franco-Santos RM, Auel H, Boersma M, De Troch M, Meunier CL, Niehoff B

Published in Journal of Plankton Research, 40(4), doi:10.1093/plankt/fby016, in May 2018.

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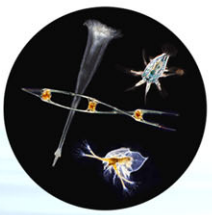

J. Plankton Res. (2018) 40(4): 420–435. First published online May 21, 2018 doi:10.1093/plankt/fby016

Bioenergetics of the copepod *Temora longicornis* under different nutrient regimes

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The copepod *Temora longicornis* depends on constant prey availability, but its performance also depends on how efficiently it utilizes its food sources. Our research goal was to understand copepod energy allocation in relation to diet quality. The working hypothesis was that *Temora* performs better on the diet whose elemental ratio is closest to its own. Diatoms (Diat) and dinoflagellates (Dino) cultured in nutrient-replete (+) and nitrogen-depleted (−) conditions were fed to the copepods. Ingestion, respiration, excretion and egg and fecal pellet production rates were measured. Carbon (C) and nitrogen (N) budgets were built to investigate differences in dietary C and N partitioning. Copepods fed nitrogen-depleted diatoms (Diat−), which had the most different C:N ratio to that of *Temora longicornis*, had high metabolic losses and low growth. Copepods fed nitrogen-rich dinoflagellates (Dino+) with a more similar C:N ratio to their own also had high metabolic losses, but displayed the highest investment into somatic growth and egg production. The results indicate that dinoflagellates are a better food source for *T. longicornis*. Furthermore, consumption of low-quality food leads to higher respiration rates and faster leakage of dissolved organic carbon from copepod fecal pellets; and egestion is a main pathway in copepods for eliminating unabsorbed and non-metabolized carbon.

KEYWORDS: bioenergetics; budget; carbon; nitrogen; stoichiometry

INTRODUCTION

Copepods are an important link in the energy transfer between primary producers and higher trophic levels, and contribute to the cycling of organic matter (Juul-Pedersen *et al.*, 2006; Castellani and Altunbas, 2014). The small calanoid copepod *Temora longicornis* (Müller, 1785) is one of the dominant species in the coastal

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zooplankton community in the North Atlantic and North Sea, reaching peak densities during the spring and early summer (Hickel, 1975; Castellani and Altunbas, 2014). Off Helgoland, this omnivorous species is abundant all year round and its grazing may have a substantial impact on the phytoplankton standing stock (Gentsch *et al.*, 2009; Maar *et al.*, 2004). *Temora longicornis* has high metabolic turnover rates, but is unable to accumulate significant amounts of energy reserves (Kreibich *et al.*, 2008, 2011). The species is, thus, dependent on a constant availability of prey and is vulnerable to fluctuations in food supply (Helland *et al.*, 2003; Kreibich *et al.*, 2008, 2011). This can be particularly problematic in systems such as the North Sea, where plankton community composition can change rapidly (Kiørboe and Nielsen, 1994). As *T. longicornis* inhabits dynamic systems, it must be able to quickly react to changes in trophic conditions (Gentsch *et al.*, 2009; Kreibich *et al.*, 2008), and efficient food utilization is of paramount importance for its survival.

Food quantity and quality influence the physiology of copepods, their efficiency in nutrient uptake, and their ability to both convert food into energy and channel stored energy into reproduction (Møller, 2007; Hessen and Anderson, 2008; Jónasdóttir *et al.*, 2009), and changes in these prey characteristics could impact not only copepod populations but also the recruitment of their predators (Boersma *et al.*, 2015). Zooplankton are often limited by food quantity in coastal regions (Hirst and Bunker, 2003), and some food sources are also of lower nutritional value (Sterner and Schulz, 1998). It has been suggested, for example, that protozoans are qualitatively important to copepod diet (Stoecker and Capuzzo, 1990), and that egg hatching success is dependent upon the ingestion of essential fatty acids (Broglio *et al.*, 2003). Elemental composition, digestion resistance and biochemical composition are important factors determining the nutritional value of food particles (Sterner and Schulz, 1998).

A vast body of literature is available on the functional responses of calanoids to diet quality (e.g. Dam and Lopes, 2003; Arendt *et al.*, 2005; Jónasdóttir *et al.*, 2009; Nobili *et al.*, 2013), but few address all important vital rates at once (e.g. Abou Debs, 1984). This comprehensive approach would allow for the assessment of energy allocation to different processes in copepods, and is necessary to come to a better understanding of the potential responses of *T. longicornis* to climate change-induced food regime shifts. The present work investigated metabolism, feeding, growth and reproduction in *T. longicornis* females in light of different prey elemental compositions/limitations. Based on the concepts of ecological stoichiometry, homeostasis and trophic upgrading (Klein Breteler *et al.*, 1999; Sterner and Elser, 2002; Malzahn *et al.*, 2010), specifically on how prey elemental composition affects consumer performance and energy utilization, the research goal was to investigate the partitioning of dietary carbon (C) and nitrogen (N) in relation to food quality. The working hypothesis was that copepods feeding on a diet with a C:N ratio close to their own body composition would perform best, i.e. would have the highest possible growth and reproduction rates and the lowest possible egestion, respiration and excretion rates. The opposite pattern would thus be observed for copepods feeding on prey with a C:N ratio as different from their own as possible (higher or lower).

METHOD

Field sampling

Zooplankton were collected by horizontal hauls with a 500-μm mesh-size CalCOFI net, which were conducted for 15 minutes at 5 m depth off the German island of Helgoland (54°11′N, 07°54′E), in the southern North Sea. The quantity of females needed for the full experimental design required that the experiment be split into two parts. Samplings were performed as close in time as environmental conditions allowed on 17 (Experiment I) and 30 (Experiment II) May 2016. The samples were taken to the laboratory, where intact and active adult females of *T. longicornis* were immediately sorted under an Olympus SZX16 stereoscopic microscope. A total of 1150 females were sorted at each date (t_{0h}), 1080 for the experiments, 30 for determination of *in situ* body C and N contents and another 40 were fixed in 4% formalin buffered with hexamethylenetetramine for measurement of prosome length (PL) under a Leica M205C (Fig. 1).

Prey culture

Three prey species were cultured in the laboratory, the cryptophycean *Rhodomonas salina* (Wislouch) (Hill and Wetherbee, 1989), the heterotrophic dinoflagellate *Oxyrrhis marina* (Dujardin, 1841) and the diatom *Conticribra weissflogii* (Grunow) (Stachura-Suchoples and Williams, 2009). *Rhodomonas salina* was used solely as food for *O. marina*, whereas the diatoms and dinoflagellates, which are both common prey for *T. longicornis* (Evans, 1981), were used to feed copepods.

A stock solution was maintained for each of the species. New cultures were created daily by diluting part of the stock solution with fresh medium. Two types of medium were used: nutrient-replete *f*/2 (after Guillard, 1975) and N-depleted (*f*/2 without the addition of

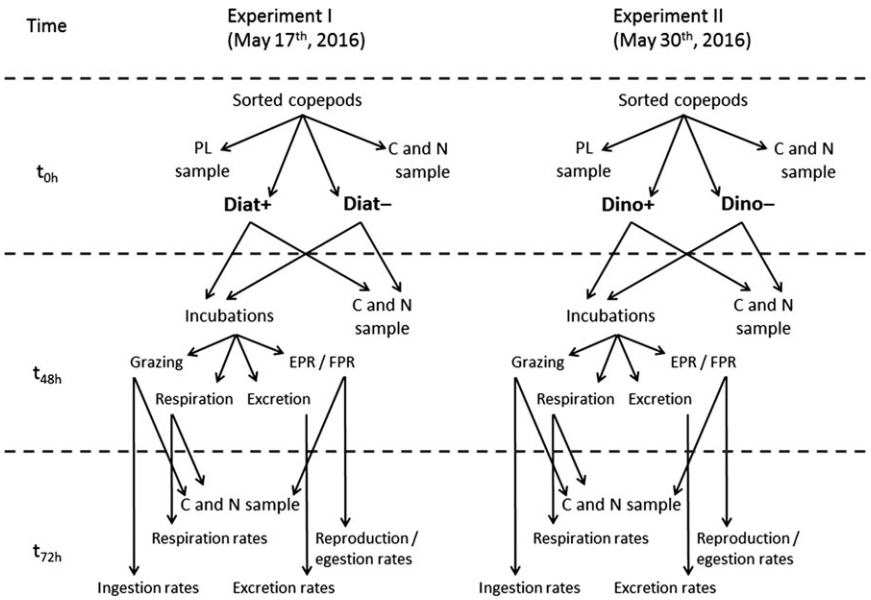

Fig. 1. Experimental design used in the present study with females of the copepod *Temora longicornis*. The two zooplankton sampling dates in May 2016 are indicated, after which Experiments I and II immediately took place. The experimental design was almost equal between these, except that the former was conducted with diet treatments (names in bold) consisting of diatoms cultured in nutrient-rich (Diat+) and N-limited (Diat-) conditions, whereas the latter was conducted with dinoflagellates fed with nutrient-rich (Dino+) and N-limited (Dino–) prey. The timing of experimental phases (acclimation period, between t_{0h} and t_{48h}, and incubations, between t_{48h} and t_{72h}) is shown in the left column. Copepods were acclimated and incubated in triplicates. Sampling of copepods for C and N analysis and PL measurement are included in the diagram, as are the estimations of vital rates.

nitrate). Silicate was only added to the medium used for diatoms. Cultures were kept in a temperature-controlled room at 18°C. *Rhodomonas salina* and *C. weissflogii* were provided constant light, while *O. marina* was kept in the dark. Aeration was provided to *R. salina*, and *C. weissflogii* and *O. marina* cultures were stirred twice a day to keep cells suspended. Cultures were grown for 5 days and then used as food suspension for copepods during experiments. Daily feeding of *O. marina* with *R. salina* was planned such that prey cells were depleted by the dinoflagellate on Day 5. Cell densities were determined with a BD Accuri C6 Flow Cytometer. *Conticribra weissflogii* and *O. marina* cultures were sampled daily for the determination of cell C and N contents by filtering known cell concentrations through Whatman GF/F filters. The remaining volume was then used to feed copepods.

Experimental design

Experiments were initiated right after field sampling. Figure 1 depicts the distribution of different diet treatments, replication and the sampling scheme. Copepods from Experiment I were fed diatoms cultured in nutrient-replete (Diat+) and N-depleted (Diat–) medium. In Experiment II, the copepods were fed with dinoflagellates cultured with nutrient-replete (Dino+) and N-depleted (Dino–) food. The effects of food quality on predators are more evident when food is abundant than when prey quantity is low (Sterner, 1997). In order to better observe the effects of food quality on the copepods, and to be able to distinguish them from the effects of food quantity, prey were offered to copepods *ad libitum* (>350 µg C L^{-1}, 8×10^3 diatom and 2×10^3 dinoflagellate cells mL^{-1}) during the entire experiment. The only exceptions were the respiration and excretion incubations, which were performed without any food, as described below. Copepod density varied in the different experimental units for practical reasons, such as the amount of copepods available for performing the experiments and the volumes of the different experimental units themselves. Visual observations of female behavior during the experiments and the results obtained indicate that these differences in density did not affect copepod feeding.

Copepods were incubated in groups of 180 females in partially filled 3 L plastic beakers (75 females L^{-1}) fitted with a 300 µm meshed-bottom cylinder to keep the copepods from feeding on eggs and fecal pellets (FP). Although the *in situ* temperature was different between Experiments I and II (10 and 12°C, respectively), all experiments were conducted in the same temperature, and copepods were kept in a dark temperature-controlled room at 10 ± 0.3°C. The water was gently stirred three times a day for food resuspension. Partial

water exchanges (66%) were performed daily in order to remove eggs and FP and to renew copepod food. The copepods were acclimated under these conditions for 48 h. Subsequently, 10 females were sampled from each replicate to determine body C and N contents (samples referred to as t_{48h}). The remaining individuals were distributed among four experimental incubations in order to separately measure grazing, respiration, excretion and egg and FP production rates. Experimental units were kept in the same temperature-controlled room as the beakers.

Grazing

Ingestion rates were estimated based on changes in prey cell concentrations in the absence and presence of copepods (Frost, 1972). The prey cell concentrations at the beginning and end of the grazing incubation were measured from triplicate 1 L glass bottles containing filtered seawater (FSW) + the respective food suspension ("start bottles") and FSW + food suspension + 10 copepods ("grazed bottles"), respectively. The start bottles were fixed immediately with 4% buffered formalin. Prey growth during the incubation was accounted for by creating another set of three 1 L glass bottles with FSW + food suspension ("control bottles"). The control and grazed bottles were attached onto a plankton wheel rotating with speed between 0.5 and 1 rpm for 24 h, after which their contents were fixed with formalin as described above. The copepods were retained in 300 µm mesh-sized sieves prior to fixation and sampled to measure body C and N contents. Cell densities of the preserved food suspension were determined within 6 to 8 days of fixation with a BD Accuri C6 Flow Cytometer. These were used to calculate grazing rates as number of prey cells ingested per female and per day.

Respiration

The sealed chamber method was applied to measure oxygen consumption rates (Harris *et al.*, 2000) with a NTH oxygen microsensor (PreSens GmbH, Regensburg, Germany) connected to a 4-channel oxygen meter (Microx TX3, PreSens GmbH). Calibration of the microsensor was performed with aerated artificial seawater (ASW, salinity 32) as the 100% O_2 reference and with a saturated Na_2SO_3 solution as the 0% O_2 reference. In order to avoid complicated corrections for O_2 production or consumption by prey (Ikeda, 1976), this incubation was conducted without prey. For each treatment, ASW was added to six 60-mL Winkler bottles. Three of these were replicate control bottles and contained only ASW and the other three were replicates with ASW and 10 copepods each. Females were placed inside the bottles and allowed to acclimate for an hour with the lid open. The O_2 saturation of water was then measured, and the lid was closed. After 24 h, it was measured again, and the copepods were sampled to measure body C and N contents. O_2 consumption rates (under starvation conditions) were obtained per female and per day. The use of control bottles allowed for correction for potential oxygen consumption or production by microbes during incubation. Air pressure values for Helgoland were obtained from www.wetter.com.

Excretion

Daily, individual excretion rates were also calculated following the sealed chamber method, and the equation from Miller and Glibert (1998) was used to calculate the total dissolved nitrogen (TDN) and the non-purgeable organic carbon (NPOC) content of filtrate samples. Non-feeding conditions were also employed in this incubation in order to create a low background against which to measure and compare changes in dissolved organic carbon (DOC) and nitrogen (DON). For each treatment, triplicate 100-mL (pre-combusted) glass bottles were prepared with only ASW (controls) and with ASW + ca. 15 copepods. After 24 h of incubation, ASW and copepods were filtered through pre-cleaned (10% HCl) syringes fitted with Whatman GF/F w/GMF (0.7 µm pore size, 25 mm diameter) syringe filters. Filtrates were immediately frozen at −20°C in pre-cleaned high-density polyethylene bottles. DOC and TDN in the filtrate were determined by high temperature catalytic oxidation (HTCO) and subsequent non-dispersive infrared spectroscopy and chemiluminescence detection using a Shimadzu TOC-VCPN analyzer. In the autosampler, the samples (6.5 mL) were acidified with HCl and sparged with oxygen (100 mL min^{-1}) for 5 min to remove inorganic carbon. A 50-µL sample volume was injected directly on the catalyst (heated to 680°C). Final DOC concentrations were averaged values of triplicate measurements. If the standard variation or the coefficient of variation exceeded 0.1 µM or 1%, respectively, up to two additional analyses were performed and outliers were eliminated. After each batch of six samples, one DSR (Deep Sea Water Reference Material, Hansell Research Lab, University of Miami, US), one Milli-Q blank, and one potassium hydrogen phthalate standard were measured. The limit of quantification was 7 µM for DOC and 11 µM for TDN, and the accuracy was ±5%. The copepods were trapped inside the syringe filters and could not be sampled for C and N content analysis. Since both the excretion and the respiration incubations were conducted under similar conditions, the C and N contents of the copepods from the latter were also used for calculating the excretion rates.

Egg and FP production

For each diet treatment, females were placed individually in triplicate 12-well cell culture plates (12 females per replicate) filled with FSW and food suspension to 4 mL volume. The production of eggs and FP was checked under a binocular 1, 6, 12, 18 and 24 h after the beginning of the experiment. After eggs and feces were counted, they were carefully pipetted out of the well, at times being collected in filters for determination of their C and N contents. Due to the high amount of eggs and feces required for C and N analysis, only one pooled sample was taken from each of the different treatments, with a minimum of 400 eggs and 379 FP. Due to time limitations, eggs and FP were not washed in distilled water before sampling, but the presence of algal cells in the samples was unlikely, since most of the food had been eaten by copepods at the time of collection. Food was immediately replenished until the next count, and plates were left undisturbed. Copepods were sampled after 24 h for determination of body C and N contents. Individual egg production rates (EPRs) were calculated as a daily estimate based on the production observed over 24 h. FP production rates (FPRs) were also observed during 24 h, but due to problems during the last 6 h period, only the data from the first 18 h of incubation were used to estimate daily rates.

C and N content analysis

Copepods, prey cultures, eggs and FP were sampled for the determination of their C and N contents. Copepods were gently washed in distilled water, placed into preweighed tin cartridges and stored at −80°C until further analysis. *In situ* water samples and prey cultures were filtered onto pre-combusted (500°C for 24 h) Whatman GF/F filters (0.7 µm pore size, 25 mm diameter). Eggs and FP were counted and pipetted onto pre-combusted Whatman GF/F filters. Both tin cartridges and filters with samples were dried at 60°C for 48 h, folded (filters inside aluminum foil), and stored in a desiccator. The C and N contents of all samples were later measured with an elemental analyzer (detection limit: 2 µg C / 0.5 µg N; maximum error: ± 3%, Euro EA 3000, EuroVector S.P.A., Milan, Italy) using acetanilide as a standard.

C and N budgets

The amount of energy ingested by an organism should equal the sum of the amounts of energy egested and used for growth and metabolism. In that sense, the vital rates of an individual can also be expressed as a balanced equation, such that $I = G + R + U + E + F$, where I is ingestion, G is somatic growth, R is respiration, U is excretion, E is egg production and F is FP production. In order to do so, the vital rates were converted to C and N units. The number of prey cells ingested and of eggs and FP produced were multiplied by their respective C and N contents. Respiratory quotients (RQs), which ranged from 0.74 to 0.76, were estimated for each replicate (as described by McConnaughey, 1978) and used to convert the O_2 consumption into C-equivalent respiration (Harris *et al.*, 2000). The excretion incubations did not allow for the differentiation between C properly excreted (in the form of urea and amino acids, for example) and C leaked from the FP before absorption by the copepods. The majority of the DOC measured was likely leaked from FP, and thus added to the calculation of F. The G term was obtained by the formula $G = (X_{t48h} - X_{t0h}) / (N * \Delta t)$, where X_{t48h} and X_{t0h} are the C or the N content of the copepod samples from t_{48h} and t_{0h}, respectively, N is the number of females in the sample and Δt is the time in days between t_{48h} and t_{0h}. Values were compared in the format µg C or N female^{-1} day^{-1}. Some organisms have a limited ability to store C relative to other elements, which is especially true for many copepod species (Meunier *et al.*, 2014), and will be in excess of this element when they are supplied a diet with more C than they can absorb and/or utilize (Hessen and Anderson, 2008). Therefore, the discussion on the C budget also approaches the topic of excess C and the pathways for returning this excess C to the environment (i.e. eliminating it).

Turnover rates

The budget data were further standardized to daily C and N turnover rates (% body C or N day^{-1}). This was achieved by dividing the budget values by the median C or N contents of the copepods sampled at t_{48h} and at the end of each incubation (to account for possible weight losses during the incubations). Somatic growth was derived from weight differences between $t_{0\,h}$ and t_{48h}, so the budget values for this specific turnover rate were divided by the median C or N contents of the copepods sampled at t_{0h} and t_{48h} (to account for possible weight gain during the acclimation). An arcsine square root transformation was then applied to the calculated percentage values. Given that there were negative values in the N growth term, and that the transformation is not possible for negative numbers, a fixed, minimum value was added to all treatments in that category to ensure values above zero.

Efficiencies

Different physiological efficiencies were calculated to evaluate copepod performance in relation to food quality. Absorption efficiency (AE), the percentage of ingested

carbon absorbed in the gut of copepods, was calculated as AE = 1 – (*F*/*I*), where *F* is the egestion and *I* is the ingestion (Harris *et al.*, 2000). The DOC excreted by copepods was added to the *F* term in the carbon AE to account for leakage from FP. The net somatic growth efficiency (NSGE) and the net egg production efficiency (NEPE) indicated the percentage of absorbed food converted into somatic growth and egg production, respectively. The formulas were adapted from Kiørboe *et al.* (1985) and Wendt and Thor (2015) as NSGE = *G*/(*G* + *E* + *R* + *U*) and NEPE = *E*/(*G* + *E* + *R* + *U*), where *G* is the somatic growth, *E* is the egg production, *R* is the respiration and *U* is the excretion.

Statistical analysis

Differences in seston and in copepod *in situ* elemental composition and PL were verified with *t*-tests. Differences in vital rates, turnover rates and efficiency calculations were tested with one-way analysis of variance (ANOVA). Differences in copepod elemental composition during the experiments were tested with a two-way ANOVA, in order to account for interactions between diet treatment and sampling time. When these results were significant, the Tukey HSD *post hoc* test was used at 95% confidence limits to further identify origin of differences. Prior to the ANOVA, the data were tested for normality and homogeneity of variances with Shapiro–Wilk and Bartlett tests, respectively. If the data were non-normal and/or heteroscedastic, they were analyzed with a Kruskal–Wallis test and with the *post hoc* Nemenyi test (with *P*-value being determined with the Tukey method). Analyses were performed using R ver. 3.2.5 (Ihaka and Gentleman, 1993).

RESULTS

C and N contents

Prey culture

The C and N contents and molar C:N ratio of prey cultures are presented in Table I. The average C content was always above 500 µg C L^{-1}, confirming the *ad libitum* feeding condition. The average N content was always above 100 µg N L^{-1}, with the exception of the Diat− treatment, which was clearly N-limited and contained less than half this amount. The molar C:N ratio was also similar between all treatments with the exception of the Diat−, such that the approximate values were 6 and 19 for the former and the latter, respectively. Cultures were thus significantly different from one another (Supplementary Tables S1 and S2).

Copepods

The PL of *in situ* females was significantly greater in Experiment II (Table II, Supplementary Table S1), but their elemental compositions were not statistically different.

Copepod C and N contents varied between 13 and 21 µg C copepod^{-1} and between 3 and 5 µg N copepod^{-1}, and molar C:N ratios ranged from 4.0 to 5.6 (Table II). Although no significant differences were found between the t_{0h} samples from Experiments I and II, the C and N contents and the molar C:N ratio were significantly different when comparing t_{0h} with t_{48h} and t_{72h}, both within and between experiments (Supplementary Tables 1 and 2). A significant increase in C and N contents was observed between t_{0h} and t_{48h}, except in the Diat− treatment, for which the N content remained the same throughout the experiment. C and N contents of copepods sampled between t_{48h} and the end of the incubations remained constant in the grazing incubation (Fig. 2, Table II, Supplementary Table S2), for which surplus conditions were maintained. Some level of food limitation was present in the egg and FP production rate incubations, given the small volume of the experimental unit, and copepods in the respiration incubation were food-deprived. In both incubations, the C and N contents of copepods decreased (Table II, Supplementary Table S2). The highest increase in molar C:N ratio was observed for the Diat − treatment (from 4.7 at t_{0h} to 5.6 at the end of the grazing incubation), which was always significantly different from all other treatments (Fig. 2, Table II, Supplementary Table S2).

Table I: C and N contents and molar C:N ratio of the prey cultures (nutrient-replete and N-depleted C. weissflogii, *Diat+ and Diat−, and* O. marina, *Dino+ and Dino−, respectively). Values are mean ± standard deviation from the three batches (temporal replicates) used for feeding in each experiment*

		Prey culture				
Experiment	Treatment	µg C L^{-1}	pg C $cell^{-1}$	µg N L^{-1}	pg N $cell^{-1}$	C:N ratio
I	Diat+	572 ± 55	72 ± 7	105 ± 18	13 ± 2	6.4 ± 0.5
	Diat−	696 ± 46	87 ± 6	43 ± 4	5 ± 1	19.1 ± 2.2
II	Dino+	906 ± 179	453 ± 90	179 ± 26	89 ± 13	5.9 ± 0.3
	Dino−	534 ± 44	267 ± 22	108 ± 4	54 ± 2	5.8 ± 0.4

Table II: C and N contents (in µg C copepod^{-1} and µg N copepod^{-1}), and molar C:N ratio of T. longicornis. *Values presented for samples obtained at t_{0h}* (in situ *condition), t_{48h} (after 48h of acclimation) and after the experimental incubations on Day 3 (grazing = G; respiration = R; and egg and FP production = EF). PL (in mm) are shown for t_{0h} copepods. Diet treatments: single cultures of* C. weissflogii *(Diat) and* O. marina *(Dino) in nutrient-replete (+) and N-depleted (−) conditions. Values presented are mean ± standard deviation of triplicate samples containing between 10 and 12 copepods each (except for PLs, which had 40 replicates each)*

	Carbon					Nitrogen					C:N					PL
Treatment	t_{0h}	t_{48h}	G	R	EF	t_{0h}	t_{48h}	G	R	EF	t_{0h}	t_{48h}	G	R	EF	t_{0h}
Diat+	16 ± 1	20 ± 0	20 ± 1	18 ± 1	18 ± 1	4 ± 0	5 ± 0	5 ± 0	4 ± 0	4 ± 0	4.7 ± 0.2	4.9 ± 0.1	5.0 ± 0.0	4.8 ± 0.1	4.8 ± 0.0	0.99 ± 0.1
Diat−		19 ± 1	20 ± 0	17 ± 1	17 ± 2		4 ± 0	4 ± 0	4 ± 0	4 ± 0		5.4 ± 0.2	5.6 ± 0.1	5.1 ± 0.1	5.5 ± 0.1	
Dino+	15 ± 0	20 ± 0	20 ± 1	15 ± 2	18 ± 2	4 ± 0	5 ± 0	5 ± 0	4 ± 1	4 ± 0	4.4 ± 0.1	4.8 ± 0.0	4.8 ± 0.0	4.6 ± 0.0	4.9 ± 0.0	1.05 ± 0.1
Dino−		18 ± 1	18 ± 1	15 ± 1	15 ± 1		5 ± 0	5 ± 0	4 ± 0	4 ± 0		4.6 ± 0.1	4.7 ± 0.0	4.6 ± 0.1	4.6 ± 0.0	

Eggs and FP

Diatom-fed copepods produced eggs with the highest C content and the highest and the lowest N content (72 and 69 ng C egg^{-1} and 13 and 9 ng N egg^{-1} for nutrient-replete and N-depleted treatments, respectively). Dinoflagellate-fed copepods produced eggs with similar C and N contents (60 ng C egg^{-1} and 12 and 11 ng N egg^{-1} for Dino+ and Dino−, respectively) The molar C:N ratio of eggs was similar between the Diat+ (6.5) and Dino− (6.4) diets, and lower and higher in the Dino+ (5.8) and Diat− (8.9) treatments, respectively.

The FP from diatom-fed copepods had the lowest C and N contents (26 and 20 ng C FP^{-1} and 4 and 2 ng N FP^{-1} for nutrient-replete and N-depleted treatments, respectively). The C and N contents of the FP from dinoflagellate-fed copepods were almost twice as high (52 and 48 ng C FP^{-1} and 9 and 7 ng N FP^{-1} for Dino+ and Dino−, respectively). The molar C:N ratio of FP was lower in the Dino+ (6.7), Diat+ (7.6) and Dino− (8.0) diets than in the Diat− treatment (11.7). It was not possible to measure FP sizes, however, visual observations indicate that FPs from copepods fed with diatoms were slightly longer and thicker than those produced by copepods fed with dinoflagellates.

Vital rates and turnover rates

Diatom-fed copepods had significantly higher average ingestion rates (8.6 ± 0.5 × 10^4 and 10.1 ± 1.2 × 10^4 cells female^{-1} day^{-1} for Diat+ and Diat−, respectively) than the dinoflagellate-fed copepods (2.4 ± 0.8 × 10^4 and 1.6 ± 0.5 × 10^4 cells female^{-1} day^{-1} for Dino+ and Dino−, respectively) (Supplementary Tables S1 and S3). C ingestion, which ranged from 17 to 86% body C day^{-1}, was significantly higher in the Dino+ treatment than in the Dino−, whereas N ingestion, which ranged from 12 to 66% body N day^{-1} (Table III), was significantly higher in the Dino+ diet than in the N-depleted treatments (Supplementary Tables S1 and S3).

The average oxygen consumption rates were lower in the diatom treatments (3.4 ± 0.3 and 4.3 ± 0.1 mL O_2 female^{-1} day^{-1} for Diat+ and Diat−, respectively) than in the dinoflagellate treatments (6.0 ± 1.3 and 4.2 ± 0.4 mL O_2 female^{-1} day^{-1} for Dino+ and Dino−, respectively). They corresponded to a C-equivalent respiration of 6–18% body C day^{-1} (Table III). A significant difference in O_2 consumption and turnover of respired C was only found between the nutrient-replete treatments, with higher values in the Dino+ diet (Supplementary Tables S1 and S3 and Table III). Surprisingly, the average N excretion values were higher in the N-depleted treatments (1.0 ± 0.3 and 0.6 ± 0.1 µg N

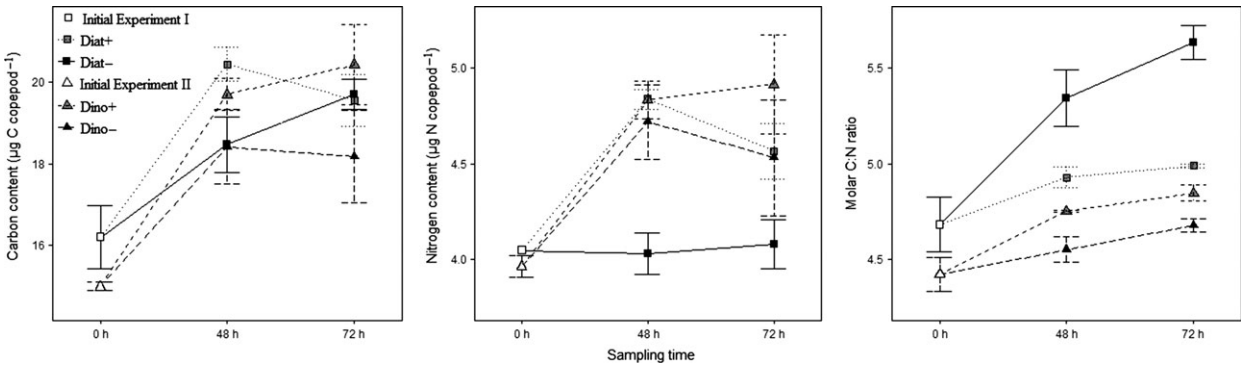

Fig. 2. C and N contents and molar C:N ratio of *T. longicornis* females during Experiments I and II. Experiment I tested diet treatments consisting of diatoms cultured in nutrient-rich (Diat+, gray squares and dotted lines) and N-depleted (Diat−, black squares and full lines) conditions, whereas Experiment II tested diets consisting of dinoflagellates fed with nutrient-rich (Dino+, gray triangle and dotted lines) and N-limited (Dino −, black triangle and dotted lines) prey. Samples were taken of *in situ* conditions (t_{0h}, empty symbols), after 48h of acclimation (t_{48h}), and after 24h of incubations (t_{72h}). Bars represent mean values ± one standard deviation.

female^{-1} day^{-1} for the Diat− and Dino−, respectively) than in the nutrient-replete diets (0.2 ± 0.2 and 0.3 ± 0.1 µg N female^{-1} day^{-1} for the Diat+ and Dino+, respectively). They corresponded to 5–32% body N day^{-1} (Table III) and were significantly lower in the Diat− treatment (Supplementary Tables S1 and S3).

The average FP production rates were not significantly different between the Diat+ (89 ± 4 pellets female^{-1} day^{-1}) and Diat− (86 ± 8 pellets female^{-1} day^{-1}) treatments. FP production rates recorded for copepods fed with dinoflagellates were lower, 72 ± 5 and 49 ± 1 pellets female^{-1} day^{-1} for the Dino+ and Dino− treatments, respectively (Supplementary Tables S1 and S3). The average amount of DOC leaked from FP, which was significantly different between all treatments (Supplementary Table S1), was higher in the N-depleted diets (1.6 ± 0.2 and 1.1 ± 0.1 µg C female^{-1} day^{-1} for Diat− and Dino−, respectively) than in the nutrient-replete treatments (0.2 ± 0.1 and 0.7 ± 0.1 µg C female^{-1} day^{-1} for Diat+ and Dino+, respectively). The C and N turnover rates for FP production, which varied between 12 and 24% body C day^{-1} and between 4 and 14% body N day^{-1} (Table III), were significantly different between almost all treatment pairs (Supplementary Tables S1 and S3).

Somatic growth was significantly different between treatments in terms of both C and N (Supplementary Table S1). It ranged from 4 to 14% body C day^{-1} and from 0 to 11% body N day^{-1} (Table III), with the lowest values recorded for the N-limited diatom treatment (Supplementary Table S3). The average EPRs were significantly higher in the Dino+ treatment (61 ± 4 eggs female^{-1} day^{-1}) than for copepods fed with the other diets (39 ± 9, 36 ± 4, and 41 ± 5 eggs female^{-1} day^{-1} for Diat +, Diat− and Dino−, respectively) (Supplementary Tables S1 and S3). The egg C turnover (13–21% body C day^{-1}) was similar between treatments (Table III), but the N turnover (7–16% body N day^{-1}) was significantly different between the Dino+ and Diat− treatments (Supplementary Tables S1 and S3).

C and N budgets

The C and N budgets obtained for each of the treatments are represented in Fig. 3. They were mostly unbalanced, which is represented in the figure by the equation symbols "−" and "+". The "*I*" values are represented on the left side of the copepod antenna, whereas the right side represents the sum of "$G + R + U + E + F$". When the gain of energy via feeding was higher than growth and energy expenditure (e.g. Fig 3B and G), the "+" and "−" signs are placed on the left and right sides of the antenna, respectively. When the gain of energy was lower than growth and energy expenditure (e.g. Fig 3D and F), the "+" and "−" signs are placed on the right and left sides of the antenna, respectively. The symbol "=" was used for the C budget which was nearly balanced (Fig. 3C). To the right of these symbols is a percentage, which indicates the percentage of energy gained via feeding that was recorded as being used. The budgets recorded for the Dino+ treatment seem to have the best estimates (percentages close to 100%). The budgets for the other treatments mostly point to higher growth and energy expenditure. The Dino− treatment had the most deviating results, with expenses amounting to twice as much the recorded energy ingested, a pattern visually represented by the use of double equation symbols (Fig. 3D and H). The Diat+ budgets also show higher expenses than energy gain (Fig. 3A and E), whereas the Diat− budgets show a mixed response, with an

Table III: C and N turnover rates and AE, NSGE and NEPE efficiencies from experimental incubations with T. longicornis. *Diet treatments: single cultures of* C. weissflogii *(Diat) and* O. marina *(Dino) in nutrient-replete (+) and N-depleted (−) conditions. Mass-specific values for ingestion (I), growth (G), carbon-equivalent respiration (R), excretion (U), egg production (E) and FP production (F) turnover rates are expressed as % body C or N day^{-1}, and efficiencies as %. Values presented are mean ± standard deviation.*

	Carbon							Nitrogen						
Treatment	*I*	*G*	*R*	*E*	*F*	AE	NSGE	NEPE	*I*	*G*	*U*	*E*	*F*	AE	NSGE	NEPE
Diat+	32 ± 1	11 ± 1	7 ± 1	15 ± 4	13 ± 1	60 ± 3	33 ± 4	45 ± 5	24 ± 1	9 ± 1	5 ± 9	11 ± 3	8 ± 0	68 ± 1	36 ± 11	48 ± 6
Diat−	49 ± 6	6 ± 2	10 ± 0	14 ± 1	19 ± 2	64 ± 7	20 ± 5	47 ± 2	14 ± 2	0 ± 1	27 ± 5	8 ± 1	5 ± 0	67 ± 7	−1 ± 4	23 ± 4
Dino+	65 ± 23	13 ± 1	14 ± 3	19 ± 1	23 ± 1	64 ± 12	27 ± 2	44 ± 4	50 ± 17	10 ± 1	8 ± 4	15 ± 1	13 ± 1	73 ± 9	29 ± 3	48 ± 7
Dino−	27 ± 10	10 ± 2	10 ± 1	14 ± 1	20 ± 1	36 ± 5	29 ± 5	42 ± 2	21 ± 8	9 ± 2	15 ± 3	11 ± 1	8 ± 0	60 ± 12	25 ± 6	32 ± 3

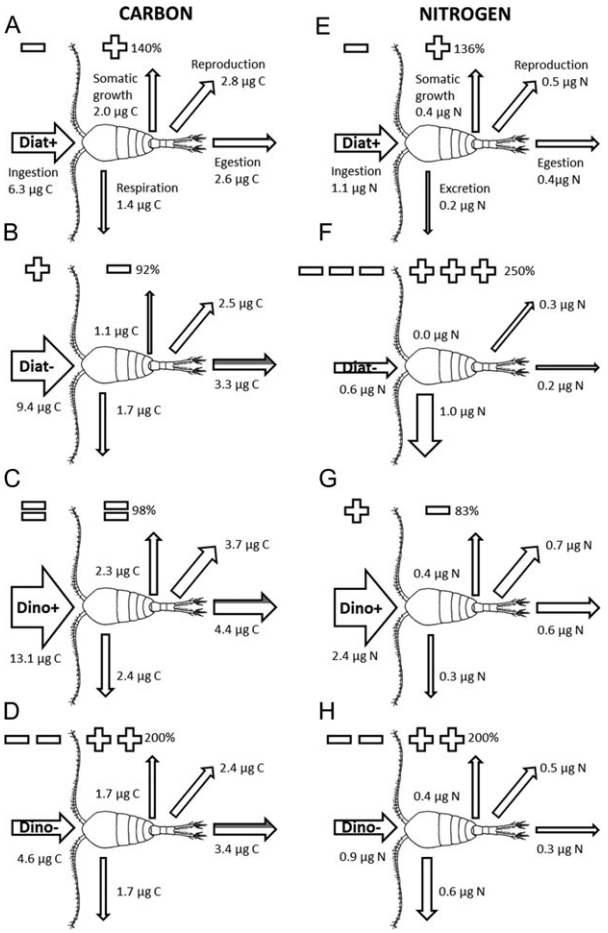

Fig. 3. Carbon (**A**–**D**) and nitrogen (**E**–**H**) budgets obtained for *T. longicornis* females feeding on diatoms cultured in nutrient-rich (Diat+) and N-depleted (Diat−) conditions and on dinoflagellates fed with nutrient-rich (Dino+) and N-limited (Dino−) prey. Values given represent daily ingestion, egestion, respiration, excretion, reproduction and somatic growth rates for one individual copepod, and are visually represented by proportionately sized arrows. The comparison between the amount of C and N ingested by copepods ("*I*" values on the right side of the copepod antenna) and the amount used by them (the sum of "$G + R + U + E + F$" values on the left side of the copepod antenna) is represented in the figure by the equation symbols "+", "−" and "=". When the gain of energy via feeding was higher than growth and energy expenditure (B and G), the "+" and "−" signs are placed on the left and right sides of the antenna, respectively. When the gain of energy was lower than growth and energy expenditure (A, D, E, F and H), the "+" and "−" signs are placed on the right and left sides of the antenna, respectively. The symbol "=" was used for the C budget which was nearly balanced (C). To the right of these symbols is a percentage, which indicates the percentage of energy gained via feeding that was recorded as being used. The closer to 100%, the more balanced the measurements; values below and above 100% indicated higher energy gain from feeding than energy expenditure and higher energy expenditure than energy gain from ingestion, respectively. The DOC and FP components of the egestion term of the C budget (percentage of total "*F*") are indicated in gray and white colors (respectively) in the corresponding arrows.

accurate estimate for the C budget (Fig. 3B) but an inaccurate estimate for the N budget (Fig. 3F). The DOC and FP components of the egestion term of the

C budget (percentage of total "*F*") are indicated in gray and white colors (respectively) in the corresponding arrows in Fig. 3.

Efficiencies

The AEs varied between 33% and 75% and between 46% and 81% of C and N ingested, respectively (Table III), with the carbon AE for Dino− being significantly lower than that of Diat− and Dino+ (Supplementary Tables S1 and S3). The carbon NSGEs varied between 15% and 36%, and were significantly different between the diatom treatments, while the nitrogen NSGEs varied from −4% to 48% and were significantly lower in Diat− (Table III, Supplementary Tables S1 and S3). No significant differences were found between the carbon NEPE, which ranged from 39% to 50%, whereas the nitrogen NEPEs, which varied from 19% to 56% (Table III), were significantly higher in the nutrient-replete treatments (Supplementary Tables S1 and S3).

DISCUSSION

To our knowledge, this is the first study of energy budgets of *T. longicornis* females feeding on prey items of different qualities, which enabled the comparison of copepod performance under different food regimes. Our working hypothesis was that the highest growth and reproduction rates and the lowest catabolic rates would be recorded for copepods feeding on a diet with a C:N ratio as close as possible to their own body composition. Our study was able to partially confirm the working hypothesis. The highest investment in somatic and reproductive growth was indeed shown by copepods fed with a food source of C:N ratio close to their own (Dino+), but so were the highest expenditures with egestion and catabolism, contrary to what had been postulated. Performance entails the amount of energy ingested and used by an individual for different vital activities, but is ultimately defined by what is invested in reproduction and how successful this process is. Thus, the major finding of this study is that dinoflagellates are a food source for copepods of superior or similar quality to diatoms under nutrient-replete or N-depleted conditions, respectively. This is, however, only valid for the temperatures investigated in the present study, as individual metabolism and C requirements are affected by temperature (e.g. Boersma *et al.*, 2016). Results also revealed that egestion is a major pathway for *T. longicornis* females to eliminate the excess C; and that low food quality can influence copepod respiration (regardless of its C-to-nutrient ratio) and the intensity and speed with which DOC leaks from FP. The fact that results only partially agreed with predictions from stoichiometric theory indicates the need for further investigations into copepod ecophysiology and adaptive capacity to shifting food regimes.

The level of N-limitation achieved in the Diat− treatment was not expected for the Dino− diet, as it has been shown that *O. marina* can regulate its body composition to incorporate the stoichiometric imbalances of its prey in an attenuated form (Malzahn *et al.*, 2010; Meunier *et al.*, 2014). Although the elemental composition of the Dino− treatment was similar to that of the Dino+, its fatty acid profile was not (unpublished data). This could explain why performance was different for copepods fed with these two diets, and why the former constituted a food source of lower quality.

Copepod feeding

The C ingestion rates reported by Arendt *et al.* (2005) and Jónasdóttir *et al.* (2009) for *T. longicornis* feeding on *C. weissflogii* are twice as high as those reported herein, despite their use of lower prey concentrations (Table IV). The values from our study might be underestimated, as indicated by the comparison of the amount of energy ingested and used by copepods (symbols on the top left and right sides of the copepod antenna in Fig. 3, respectively). Despite measurements being recorded in the same fashion for all treatments, some show a nearly balanced budget (e.g. Fig. 3C) while others reveal unbalanced budgets (e.g. Fig. 3F). We speculate that the ingestion rates are underestimated and that this might be due to copepods feeding on food sources that were unaccounted for, which were ingested in quantities inversely proportional to the quality of the diet treatments. Coprophagy and filial cannibalism are known to occur among copepods, regardless of availability of alternative food, and can reach values of up to 50% of produced FP and 60 eggs female^{-1} day^{-1} (e.g. Lampitt *et al.*, 1990; Noji *et al.*, 1991; Dam and Lopes, 2003; Boersma *et al.*, 2014) in starved or food-limited conditions. The glass bottles used for the grazing incubation in the present study did not allow for the separation of copepods from eggs and FP. The eggs had a similar or higher nutritional quality (in terms of elemental composition/molar C:N ratio) than the prey items, and the microbiota associated with the peritrophic membrane of the FPs might be a valuable food source for copepods (as suggested by Lampitt *et al.*, 1990), so by feeding on its own eggs and FPs, copepods could have complemented nutritionally inadequate diets. This response would be a form of compensatory behavior for this copepod, and has been identified in other studies (Augustin and Boersma, 2006; Siuda and Dam, 2010) as

Table IV: Comparative values between prey species and quantity used in laboratory experiments and the ingestion and EPR of T. longicornis *recorded for that prey in the present study and elsewhere in the literature*

Copepod	Prey		Ingestion			EPR	Reference
	Type	µg C L^{-1}	Cells ind^{-1} day^{-1}	µg C ind^{-1} day^{-1}	% body C day^{-1}	Eggs ind^{-1} day^{-1}	
Temora longicornis	Natural prey assemblage	200–800[a]		1–4[a]		2–55	Dam and Peterson (1991) and Peterson and Dam (1996)
	Phytoplankton					50	Castellani and Altunbas (2014)
							Jansen *et al.* (2006)
	Dinoflagellates					3	Jansen *et al.* (2006)
	Conticribra weissflogii	349	204 000 ± 70 000	15.6 ± 4.8	124	27.8 ± 7.3	Arendt *et al.* (2005)
		349		6.5	70	11	Dam and Lopes (2003)
		225–276			68	5–19	Jónasdóttir *et al.* (2009)
						20–60	Niehoff *et al.* (2015)
		274		2.2 ± 0.2	25		van Someren Gréve (2013)
		572 ± 55	86 000 ± 500	6.3 ± 0.2	32 ± 1	39 ± 9	This study
	Oxyrrhis marina					20–80	Niehoff *et al.* (2015)
		286		4.7 ± 0.4	51		van Someren Gréve (2013)
		906 ± 179	24 000 ± 800	13.1 ± 4.6	65 ± 23	61 ± 4	This study

[a]Using a carbon:chlorophyll *α* ratio of 50, according to Dam and Peterson (1991).

a mechanism for other small calanoids to overcome the elemental limitation of their prey.

Copepod waste production (or catabolic activities)

Few studies are available on the metabolic activities of small copepods such as *T. longicornis* (e.g. Berner, 1962; Dam and Peterson, 1993; Nobili *et al.*, 2013; Castellani and Altunbas, 2014), all reporting lower O_2 consumption and C-equivalent respiration than the ones described herein (1–2 µL O_2 ind^{-1} day^{-1} and 1% body C day^{-1}). Our respiration and excretion incubations were performed without feeding the copepods, and probably underestimate the rates of fed copepods (Miller and Glibert, 1998; Thor, 2002a, 2002b; Nobili *et al.*, 2013). High respiration rates have been suggested as a mechanism for removal of excess C in unbalanced nutrient conditions (Anderson *et al.*, 2005; Hessen and Anderson, 2008), even though the mechanism itself is yet to be described (Malzahn *et al.*, 2010). The results of the present study partially agree with this assumption. The second highest respiration rates were reported for copepods fed with the N-poor diets, but one of them was enriched in C in relation to N (Diat−) and the other had a C:N ratio close to that of the copepods (Dino−). Furthermore, the highest rates were measured for another one of the diets whose C:N more closely resembled that of the copepods (Dino+). The type of substrate catabolized by starved copepods in order to obtain energy influences respiration (Castellani and Altunbas, 2014) and excretion (Anderson, 1992) rates. Proteins are the main substrates catabolized in small calanoids (Thor, 2002). The RQ of proteins is higher than that of lipids (Gnaiger, 1983) and would, thus, generate higher C-equivalent respiration rates in starved individuals that were previously fed N-rich diets, as observed for *O. marina* by Meunier *et al.* (2012). Our results thus suggest that O_2 consumption increases with decreasing food quality, regardless of an excess of C or not. If prey is of high nutritional quality, then O_2 consumption seems to be modulated by the amount of food ingested.

The preferential utilization of proteins by copepods previously fed with a N-rich diet should also result in higher ammonia excretion rates than those of copepods previously fed N-poor diets, but the opposite was recorded. It is possible that the high food C:N ratio of 19 in the Diat− treatment led to an increase in ingestion rates and a decrease in gut passage time. This, in turn, reduced the efficiency with which nutrients were absorbed and resulted in higher excretion rates (Plath and Boersma, 2001).

FP production by copepods is an important source of POM and DOC in the epipelagic (Møller *et al.*, 2003;

Thor *et al.*, 2003), contributing to microbial production with recycled nutrients (Smetacek, 1980; Strom *et al.*, 1997). Although it has been claimed that undisturbed FP do not release DOC (Strom *et al.*, 1997), it has been shown that 50% of the total C content of FP is released as DOC at the onset of defecation (Thor *et al.*, 2003). DOC can be generated by copepods before absorption, as leakage from egested FP, and after absorption, as excretion in the form of nitrogenous organic compounds (Frangoulis *et al.*, 2004). The methodology did not allow for the separation of both components. Even though some DOC may have been excreted, it was considered that the majority of DOC measured was leaked from FP, which is why DOC values were added to the C content of FP to calculate the egestion term ("*F*") of the C budget. In our study, the percentage of total C egested ("*F*") that corresponded to DOC varied widely between treatments, going from approximately 8% in Diat+ to 16% in Dino+, 31% in Dino− and 50% in Diat−. It would seem, thus, that the level of DOC leakage from FP is directly or indirectly influenced by food quality.

Small calanoids such as *T. longicornis* are unable to store significant amounts of energy reserves, and must return to the environment any C consumed in excess of its needs and ability to absorb. Our results suggest that the primary pathway for *T. longicornis* to eliminate excess C was egestion, which removed twice as much C as that burnt via respiration (Fig. 3A–D). Furthermore, if all C had been assimilated and metabolized and no excess was left, assuming that all N was also assimilated, the molar C:N ratio of FP would not have differed from that of the diet treatments (Checkley, 1980). The fact that it was higher in the FP (except for Diat −) indicates that N was used constantly and efficiently for production and that the excess C was egested (Checkley, 1980). This pattern is further supported by the statistically similar nitrogen AE in the treatments, contrary to what stoichiometric theory would have predicted. The carbon AE, on the other hand, was significantly lower in the Dino− treatment. It could be that the resulting biochemical composition of the Dino− diet indirectly caused an inefficient absorption of C in copepods and led to an egestion similar to that of the other treatments despite a lower ingestion rate. Our estimates for the carbon AE were greater than the assimilation efficiency estimates for another small copepod (*A. tonsa*) feeding on the same prey species (Besiktepe and Dam, 2002).

Copepod growth and reproduction (or anabolic activities)

Most studies assume negligible increases in body weight in adult copepods which do not store energy reserves and only investigate reproductive investment as a measure of growth (Hirst and McKinnon, 2001). The C and N somatic growth rates observed in the present study, though lower than the EPRs, contest this assumption, as do other studies (Hirst and McKinnon, 2001; Dam and Lopes, 2003). A comparison between the net efficiencies for somatic growth (NSGE) and egg production (NEPE) emphasizes how the majority of assimilated energy is used in reproduction, but also that the portion destined to somatic growth is not negligible. The only absence of somatic growth was observed in terms of N for the Diat− treatment, as expected.

The EPR recorded for *T. longicornis* in this study were within the range of those obtained when this copepod was fed with natural plankton (Peterson and Dam, 1996), similar prey species (Niehoff *et al.*, 2015) and with water from the spring phytoplankton bloom (Castellani and Altunbas, 2014; Peterson and Kimmerer, 1994), but higher than when it was fed water from a dinoflagellate bloom (Jansen *et al.*, 2006) (Table IV). The highest EPR in our study was obtained with the nutrient-rich dinoflagellate diet, as opposed to results from other studies (Turner *et al.*, 2001; Dam and Lopes, 2003; Jónasdóttir *et al.*, 2009). It must be noted that great controversy involves the topic of diatoms being good or bad quality diet items (for reviews, see Jónasdóttir *et al.*, 1998; Paffenhöfer *et al.*, 2005), with many suggesting its suitability for egg production but reduced success for hatching (Miralto *et al.*, 1999; Ban *et al.*, 1997).

The quantity of body C invested into reproduction was similar across treatments, but body N investment was significantly different between the Diat− and the Dino+ treatments. This indicates N-limitation in egg production, as also suggested by Nobili *et al.* (2013) for *T. longicornis*. The nitrogen NEPE observed in our study further indicates a clear limitation of this element in copepods fed with the N-depleted diets. This contrasts the assumption that nutrients in limited amounts should be used with higher efficiency. Other studies have reported similar trends, with lower egg production efficiencies associated with higher food C:N ratios and with P-limitation (Anderson *et al.*, 2005; Nobili *et al.*, 2013). This pattern might be due to increased maintenance requirements for copepods fed with nutrient-limited diets (Anderson *et al.*, 2005; Wendt and Thor, 2015). Even so, the NEPE values reported herein seem to be mostly higher than those obtained from the literature as either egg production efficiency or gross growth efficiency (e.g. Dam and Lopes, 2003; Thor *et al.*, 2007; Nobili *et al.*, 2013; Wendt and Thor, 2015). Few studies have approached the effect of N-limitation on EPR, which has been said to be both deleterious (Koski *et al.*, 2006; Nobili *et al.*, 2013) and advantageous (Augustin and Boersma, 2006). EPR does not provide information

on egg viability (hatching success), so it would be necessary to conduct further investigations in order to understand the importance of diet quality for secondary production by *T. longicornis*.

Climate change and marine C and N cycles

Long-term monitoring data from Helgoland have shown a drastic decrease in calanoid copepod densities since 1985, a decline most likely caused by the decrease in nutrient (N and P) loading coupled to the increase in light penetration in the region (Boersma *et al.*, 2015). These would have resulted in phytoplankton with higher C:nutrient ratios and, thus, of lower nutritional value for zooplankton (Boersma *et al.*, 2015). Recent studies show controversial results regarding the relative dominance in recent years of diatoms and dinoflagellates in the southern North Sea (Wiltshire *et al.*, 2010; Alvarez-Fernandes *et al.* 2012; Hinder *et al.*, 2012; Boersma *et al.*, 2015). Changes in the abundance of these prey species can affect secondary production, and a further increase in their C:N ratio could have major consequences for food web processes (Jones and Flynn, 2005; Malzahn and Boersma, 2011; Nobili *et al.*, 2013; Boersma *et al.*, 2015). On the other hand, increasing local temperatures (Wiltshire *et al.*, 2010) could result in higher metabolic costs for copepods (Castellani and Altunbas, 2014), which can only be met by an increased C consumption (Boersma *et al.*, 2016; Malzahn *et al.*, 2016). In that scenario, increasing temperatures would dampen the effects of prey with higher C:nutrient ratios (Boersma *et al.*, 2016; Malzahn *et al.*, 2016). In N-limitation conditions (e.g. late spring bloom), dinoflagellates are still of better nutritional value than diatoms (Jones and Flynn, 2005; this study), but at higher temperatures, copepods might need to preferentially consume autotrophs to supply metabolic demand, as observed for *T. longicornis* (Boersma *et al.*, 2016) and *A. tonsa* (Malzahn *et al.*, 2016). All of these variables make it difficult to say at this point how *T. longicornis* (and possibly other calanoid copepods) will react to future changes in prey composition, even though its omnivorous strategy and related adaptations allow it to switch food sources depending on their availability (Daan *et al.*, 1988; Gentsch *et al.*, 2009).

CONCLUSIONS

The results obtained in the present study indicate that, under nutrient-replete conditions, dinoflagellates such as *O. marina* are a better food source for *T. longicornis* than diatoms. This major finding is, however, dependent upon environmental conditions. Furthermore, low-quality food also leads to higher respiration rates, regardless of its C-to-nutrient ratio, and to faster leakage of DOC from copepod FP. In addition, egestion seems to be the main pathway for eliminating excess C, contrary to the common belief of excess C being mostly respired. The vital rates measured herein for *T. longicornis* provide important information on this species' food utilization efficiency and maintenance costs in relation to diet quality. The budget approach used herein is not free of flaws, and future research in the field should investigate more complex scenarios (mixed diets, different temperatures, varying food concentrations, include all life stages).

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Plankton Research* online.

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DATA ARCHIVING

The laboratory experimental raw data and metadata for this study are available through the PANGAEA repository under https://doi.pangaea.de/10.1594/PANGAEA.886050.

Ethical standards

The experiments described in the present study comply with the current laws of the country in which they were performed.

Conflict of interest

The authors declare that they have no conflict of interest.

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CHAPTER II

Life history strategies of the copepod *Temora longicornis* as inferred from lipid carbon assimilation experiments

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Life history strategies of the copepod *Temora longicornis* as inferred from lipid carbon assimilation experiments

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Abstract

1. The calanoid copepod *T. longicornis* needs to cope with rapidly changing plankton community composition in the North Sea. These changes can be further aggravated by recent and ongoing changes in the nutrient regime in the area, so efficient food utilization by copepods is of paramount importance for their survival.

2. The present work aimed at describing qualitatively and quantitatively how differences in the biochemical composition of prey grown under different nutrient regimes affects the dynamics of lipid utilization and assimilation and the reproductive output by *T. longicornis* females.

3. Copepods were fed prey cultures isotopically enriched with ^{13}C; the transfer of lipid C from prey to consumer was followed; and FA-specific C assimilation and turnover rates were calculated.
4. Absolute C assimilation was higher in copepods fed prey whose molar C:N ratio was the most different (higher) from their own but, due to differences in ingestion rates, relative C assimilation (as % food ingested) was similar between copepods fed with different diets.
5. The assimilation of lipid C by copepods was inversely related to their C investment in egg production. The copepods which showed highest egg production rates also displayed the highest levels of the FAs 18:3(n-3) and DHA.
6. High lipid C turnover rates indicate an opportunistic feeding strategy for *T. longicornis*, which would accumulate readily available dietary C with a speed that decreases as saturation is approached and its lipid storage is replenished. This strategy would enable them to cope with environmental changes in feeding conditions and to invest in reproduction year round.
7. These results are an important addition to a small, yet growing, body of literature which estimates the transfer of lipid C between prey and predator, and to the field of copepod feeding ecology.

Keywords: assimilation, carbon, compound-specific stable isotope analysis, copepod, fatty acid, feeding ecology, life history strategies, turnover

INTRODUCTION

Temora longicornis (Müller, 1785) is a small calanoid copepod (approx. 1 mm prosome length) abundant all year round in the southern North Sea off the German island of Helgoland, where its grazing can have a substantial impact on the phytoplankton standing stock (Gentsch, Kreibich, Hagen, and Niehoff, 2009; Maar et al., 2004). It is one of the dominant copepod species in the North Sea, and plays a pivotal role in the energy transfer from primary producers to higher trophic levels. Because *T. longicornis* has high metabolic turnover rates and is unable to accumulate

significant amounts of energy reserves, it is dependent on a constant availability of prey and vulnerable to fluctuations in food supply (Helland, Terjesen, and Berg, 2003; Kreibich, Saborowski, Hagen, and Niehoff, 2008, 2011). Efficient food utilization is, thus, of paramount importance for the survival of *T. longicornis*, as this species needs to cope with rapidly changing plankton community composition in the North Sea (Gentsch, Kreibich, Hagen, and Niehoff, 2009; Kiørboe and Nielsen, 1994; Kreibich, Saborowski, Hagen, and Niehoff, 2008), which can be further aggravated by recent changes in the nutrient regime in the area (Boersma, Wiltshire, Kong, Greve, and Renz, 2015).

Several studies have been conducted on how diet quality can influence copepod physiology, reproduction success, nutrient uptake efficiency, and ability to produce and metabolize energy (Hessen and Anderson, 2008; Jónasdóttir, Visser, and Jespersen, 2009; Klein Breteler, Schogt, Baas, Schouten, and Kraay, 1999; Møller, 2007). *Temora longicornis* has been reported to selectively graze according to its physiological requirements, choosing its prey by their biochemical composition and nutritional value (Cotonnec, Brunet, Sautour, and Thoumelin, 2001). The nutritional value of a food source can be defined, a.o., by its elemental and biochemical composition (Sterner and Schulz, 1998).

A gap in knowledge still exists on the role and importance of lipids in marine zooplankton, and on how species cope with inadequate food supply, especially during reproductive periods (Lee, Hagen, and Kattner, 2006). Studies which apply isotope labelling to specific compounds such as lipids or proteins (compound-specific stable isotope analysis, CSIA) can obtain rates of carbon (C) transfer, accumulation, and turnover and information on lipid biosynthesis (Boissonnot, Niehoff, Hagen, Søreide, and Graeve, 2016; Dalsgaard, John, Kattner, Müller-Navarra, and Hagen, 2003; De Troch et al., 2012; Graeve, Albers, and Kattner, 2005). FA dynamics in marine invertebrates have been mostly characterized for larger, often herbivorous, calanoid copepods from polar regions, whereas few studies are available on the lipid biochemistry of smaller copepod species (Boissonnot,

Niehoff, Hagen, Søreide, and Graeve, 2016; Dalsgaard, John, Kattner, Müller-Navarra, and Hagen, 2003; Fraser, Sargent, and Gamble, 1989).

The present work thus aimed at describing qualitatively and quantitatively how the biochemical composition of prey (diatoms and dinoflagellates) grown under different nutrient regimes affects the dynamics of lipid utilization and assimilation and the reproductive output by *T. longicornis* females. We assumed that lipid C assimilation would be inversely related to reproductive output and that these are, in turn, affected by the nutritional quality (elemental and biochemical composition) of the diet. In order to obtain data on lipid dynamics and on reproductive output, copepods were fed with prey cultures previously labelled with ^{13}C, which enabled the calculation of egg production rates, the monitoring of lipid C transfer between prey and predator, and the calculation of FA-specific C assimilation and turnover rates.

METHODS

Field sampling

Zooplankton were sampled with a 500 µm mesh-size CalCOFI net towed horizontally for 15 minutes at 5 m depth off the German island of Helgoland (54°11'N, 07°54'E), in the southern North Sea. Samples were taken to the laboratory and intact and active adult females of *T. longicornis* were immediately sorted under an Olympus SZX16 stereoscopic microscope. A total of 1260 females were sorted during each sampling date, 1080 for feeding experiments (with diets of varying nutritional qualities) and 180 for determination of *in situ* body C, N, and lipid contents, FA composition, and FA-specific ^{13}C isotopic enrichment. Samples for *in situ* conditions were collected in triplicates of 10 and 50 individuals each for elemental and biochemical analyses, respectively. Due to an insufficient number of females during the first sampling date, the sorting procedure was performed twice, on May 17th (Experiment I) and 30th (Experiment II), 2016. The effects of the nutrient-replete and N-depleted diatom treatments (Diat+ and Diat–, respectively) on copepod

elemental and biochemical composition were investigated in Experiment I, and the effects of the nutrient-replete and N-depleted dinoflagellate treatments (Dino+ and Dino–, respectively) were tested in Experiment II.

Prey culture

Common prey species for *T. longicornis* (Evans, 1981) were used in the feeding experiments, the diatom *Conticribra weissflogii* (Grunow) (Stachura-Suchoples and Williams, 2009) and the dinoflagellate *Oxyrrhis marina* (Dujardin, 1841). Batch cultures were created daily for *C. weissflogii* and *O. marina* for five consecutive days under two nutrient treatments. The cryptophycean *Rhodomonas salina* (Wislouch) (Hill and Wetherbee, 1989) was also cultured in batches (created on a daily basis) for five consecutive days to serve as food for the dinoflagellates. *Conticribra weissflogii* and *R. salina* batches were created by diluting stock solutions with fresh nutrient replete (f/2) medium and with fresh N-depleted medium (f/2 without nitrate). The two different types of medium were created (after Guillard, 1975) with seawater filtered in cellulose acetate filters (0.2 µm pore size). Diatom and cryptophycean cultures were labelled by adding ^{13}C-enriched sodium bicarbonate ($NaH^{13}CO_3$) to the medium at concentrations of 4 and 12 mg L^{-1}, respectively. *Oxyrrhis marina* was labelled via daily feeding on ^{13}C-labelled *R. salina*. Cell quantities were adjusted so that cryptophycean cells were depleted by the dinoflagellate on day 5. Cultures were grown for five days (details can be found in Franco-Santos et al., 2018) and then used as food suspension for copepods at a concentration of 8 and 2 $*10^3$ cells mL^{-1} for diatom and dinoflagellate treatments, respectively.

Conticribra weissflogii and *O. marina* cultures were sampled daily for determination of cell C, N, and FA content and of FA-specific ^{13}C isotopic enrichment by filtering subsamples of known cell concentrations through pre-combusted (500^{o}C for 24h) Whatman GF/F filters (0.7 µm pore size, 25 mm diameter). Samples for FA analyses were placed into pre-combusted lipid vials and stored at -80^{o}C. Filters for determination of prey C and N content were dried at 60^{o}C for 48 h,

folded inside aluminum foil, and stored in a desiccator until analysis. Cell densities were determined with a BD Accuri C6 Flow Cytometer.

Experimental design

Feeding experiments with *T. longicornis* were initiated immediately after sorting of the copepods. Copepods were fed each diet for five days in triplicate plastic beakers fitted with a 300 µm meshed-bottom cylinder (75 females L^{-1}). Food was provided on a daily basis after partial water exchanges (66%). Females from Experiment I were fed with the Diat+ and Diat– diets, and copepods from Experiment II were fed with the Dino+ and Dino– diets. The copepods were kept at 10 ± 0.3°C in a dark temperature-controlled room. This temperature was similar to that recorded in the field (surface water) during sampling for Experiment I but was approximately 2°C lower than that recorded *in situ* during sampling for Experiment II. The beakers were gently stirred three times a day for food resuspension in the water.

Copepods were sampled from each replicate of each treatment for body C and N content determination and for FA profiling and FA-specific ^{13}C isotopic enrichment (10 and 50 individuals per sample, respectively). Sampling was conducted before (day 1, *in situ* condition, t_{0h}), during (day 3, t_{48h}), and at the end (day 6, t_{120h}) of the experiment. Copepods were gently washed in distilled water, placed into either pre-weighed tin capsules (5*9 mm, IVA Analysentechnik) for body C and N content determination or pre-combusted lipid vials for FA analyses, and stored at -80°C until further analysis. Tin capsules with copepods were dried and stored in a desiccator until analysis.

Carbon, nitrogen and fatty acid content analyses

The C and N contents of all copepod and prey culture samples were measured with an elemental analyzer (detection limit: 2 µg C / 0.5 µg N; maximum error: ± 3%, Euro EA 3000, EuroVector S.P.A., Milan, Italy) using acetanilide as a standard.

Fatty acid methyl esters (FAMEs) were extracted and identified as described by Boissonnot, Niehoff, Hagen, Søreide, and Graeve (2016). Samples were homogenized in a dichloromethane:methanol (2:1, v:v) solution, from which total lipids were extracted. A known amount of an internal standard, the tricosanoic acid methyl ester (23:0), was added to each sample. Potassium chloride (0.88% solution) was added to create a biphasic system and aid in extraction. Lipid extracts were transesterified by heating samples with 3% sulfuric acid in methanol at 80^{o}C under nitrogen atmosphere for 4 hours. FAMEs were then extracted with cyclohexane and determined with a gas chromatograph (HP 6890 N, Agilent Technologies Deutschland GmbH & Co. KG) equipped with a 60m × 0.25mm i.d. wall-coated open tubular capillary column (film thickness: 0.25 μm; liquid phase: DB-FFAP), a split/splitless injector (250°C) and a flame ionization detector (280°C). The chromatograms generated were evaluated with the ChemStation software (Agilent). The A:B(n-X) shorthand notation was used to refer to FAs, where A is the number of carbon atoms, B is the number of double bonds, and (n-X) gives the position of the double bond closest to the terminal methyl group. *T. longicornis* does not have major storage reserves and exhibits TAG as its primary neutral lipids (Fraser, Sargent, and Gamble, 1989); therefore lipid classes were not separated in the present study. Total lipid contents of copepods and prey cultures were calculated as the sum of the mass from of all FAs.

Compound-specific stable isotope analysis (CSIA)

The FA-specific stable isotope composition of carbon in FAMEs extracted was obtained according to Boissonnot, Niehoff, Hagen, Søreide, and Graeve (2016) with a Thermo gas chromatography combustion-isotope ratio mass spectrometry (GC-c-IRMS) system, equipped with a Trace GC Ultra gas chromatograph, a GC Isolink, and a Delta V Plus isotope ratio mass spectrometer connected via a Conflo IV interface (Thermo Scientific Corporation, Bremen, Germany). The chromatograms containing peak areas and C isotope ratios were obtained with the

(instrument-specific) Isodat 3.0 software. The 14:0 and 18:0 FAME reference standards (Iowa University) with known δ-values were used for further calculations.

The equations used by Boissonnot, Niehoff, Hagen, Søreide, and Graeve (2016) to calculate carbon assimilation were also applied in the present study, and are:

Isotopic ratios of FAs

$$\delta^{13}\mathrm{C}\ (‰) = \left[\left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}}\right) - 1\right] * 1000, \quad (1)$$

where R_{sample} and R_{standard} are the ratio of $^{13}C/^{12}C$ in the sample and reference standard, respectively;

Atom percent (atom%), converted δ-values which express isotope data in terms of concentrations to inform on the ^{13}C enrichment in each FA

$$\mathrm{atom\%} = \left(\frac{R_{\mathrm{sample}}}{(R_{\mathrm{sample}}+1)}\right) * 100, \quad (2)$$

where R is the ratio $^{13}C/^{12}C$ in the sample;

Atom percent excess (APE), the isotopic enrichment in experimental copepods (in comparison to field individuals) due to the assimilation of ^{13}C-enriched prey cultures

$$\mathrm{APE} = \mathrm{atom\%}_{\text{experimental copepods}} - \mathrm{atom\%}_{\text{field copepods}} \quad (3)$$

Carbon mass of each FA (C_{mass}, in µg C ind^{-1})

$$\mathrm{C_{mass}} = \frac{\{(\mathrm{atom\%} / 100 * \mathrm{A_{13C}}) + [(100 - \mathrm{atom\%}) / 100 * \mathrm{A_{12C}}]\} * \mathrm{C_{FA}} * \mathrm{FA_{mass}}}{\{[(\mathrm{atom\%} / 100 * \mathrm{A_{13C}}) + ((100 - \mathrm{atom\%}) / 100 * \mathrm{A_{12C}})] * \mathrm{C_{FA}}\} + \mathrm{A_{12C}} + (\mathrm{H_{FAME}} * \mathrm{A_H}) + (\mathrm{O_{FAME}} * \mathrm{A_O})}, \quad (4)$$

where A_{12C}, A_{13C}, A_H, and A_O are the atomic masses of ^{12}C, ^{13}C, H and O, respectively, C_{FA} is the number of carbon atoms in the FA, H_{FAME} and O_{FAME} are the number of hydrogen and oxygen atoms in the FAME, and FA_{mass} is the mass (in µg ind^{-1}) of the FA;

Proportion of carbon assimilated (PA)

$$PA = \frac{APE}{L}, \quad (5)$$

where L is the atom% averaged for all prey FAs except for 18:0. The averaging is to account for the elongation and/or desaturation of small amounts of dietary FAs when they are assimilated by the copepods (Dalsgaard, John, Kattner, Müller-Navarra, and Hagen, 2003), and the exclusion of 18:0 is due to it not labelling as well as all other FAs in prey cultures (present study; Boissonnot, Niehoff, Hagen, Søreide, and Graeve, 2016 and references therein). When calculating the PA for FA 18:0 in the copepod, L was the average of labeling in prey FA 18:0. For copepods sampled at t_{48h}, L was calculated from the first two batches of prey cultures used for feeding, whereas for copepods sampled at t_{120h}, L was calculated from all prey culture batches;

FA-specific carbon assimilation (C_{assim}, in µg ^{13}C ind^{-1})

$$C_{assim} = C_{mass} * PA \quad (6)$$

Total C assimilation into lipids was obtained by summing the C_{assim} values for all FAs, and C assimilation as % of food ingested was obtained with the grazing rates reported in Franco-Santos et al. (2018). Assimilation rates were obtained by dividing C_{assim} by the number of experimental days passed when sample was collected;

Carbon turnover rates (C_T in % day^{-1})

$$C_T = \frac{\frac{C_{assim(t)}}{B_{(t)}}}{\Delta t}, \quad (7)$$

where $_{(t)}$ indicates the sampling time for which the values of C_{assim} and B should be used and Δt the number of experimental days when the sample was collected.

Statistical analysis

The statistical significance of differences in C, N, and total lipid contents of prey culture, their C:N molar ratio and atom% was investigated with one-way analyses of variance (ANOVA).

Differences in atom% were tested (a) between the different diets; and (b) within each diet between the different sampling days. When the results were significant, the origin of differences was identified by applying the Tukey HSD (Honestly Significant Difference) post-hoc test with a 95% confidence limit. Prior to the ANOVAs, the data were tested for normality and homogeneity of variances with Shapiro-Wilk and Bartlett tests, respectively. Non-normal and/or heteroscedastic data were either analyzed with ANOVA and Tukey HSD after normalization/homogenization or with the non-parametric test Kruskal–Wallis, followed by the post-hoc Nemenyi test (with P-value being determined with the Tukey method).

Differences in copepod C, N, and total lipid contents and C:N molar ratio were investigated with two-way repeated measures ANOVA. When the results regarding the diet treatment factor were significant, the origin of differences was identified by applying one-way repeated measures ANOVA for each treatment, followed by pairwise Tukey HSD comparisons with a Bonferroni correction (*p*-value significance threshold was set at 0.0167). When the results regarding the sampling day factor were significant, the origin of differences was identified by applying one-way ANOVA for each day, followed by a Tukey HSD test. Because the *in situ* samples were shared between the treatments of Experiment I and Experiment II, these were investigated for differences with a t-test rather than with the Tukey HSD. Repeated measures ANOVA data were tested for normality with graphical methods and residuals analysis and for sphericity with Mauchly's test.

Differences between copepods with regard to ^{13}C labelling (average APE) and rates of C assimilation and turnover within each sampling period (t_{48h}-t_{0h} and t_{120h}-t_{0h}) were tested with one-way ANOVAs, and differences between treatments were identified with a Tukey HSD test. Differences in C assimilation and turnover for SFA, MUFA and PUFA were also investigated between the different diets within each sampling period with one-way ANOVAs. Differences within each diet between sampling days in C assimilation and turnover rates for SFA, MUFA and PUFA were analyzed with paired t-tests, with data previously checked for normality and homoscedasticity with Shapiro-Wilk and F tests, respectively. Problems in the labelling of the

Dino+ diet affected the isotopic enrichment (and, consequently, the calculation of C assimilation and turnover rates) in copepods fed with it, and only the data from copepods fed with the Diat+, Diat– and Dino– diets was used for the statistical tests. All univariate analyses were performed using R ver. 3.4.4 (Ihaka and Gentleman, 1993).

Multivariate analysis of FA composition (untransformed relative FA concentration data for FAs accounting for > 1% total fatty acids, TFA) for both prey cultures and copepods was performed with a non-metric multidimensional scaling (nMDS) method generated from a Bray-Curtis similarity matrix. A one-way analysis of similarity (ANOSIM) was conducted in order to identify differences in the FA profiles of the diets offered to the copepods, and followed by a similarity percentages (SIMPER) analysis to identify the specific FAs contributing to the difference. Differences in the FA profile of *T. longicornis* across factors were investigated with a permutational multivariate analysis of variance (PERMANOVA) with Monte Carlo simulations to account for small sample sizes. Significant results were followed by a test of homogeneity of dispersions (PERMDISP), the multivariate data equivalent of a sphericity test for repeated measures design. Although not a requirement for PERMANOVA, PERMDIST enables, in cases of a significant effects result, the differentiation between differences caused by the treatment and differences caused by the variation within replicates among time points due to their non-independence in repeated measures designs (Anderson et al., 2008). All multivariate analyses were performed with PRIMER 7.0 software (Clarke and Gorley, 2015) with the PERMANOVA+ add-on (Anderson et al., 2008).

RESULTS

Prey culture composition and labelling

Although the Dino+ culture had significantly higher C content, all diets used for feeding copepods contained >500 µg C L^{-1}, confirming the *ad libitum* feeding condition (Table 1). The

281 approximate N content of diet cultures was 200 µg N L^{-1} for Dino +, 100 µg N L^{-1} for Diat+ and
282 Dino–, and 50 µg N L^{-1} for Diat–. The molar C:N ratio was also similar between all treatments
283 (approx. 6) with the exception of the Diat–, which was almost three times greater. Total lipid
284 content in the diets was approximately 100 µg L^{-1} except for Dino+, which had double that amount.
285
286 **Table 1** Carbon (C), nitrogen (N), and total lipid contents, molar C:N ratios (C:N), and average ^{13}C
287 enrichment (of all FAs but 18:0, atom%) of the prey cultures used in the feeding experiment
288 (nutrient-replete and N-depleted *Conticribra weissflogii*, Diat+ and Diat–, and *Oxyrrhis marina*,
289 Dino+ and Dino–, respectively). Values are mean ± standard deviation from four to five batches
290 (temporal replicates).

Experiment	Treatment	pg C $cell^{-1}$	pg N $cell^{-1}$	C:N	pg lipid $cell^{-1}$	atom%
	Diat+	71 ± 5	13 ± 2	6.2 ± 0.6	13 ± 1	2.73 ± 0.11
I	Diat–	79 ± 12	6 ± 1	17.0 ± 3.4	13 ± 2	2.81 ± 0.09
	Dino+	517 ± 110	101 ± 18	6.0 ± 0.3	107 ± 21	1.18 ± 0.05
II	Dino–	273 ± 18	51 ± 4	6.3 ± 0.8	54 ± 9	2.03 ± 0.14

291
292 The FA composition of all prey cultures (Table 2) was mostly characterized by
293 polyunsaturated fatty acids (PUFAs), which accounted for 44 to 67% of the total fatty acid (TFA)
294 concentration. Saturated (SFA) and monounsaturated (MUFA) fatty acids were equally present in
295 the Diat+ (20% TFA) and Diat– (30% TFA) diets, but in the Dino+ and Dino– diets SFAs were
296 present in higher relative concentrations (26 and 41% TFA, respectively) than MUFAs (7 and 15%
297 TFA, respectively). The most important FAs in the Diat+ and Diat– cultures were 20:5(n-3)
298 (eicosapentaenoic acid, EPA, with 21 and 18% TFA, respectively), 16:1(n-7) (16 and 26% TFA,
299 respectively), 16:3(n-4) (20 and 12% TFA, respectively), and 16:0 (14 and 19% TFA, respectively).
300 16:0 and 22:6(n-3) (docosahexaenoic acid, DHA) were the dominant FAs in dinoflagellate cultures
301 (19 and 29% TFA in Dino+ and 31 and 30% TFA in Dino–, respectively), with a high importance
302 also of 18:3(n-3) in Dino+ (15% TFA). The FAs 16:2(n-4) and 16:3(n-4) were only detected in the

diatom cultures. The ANOSIM (global R: 0.974, p = 0.001) indicated differences in FA composition between the prey cultures, which the nMDS (Fig. 1, stress 0.06) and SIMPER (Supplementary Table S1) showed to be a clear separation between the diatom and dinoflagellate cultures. The Diat+ culture was mostly different from the Dino cultures in terms of the FAs DHA (16-18%) and 16:3(n-4) (15-16%), whereas the Diat– culture was mostly different from the Dino cultures due to the FAs 16:1(n-7) (20-22%) and DHA (19-21%).The nMDS and SIMPER results also indicated that the nutrient-replete and N-depleted treatments were similar within the diatom and dinoflagellate cultures, mostly because of the FAs 16:1(n-7) (23%) and 16:3(n-4) (23%) for the former and to the FAs 16:0 (23%), 18:4(n-3) (20%), and 18:3(n-3) (14%) for the latter. The FAs showed significantly higher ^{13}C enrichment in the diatom cultures (~ 3 atom%) and lower in the Dino+ diet (1.18 atom%). Labelling was significantly higher in the first batch cultures (i.e., days), with the exception for the Diat+ batch from day 5 (Fig. 2 and Supplementary Tables ST1 and ST2). FAs within the same diet had similar ^{13}C enrichment, with the exception of 18:0, which varied from 55 to 93% of the average atom% of all FAs in a given diet. Nevertheless, EPA, DHA, 18:3(n-3) and 18:4(n-3) were the FAs with better labelling in all diets but Dino+ (Table2). Cultures were significantly different from one another in all of these variables (Supplementary Tables S1 and S2).

Table 2 Prey culture absolute (Mass, in µg L^{-1}) and relative (% total fatty acid, TFA) fatty acid (FA) compositions (> 1% TFA) and ^{13}C enrichment (atom%). *Conticribra weissflogii* and *Oxyrrhis marina* were cultured in nutrient replete (Diat+ and Dino+, respectively) and nitrogen depleted (Diat– and Dino–, respectively) conditions. Values are means ± standard deviation from prey cultures and from the sum of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (n = 4 or 5). – : not detected.

	Diat+			Diat–			Dino+			Dino-		
FA	Mass	% TFA	atom%	Mass	% TFA	atom%	Mass	% TFA	atom%	Mass	% TFA	atom%
14:0	6.6 ± 0.6	6.7 ± 1.0	2.8 ± 0.1	6.3 ± 1.5	5.9 ± 0.6	2.9 ± 0.1	9.3 ± 2.1	4.4 ± 0.4	1.2 ± 0.1	5.0 ± 2.6	4.2 ± 1.1	2.0 ± 0.2
i 15:0	0.7 ± 0.1	0.8 ± 0.2	2.7 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	2.7 ± 0.1	1.5 ± 0.4	0.7 ± 0.2	1.2 ± 0.1	1.4 ± 0.2	1.2 ± 0.2	2.0 ± 0.3
15:0	1.0 ± 0.1	1.0 ± 0.1	2.8 ± 0.1	1.8 ± 0.5	1.7 ± 0.2	2.9 ± 0.1	1.5 ± 0.2	0.8 ± 0.2	1.2 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	1.9 ± 0.1
16:0	10.8 ± 7.2	10.4 ± 6.9	2.8 ± 0.1	19.4 ± 2.7	18.5 ± 0.5	2.9 ± 0.1	39.8 ± 6.4	19.0 ± 2.7	1.2 ± 0.1	34.3 ± 7.9	30.2 ± 0.8	2.0 ± 0.1
16:1n7	15.9 ± 0.5	16.2 ± 1.5	2.8 ± 0.1	27.3 ± 6.8	25.7 ± 2.3	2.9 ± 0.1	1.6 ± 0.4	0.8 ± 0.1	1.2 ± 0.1	1.9 ± 1.8	1.5 ± 1.0	1.7 ± 0.1
16:2n4	9.1 ± 1.6	9.1 ± 0.9	2.8 ± 0.1	4.2 ± 0.3	4.0 ± 0.3	2.9 ± 0.1	-	-	-	-	-	-
16:3n4	19.7 ± 2.4	20.2 ± 5.0	2.8 ± 0.1	11.2 ± 0.7	10.8 ± 1.9	2.9 ± 0.1	-	-	-	-	-	-
18:0	0.6 ± 0.1	0.6 ± 0.1	1.5 ± 0.2	1.0 ± 0.3	1.0 ± 0.2	1.9 ± 0.2	1.7 ±0.5	0.8 ± 0.2	1.1 ± 0.0	3.0 ± 2.3	2.4 ± 1.2	1.2 ± 0.0
18:1n7	0.7 ± 0.1	0.7 ± 0.2	2.5 ± 0.2	0.6 ± 0.1	0.6 ± 0.2	2.3 ± 0.1	11.3 ± 2.0	5.3 ± 0.3	1.2 ± 0.1	5.8 ± 1.0	5.1 ± 0.4	1.9 ± 0.1
18:1n9	0.5 ± 0.1	0.5 ± 0.1	2.6 ± 0.1	1.2 ± 0.5	1.1 ± 0.3	2.9 ± 0.1	2.2 ± 1.0	1.0 ± 0.3	1.2 ± 0.1	8.5 ± 4.2	7.1 ± 1.8	2.1 ± 0.2
18:2n6	1.0 ± 0.1	1.0 ± 0.1	2.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	2.9 ± 0.1	8.3 ± 2.7	3.8 ± 0.6	1.2 ± 0.0	6.8 ± 3.3	5.8 ± 1.4	2.1 ± 0.2
18:3n3	0.2 ± 0.0	0.2 ± 0.0	2.8 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	2.9 ± 0.1	32.9 ± 11.6	15.1 ± 3.0	1.1 ± 0.0	9.7 ± 4.8	8.2 ± 2.2	2.2 ± 0.1
18:4n3	3.7 ± 1.3	3.6 ± 1.0	2.8 ± 0.1	4.9 ± 1.5	4.6 ± 0.7	2.9 ± 0.1	28.2 ± 9.9	12.9 ± 2.5	1.2 ± 0.1	3.4 ± 2.2	2.8 ± 1.1	2.2 ± 0.1

20:4n3	0.2 ± 0.0	0.3 ± 0.1	2.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	2.8 ± 0.1	1.3 ± 0.2	0.6 ± 0.1	1.2 ± 0.0	1.0 ± 0.9	1.1 ± 1.0	2.1 ± 0.0
20:5n3	21.1 ± 2.3	21.2 ± 0.6	2.8 ± 0.1	19.2 ± 2.5	18.3 ± 0.7	2.9 ± 0.1	10.7 ± 2.4	5.0 ± 0.3	1.2 ± 0.1	3.5 ± 0.4	3.1 ± 0.4	2.1 ± 0.1
22:6n3	5.6 ± 0.5	5.6 ± 0.5	2.8 ± 0.1	4.7 ± 0.2	4.5 ± 0.5	2.9 ± 0.1	59.7 ± 5.9	28.6 ± 3.4	1.2 ± 0.1	28.7 ± 2.6	26.6 ± 7.8	2.1 ± 0.1
ΣSFA	19.7 ± 7.2	19.4 ± 5.9	-	28.9 ± 4.8	27.5 ± 0.4	-	55.1 ± 9.1	26.2 ± 3.5	-	44.7 ± 13.2	40.6 ± 5.1	-
ΣMUFA	18.3 ± 0.6	18.6 ± 1.8	-	30.0 ± 7.1	28.3 ± 2.3	-	15.1 ± 3.2	7.1 ± 0.2	-	16.2 ± 6.9	14.5 ± 3.6	-
ΣPUFA	61.2 ± 3.1	62.0 ± 4.1	-	46.3 ± 4.3	44.3 ± 2.6	-	142.7 ± 32.2	66.7 ± 3.5	-	47.6 ± 4.4	44.9 ± 8.6	-

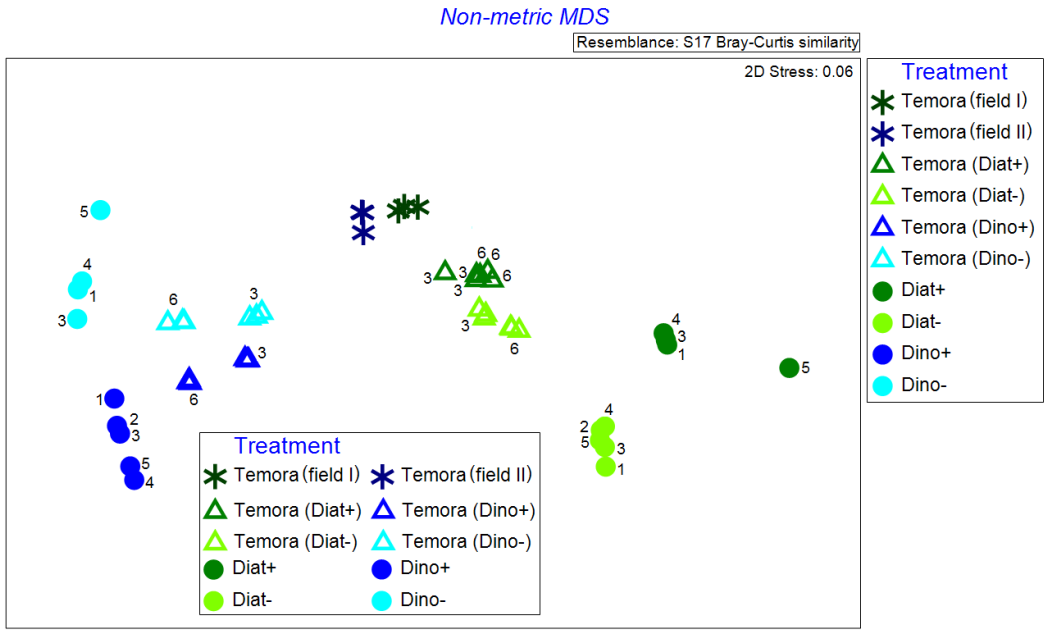

Figure 1: Non-metric multidimensional scaling (nMDS) generated with a Bray-Curtis similarity on untransformed, relative (>1% TFA) concentrations of FAs for field (Experiments I and II) and experimental (Temora (Diat+), Temora (Diat–), Temora (Dino+) and Temora (Dino–)) copepods and for the prey cultures (Diat+, Diat–, Dino+ and Dino–). Numbers represent the day the copepods and prey cultures were sampled during the experiments. Temora: *Temora longicornis*; Diat+: nutrient-replete *Conticribra weissflogii*; Diat–: Nitrogen-depleted *C. weissflogii*; Dino+: nutrient-replete *Oxyrrhis marina*; Dino–: Nitrogen-depleted *O. marina*.

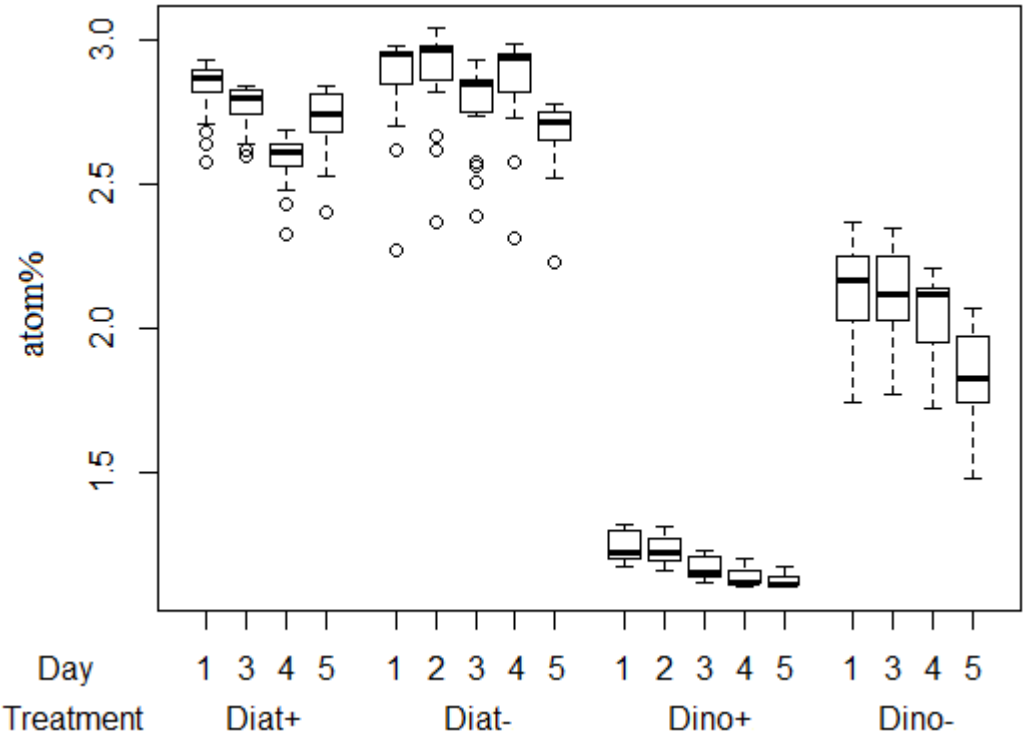

Figure 2: ^{13}C enrichment (atom%) for each prey batch culture grown for the feeding experiments. Batches were created every day consecutively for five days, and let grow for five days before being used to feed copepods. Prey used were the diatom *Conticribra weissflogii* cultured in nutrient-replete and nitrogen-depleted conditions (Diat+ and Diat–, respectively) and the dinoflagellate *Oxyrrhis marina*, fed daily with prey (*Rhodomonas salina*) cultured in nutrient-replete and nitrogen-depleted conditions (Dino+ and Dino–, respectively).

Copepods

Composition

The average C, N, and total lipid contents and molar C:N ratio of copepods at each sampling time are shown in Table 3. All values increased significantly in the first two days of the experiment (Supplementary Tables S1 and S2). Copepod C content varied between 15 and 20 µg C copepod^{-1} and was significantly higher in the copepods fed with Diat+ than in those fed with N-depleted prey at t_{48h} (Supplementary Tables S1 and S2). N content, which varied between 4 and 5 µg N copepod^{-1}, was significantly lower in the Diat– treatment at t_{48h} (Supplementary Tables S1 and S2). Lipid content varied between 2 and 4 µg copepod^{-1} and was significantly higher in the copepods feeding on Diat– (Supplementary Tables S1 and S2). The lipid C content increased throughout the experiment, from 8-9% total body C in field copepods to approximately 10 and 12% total body C at

347 t_{48h} and t_{120h}, respectively, in the Diat+ and dinoflagellate diets. Copepods fed with Diat– had the
348 highest relative lipid C content, 13 and 15% total body C at t_{48h} and t_{120h}, respectively. Molar C:N
349 ratios, which ranged from 4.4 to 5.3, were significantly higher in the Diat– copepods at t_{48h}, and
350 significantly lower in Diat+ and Dino– in comparison to Diat– and Dino+ at t_{120h} (Supplementary
351 Tables S1 and S2).
352
353 **Table 3** Carbon (C), nitrogen (N), and total lipid C contents (in µg C, N, and lipids copepod^{-1},
354 respectively), molar C:N ratios, and ^{13}C enrichment (APE) of *Temora longicornis* females. Samples
355 were obtained at t_{0h} (*in situ* condition), t_{48h} (after 48h of experiment), and t_{120h} (at the end of the
356 experiment). Diet treatments: single cultures of *Conticribra weissflogii* (Diat) and *Oxyrrhis marina*
357 (Dino) in nutrient-replete (+) and N-depleted (–) conditions. Values presented are mean ± standard
358 deviation of triplicates.

	C			N			molar C:N ratio			Lipid			APE%	
Diets	t_{0h}	t_{48h}	t_{120h}	t_{0h}	t_{48h}	t_{120h}	t_{0h}	t_{48h}	t_{120h}	t_{0h}	t_{48h}	t_{120h}	t_{48h}	t_{120h}
Diat+	16 ± 1	20 ± 0	20 ± 1	4 ± 0	5 ± 0	5 ± 0	4.7 ± 0.1	4.9 ± 0.1	4.7 ± 0.1	2 ± 0	3 ± 0	3 ± 0	0.74 ± 0.03	0.94 ± 0.03
Diat–	16 ± 1	18 ± 1	18 ± 2	4 ± 0	4 ± 0	4 ± 0	4.7 ± 0.1	5.3 ± 0.2	5.0 ± 0.1	2 ± 0	3 ± 0	4 ± 0	0.73 ± 0.01	1.02 ± 0.02
Dino+	15 ± 0	20 ± 0	20 ± 2	4 ± 0	5 ± 0	5 ± 0	4.4 ± 0.1	4.8 ± 0.0	5.0 ± 0.1	2 ± 0	3 ± 0	3 ± 0	0.10 ± 0.01	0.08 ± 0.00
Dino–	15 ± 0	18 ± 1	18 ± 1	4 ± 0	5 ± 0	4 ± 0	4.4 ± 0.1	4.6 ± 0.1	4.8 ± 0.0	2 ± 0	2 ± 0	3 ± 0	0.52 ± 0.03	0.70 ± 0.03

359
360 The relative FA composition of *T. longicornis* changed when copepods were introduced to
361 experimental diets and reflected that of their prey, though *in situ* composition seemed to indicate
362 feeding on diatoms in the field (Table 4, Figs 1 and 3). In copepods from the field, SFA, MUFA and
363 PUFA accounted for approximately 25, 10, and 65% TFA. Feeding on the Diat+ and dinoflagellate
364 diets provided a similar partition of SFA, MUFA and PUFA, but feeding on Diat– increased the
365 relative MUFA content (21% TFA) and decreased the relative PUFA content (55% TFA) in
366 copepods. EPA, DHA and 16:0 were the major FA in *T. longicornis* throughout the experiment.
367 EPA was the major FA constituent in diatom-fed copepods, whereas DHA was the major FA in

dinoflagellate-fed copepods. Together, EPA and DHA accounted for 54-72% TFA in copepods fed with diatoms, and approximately 70-75% TFA in copepods fed with dinoflagellates. The FA 16:1(n-7) accounted for another 8-16% TFA in diatom-fed copepods, and the FAs 18:3(n-3) and 18:4(n-3) together made up 11-16% TFA in the copepods fed with the Dino+ diet. The FAs 16:2(n-4) and 16:3(n-4) were only detected in copepods fed with diatoms. The difference in relative abundance for the previously mentioned major FAs between the beginning and the end of the experiments (t_{120h}–t_{0h}) can be visualized in Fig. 3, and the difference in absolute composition (for FA > 1% TFA) in Fig. 4. The relative abundance of the FAs 16:0 and 18:4(n-3) increased in all treatments, and decreased for EPA. The diatom FATMs (16:1(n-7), 16:2(n-4), and 16:3(n-4)) increased in diatom-fed copepods and decreased in dinoflagellate-fed females, whereas the dinoflagellate FATMs (18:3(n-3) and DHA) decreased in the former and increased in the latter (Fig. 3). The nMDS (Fig. 1) suggests a clear separation between diet treatments and between sampling days within some diets, showing a continuous change in FA composition through the experimental time. According to the PERMANOVA results (main effects and pairwise comparisons) all treatments are different from one another in all sampling days, and all sampling days within a treatment are different from one another, except for Diat+ at t_{48h} and t_{120h} (Supplementary Table S3). It was not possible to confirm that these differences are solely due to treatment effects, as the PERMDIST result was significant (F = 704.82, df1 = 2, df2 = 33, P(perm) = 0.001).

386 **Table 4** Absolute (in ng C ind^{-1}) and relative (% total fatty acid, TFA, in parenthesis) FA composition (>1% total FAs) of *Temora longicornis* females
387 at the beginning (t_{0h}, *in situ* condition), middle (t_{48h}, after 48h of experiment), and end (t_{120h}) of the feeding experiment. Diet treatments: single cultures
388 of *Conticribra weissflogii* (Diat) and *Oxyrrhis marina* (Dino) in nutrient-replete (+) and N-depleted (–) conditions. Values presented are mean ±
389 standard deviation of triplicates. Values are mean ± standard deviation (n = 3 each) from each FA and from the sum of all (TFA), saturated (SFA),
390 monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids (n = 4 or 5). – : not detected.

	Diat+			Diat–			Dino+			Dino–		
FA	t_{0h}	t_{48h}	t_{120h}	t_{0h}	t_{48h}	t_{120h}	t_{0h}	t_{48h}	t_{120h}	t_{0h}	t_{48h}	t_{120h}
TFA	1509 ± 92	2129 ± 116	2105 ± 129	1509 ± 92	2469 ± 221	2707 ± 365	1249 ± 170	2025 ± 111	2509 ± 91	1249 ± 170	1794 ± 226	2117 ± 146
14:0	65 ± 17 (5 ± 1)	89 ± 11 (5 ± 0)	70 ± 9 (4 ± 0)	65 ± 17 (5 ± 1)	106 ± 15 (5 ± 1)	104 ± 19 (4 ± 0)	46 ± 12 (4 ± 0)	52 ± 1 (3 ± 0)	67 ± 2 (3 ± 0)	46 ± 12 (4 ± 0)	43 ± 4 (3 ± 0)	48 ± 10 (2 ± 0)
16:0	207 ± 13 (14 ± 0)	322 ± 16 (16 ± 0)	320 ± 14 (16 ± 1)	207 ± 13 (14 ± 0)	390 ± 35 (16 ± 0)	424 ± 57 (16 ± 0)	186 ± 27 (16 ± 0)	369 ± 12 (19 ± 1)	452 ± 14 (19 ± 0)	186 ± 27 (16 ± 0)	369 ± 44 (22 ± 0)	473 ± 37 (23 ± 0)
16:1n7	78 ± 9 (5 ± 0)	162 ± 6 (8 ± 0)	155 ± 15 (8 ± 0)	78 ± 9 (5 ± 0)	326 ± 36 (14 ± 0)	429 ± 67 (16 ± 0)	29 ± 6 (2 ± 0)	18 ± 2 (1 ± 0)	13 ± 2 (1 ± 0)	29 ± 6 (2 ± 0)	20 ± 4 (1 ± 0)	18 ± 3 (1 ± 0)
16:2n4	11 ± 1 (0 ± 0)	83 ± 2 (4 ± 0)	92 ± 12 (5 ± 0)	11 ± 1 (0 ± 0)	49 ± 6 (2 ± 0)	80 ± 13 (3 ± 0)	6 ± 1 (1 ± 0)	–	–	6 ± 1 (1 ± 0)	–	–
16:3n4	6 ± 1 (0 ± 0)	88 ± 67 (4 ± 3)	156 ± 24 (8 ± 1)	6 ± 1 (0 ± 0)	88 ± 11 (4 ± 0)	158 ± 30 (6 ± 0)	5 ± 1 (0 ± 0)	–	–	5 ± 1 (0 ± 0)	–	–
18:0	47 ± 4 (3 ± 0)	45 ± 2 (2 ± 0)	45 ± 8 (2 ± 1)	47 ± 4 (3 ± 0)	61 ± 8 (3 ± 0)	55 ± 7 (2 ± 0)	53 ± 14 (4 ± 1)	48 ± 4 (3 ± 0)	39 ± 1 (2 ± 0)	53 ± 14 (4 ± 1)	49 ± 7 (3 ± 0)	33 ± 2 (2 ± 0)
18:1n7	52 ± 2 (4 ± 0)	67 ± 3 (3 ± 0)	69 ± 3 (3 ± 0)	52 ± 2 (4 ± 0)	81 ± 9 (3 ± 0)	87 ± 11 (3 ± 0)	37 ± 4 (3 ± 0)	77 ± 5 (4 ± 0)	108 ± 5 (4 ± 0)	37 ± 4 (3 ± 0)	61 ± 7 (4 ± 0)	78 ± 6 (4 ± 0)

18:1n9	23 ± 3 (2 ± 0)	17 ± 2 (1 ± 0)	19 ± 8 (1 ± 0)	23 ± 3 (2 ± 0)	32 ± 9 (1 ± 0)	29 ± 4 (1 ± 0)	13 ± 4 (1 ± 0)	11 ± 3 (1 ± 0)	16 ± 1 (1 ± 0)	13 ± 4 (1 ± 0)	47 ± 11 (3 ± 1)	84 ± 7 (4 ± 0)
18:2n6	12 ± 1 (1 ± 0)	14 ± 1 (1 ± 0)	13 ± 1 (1 ± 0)	12 ± 1 (1 ± 0)	17 ± 3 (1 ± 0)	17 ± 2 (1 ± 0)	7 ± 2 (1 ± 0)	31 ± 3 (2 ± 0)	59 ± 4 (2 ± 0)	7 ± 2 (1 ± 0)	40 ± 7 (2 ± 0)	83 ± 6 (4 ± 0)
18:3n3	8 ± 1 (1 ± 0)	7 ± 1 (0 ± 0)	6 ± 2 (0 ± 0)	8 ± 1 (1 ± 0)	10 ± 2 (0 ± 0)	9 ± 2 (0 ± 0)	7 ± 2 (1 ± 0)	122 ± 8 (6 ± 0)	234 ± 15 (9 ± 0)	7 ± 2 (1 ± 0)	60 ± 9 (3 ± 0)	121 ± 10 (6 ± 0)
18:4n3	18 ± 1 (1 ± 0)	64 ± 1 (3 ± 0)	59 ± 7 (3 ± 0)	18 ± 1 (1 ± 0)	72 ± 7 (3 ± 0)	92 ± 15 (3 ± 0)	16 ± 3 (1 ± 0)	97 ± 6 (5 ± 0)	159 ± 11 (6 ± 0)	16 ± 3 (1 ± 0)	25 ± 4 (1 ± 0)	41 ± 4 (2 ± 0)
20:5n3	589 ± 34 (38 ± 0)	717 ± 26 (33 ± 1)	686 ± 42 (32 ± 1)	589 ± 34 (38 ± 0)	761 ± 61 (30 ± 1)	752 ± 89 (27 ± 0)	457 ± 41 (36 ± 2)	337 ± 21 (16 ± 0)	270 ± 9 (11 ± 0)	457 ± 41 (36 ± 2)	353 ± 55 (19 ± 1)	249 ± 25 (12 ± 1)
22:6n3	299 ± 19 (19 ± 1)	338 ± 16 (15 ± 1)	317 ± 13 (15 ± 1)	299 ± 19 (19 ± 1)	336 ± 19 (13 ± 1)	330 ± 33 (12 ± 0)	305 ± 43 (24 ± 0)	758 ± 41 (36 ± 0)	973 ± 31 (38 ± 0)	305 ± 43 (24 ± 0)	639 ± 64 (35 ± 1)	805 ± 37 (37 ± 1)
ΣSFA	334 ± 30 (23 ± 1)	483 ± 26 (24 ± 1)	457 ± 17 (23 ± 1)	334 ± 30 (23 ± 1)	593 ± 59 (25 ± 1)	624 ± 88 (24 ± 0)	301 ± 56 (25 ± 1)	505 ± 9 (26 ± 1)	600 ± 17 (25 ± 0)	301 ± 56 (25 ± 1)	483 ± 57 (28 ± 0)	578 ± 50 (29 ± 1)
ΣMUFA	188 ± 15 (13 ± 0)	287 ± 16 (14 ± 1)	280 ± 26 (14 ± 1)	188 ± 15 (13 ± 0)	484 ± 60 (20 ± 1)	589 ± 86 (22 ± 0)	103 ± 18 (8 ± 0)	125 ± 17 (6 ± 1)	156 ± 7 (6 ± 0)	103 ± 18 (8 ± 0)	153 ± 31 (9 ± 1)	199 ± 16 (10 ± 0)
ΣPUFA	987 ± 52 (64 ± 1)	1360 ± 92 (62 ± 2)	1368 ± 95 (64 ± 1)	987 ± 52 (64 ± 1)	1391 ± 111 (55 ± 1)	1494 ± 191 (54 ± 0)	845 ± 97 (66 ± 0)	1131 ± 414 (168 ± 21)	1754 ± 70 (69 ± 0)	845 ± 97 (66 ± 0)	1158 ± 143 (63 ± 1)	1340 ± 81 (62 ± 1)

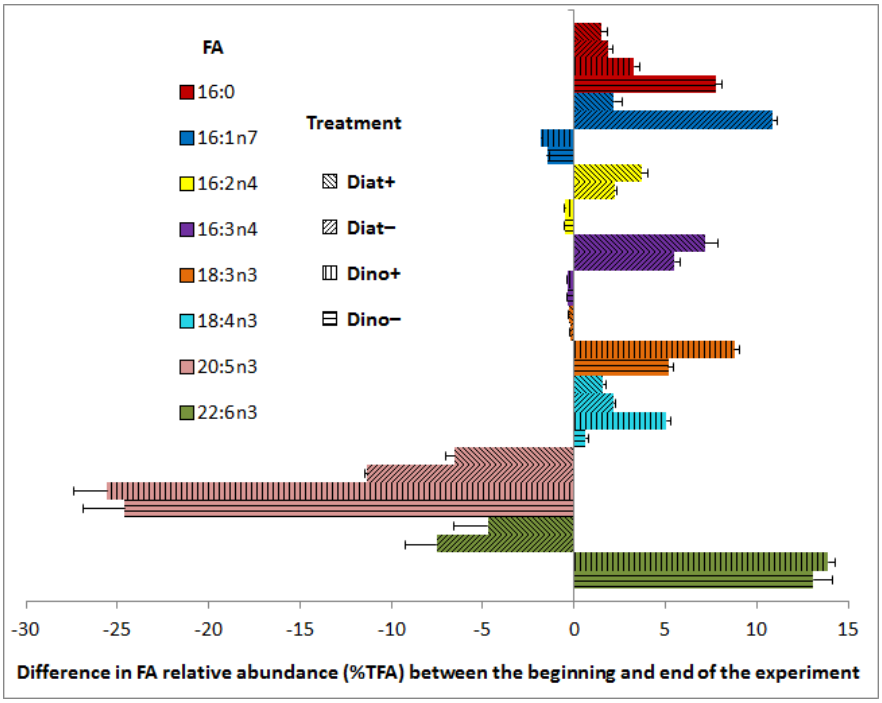

Figure 3: Difference in *Temora longicornis* fatty acid (FA) relative abundance (% total FA) between the beginning and end of the feeding experiments ($t_{120h} - t_{0h}$). Color legend represents the main FAs in the copepods, whereas fill pattern legend indicates the feeding treatment applied. Diat+ and Diat–: nutrient-replete and Nitrogen-depleted *Conticribra weissflogii*; respectively. Dino+ and Dino–: nutrient- replete and Nitrogen-depleted *Oxyrrhis marina*. Values are mean ± standard deviation of triplicate samples.

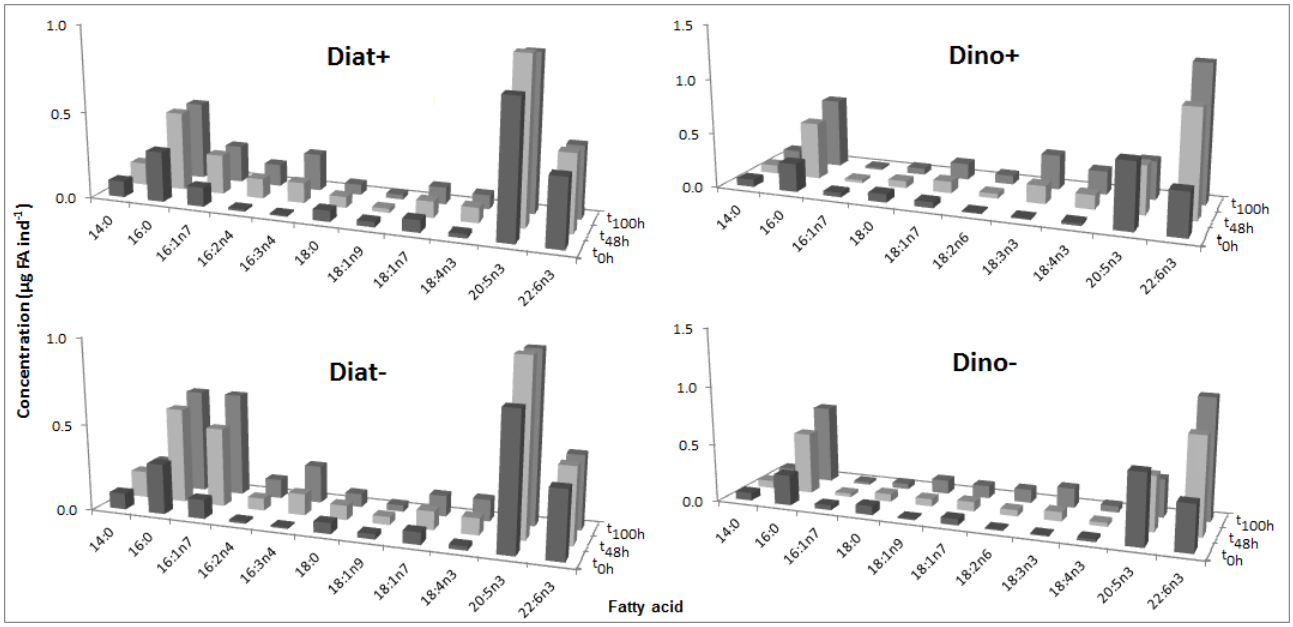

Figure 4: Change in *Temora longicornis* main fatty acids (FA, >1 % total FA) absolute abundance (µg FA ind^{-1}) between the beginning (t_{0h}), middle (t_{48h}), and end (t_{120h}) of the feeding experiments in relation to the diet treatments they received. Diets were made of monocultures of diatoms (*Conticribra weissflogii*) and dinoflagellates (*Oxyrrhis marina*) cultured in nutrient-replete (Diat+ and Dino+, respectively) and in nitrogen-depleted (Diat– and Dino–, respectively) conditions.

403 *Labelling*

404 The average ^{13}C enrichment of copepod FAs, which varied between 0.1 and 1.0 APE (Table
405 3, FA-specific APE in Supplementary Table S4), increased significantly from day 3 to day 6 in all
406 treatments but Dino+, for which it significantly decreased (Supplementary Tables S1 and S2). The
407 APE values were lowest for the Dino+ treatment. Within the same sampling day, the APE of
408 diatom-fed copepods was significantly higher than that of copepods fed with Dino–, and those fed
409 with Diat– had the highest APE at the end of the experiment (Supplementary Tables S1 and S2).
410 The FA-specific ^{13}C enrichment in copepods on days 3 and 6 is shown in Table 5. The diatom
411 FATMs 16:2(n-4) and 16:3(n-4) had some of the better labelling results in copepods fed with these
412 diets, whereas the dinoflagellate FATMs 18:3(n-3) and 18:4(n-3) showed the highest labelling in
413 Dino–. The FA 18:4(n-3) was also well labelled in the Diat– diet. FAs were homogeneously
414 labelled in copepods fed with the Dino+ diet, though DHA seemed to label slightly better.
415
416 **Table 5** Total (TFA) and fatty acid (FA)-specific lipid carbon (C) assimilation (as ng C ind^{-1} for
417 TFA and as % TFA assimilated for specific FAs) between t_{0h}-t_{48h} and between t_{0h}-t_{120h} for *Temora*
418 *longicornis* females. Value in parenthesis for TFA indicates lipid C assimilation as % C ingested
419 reported by Franco-Santos et al. (2018) between t_{0h}-t_{48h}. Diet treatments: single cultures of
420 *Conticribra weissflogii* (Diat) and *Oxyrrhis marina* (Dino) in nutrient-replete (+) and N-depleted (–
421) conditions. SFA: saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA;
422 $PUFA_3$: set of FAs 18:3(n-3), 20:5(n-3), and 22:6(n-3). Values are mean ± standard deviation (n = 3
423 each). – : not detected.

	C assimilation							
	Diat+		Diat–		Dino+		Dino–	
FA	t_{0h} - t_{48h}	t_{0h} - t_{120h}	t_{0h} - t_{48h}	t_{0h} - t_{120h}	t_{0h} - t_{48h}	t_{0h} - t_{120h}	t_{0h} - t_{48h}	t_{0h} - t_{120h}
TFA	657 ± 46 (5 ± 0)	901 ± 68	755 ± 69 (4 ± 1)	1200 ± 165	201 ± 10 (1 ± 0)	187 ± 4	498 ± 52 (6 ± 2)	842 ± 47
14:0	4 ± 0	3 ± 0	4 ± 0	4 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0

i-15:0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
15:0	1 ± 0	1 ± 0	2 ± 0	1 ± 0	1 ± 0	1 ± 0	0 ± 0	0 ± 0
16:0	16 ± 1	16 ± 0	18 ± 0	17 ± 0	17 ± 0	16 ± 0	24 ± 0	23 ± 0
16:1n5	1 ± 0	1 ± 0	1 ± 0	1 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
16:1n7	11 ± 1	9 ± 0	23 ± 0	21 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
16:2n4	7 ± 0	6 ± 0	3 ± 0	4 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
16:3n4	8 ± 6	11 ± 1	7 ± 0	8 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
16:4n1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
17:0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0	1 ± 0	0 ± 0	0 ± 0
18:0	1 ± 0	2 ± 0	1 ± 0	2 ± 0	1 ± 0	1 ± 0	2 ± 0	1 ± 0
18:1n7	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0
18:1n9	0 ± 0	0 ± 0	1 ± 0	1 ± 0	0 ± 0	0 ± 0	4 ± 0	5 ± 0
18:2n6	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	2 ± 0	4 ± 0	5 ± 0
18:3n3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	5 ± 0	5 ± 0	5 ± 3	7 ± 0
18:3n6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2 ± 3	0 ± 0
18:4n3	5 ± 0	4 ± 0	5 ± 0	5 ± 0	5 ± 0	4 ± 0	1 ± 1	2 ± 0
20:1n7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
20:1n9	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0
20:2n6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0	1 ± 0	1 ± 0
20:4n3	0 ± 0	0 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	0 ± 0
20:4n6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
20:5n3	31 ± 2	32 ± 1	24 ± 1	24 ± 1	8 ± 0	8 ± 0	6 ± 0	6 ± 0
22:1n7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
22:1n9	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
22:5n3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
22:6n3	8 ± 1	10 ± 1	6 ± 0	7 ± 0	53 ± 0	54 ± 0	44 ± 1	41 ± 1
ΣSFA	22 ± 1	20 ± 0	25 ± 1	24 ± 0	22 ± 1	21 ± 0	29 ± 0	28 ± 0
ΣMUFA	15 ± 1	13 ± 0	28 ± 0	26 ± 0	4 ± 0	5 ± 0	8 ± 0	9 ± 0
ΣPUFA	63 ± 2	65 ± 0	47 ± 1	50 ± 1	74 ± 0	75 ± 0	63 ± 0	63 ± 1

424

425

426 *Lipid C assimilation*

427 Total assimilation of C into copepod lipids, which varied between 0.2 and 1.2 µg C ind^{-1} at
428 the end of the experiment, was higher in copepods fed with diatoms than in those fed with
429 dinoflagellates (Table 5). C assimilation into SFAs was higher for copepods fed with the N-depleted
430 diets (both in absolute and relative terms), whereas assimilation into MUFAs and PUFAs was
431 higher for copepods fed with diatom diets (both in absolute and relative terms) (Table 5, Fig. 5).
432 Absolute C assimilation into PUFAs and, more specifically, into the FAs 18:3(n-3), EPA and DHA,
433 was higher for copepods fed with the Diat+ prey, but relative assimilation was higher for copepods
434 fed with dinoflagellates (Table 5, Fig. 5). Copepods assimilated a higher amount of PUFAs than
435 MUFAs and SFAs in all diet treatments (Fig. 5). The C assimilation rates were significantly higher
436 for all treatments (but for Diat–) in the first two days than for the entire duration of the experiment
437 (Supplementary Table S1). At t_{48h} the rates were significantly higher for copepods on the diatom
438 diets than for those feeding on Dino–, but by the end of the experiment significantly higher rates
439 were recorded for the copepods fed with Diat– (Supplementary Tables ST1 and ST2). C
440 assimilation in FATM on days 3 and 6 was also different between copepods fed with diatoms and
441 dinoflagellates, and reflected the FA composition of their respective diets (Table 5, Fig. 6). For
442 Diat+ and Diat– copepods, most of the total lipid C assimilated was into EPA (~30 and 25%,
443 respectively), followed by 16:0 (~15%), 16:1(n-7) (~10 and 20%, respectively), and 16:3(n-4) and
444 DHA (~10% each). For Dino+ and Dino– copepods, most of the total lipid C assimilated was into
445 DHA (~55 and 42%, respectively), followed by 16:0 (16 and 24%, respectively), EPA (8 and 6%,
446 respectively), and 18:3(n-3) (~5%). Lipid C assimilation between t_{0h} and t_{48h} accounted for between
447 1 and 6% of the food ingested (in terms of C content) at the same time period (Table 5), and was not
448 significantly different between copepods fed with diatom diets and Dino–.
449

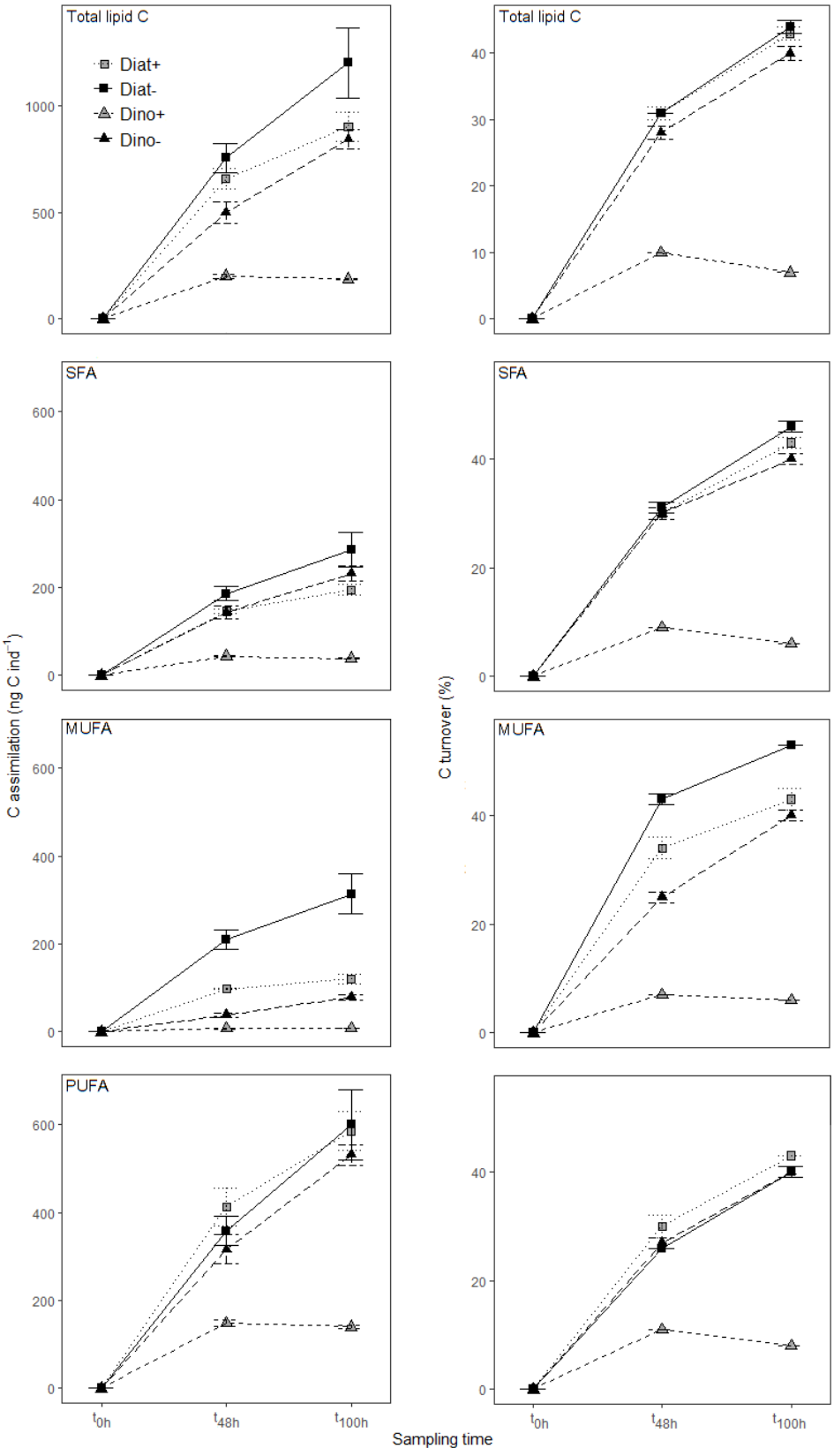

Figure 5: Carbon (C) assimilation (ng C ind^{-1}) and turnover rate (%) recorded for *Temora longicornis* at the beginning (t_{0h}), middle (t_{48h}), and end (t_{120h}) of the feeding experiments in relation to the diet treatments. Values are shown for total lipids (sum of all fatty acids identified) and for saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids. Diet treatments consisted of monocultures of diatoms (*Conticribra weissflogii*) and dinoflagellates (*Oxyrrhis marina*) cultured in nutrient-replete (Diat+ and Dino+, respectively) and in nitrogen-depleted (Diat– and Dino–, respectively) conditions. Values are mean ± standard deviation of triplicate samples.

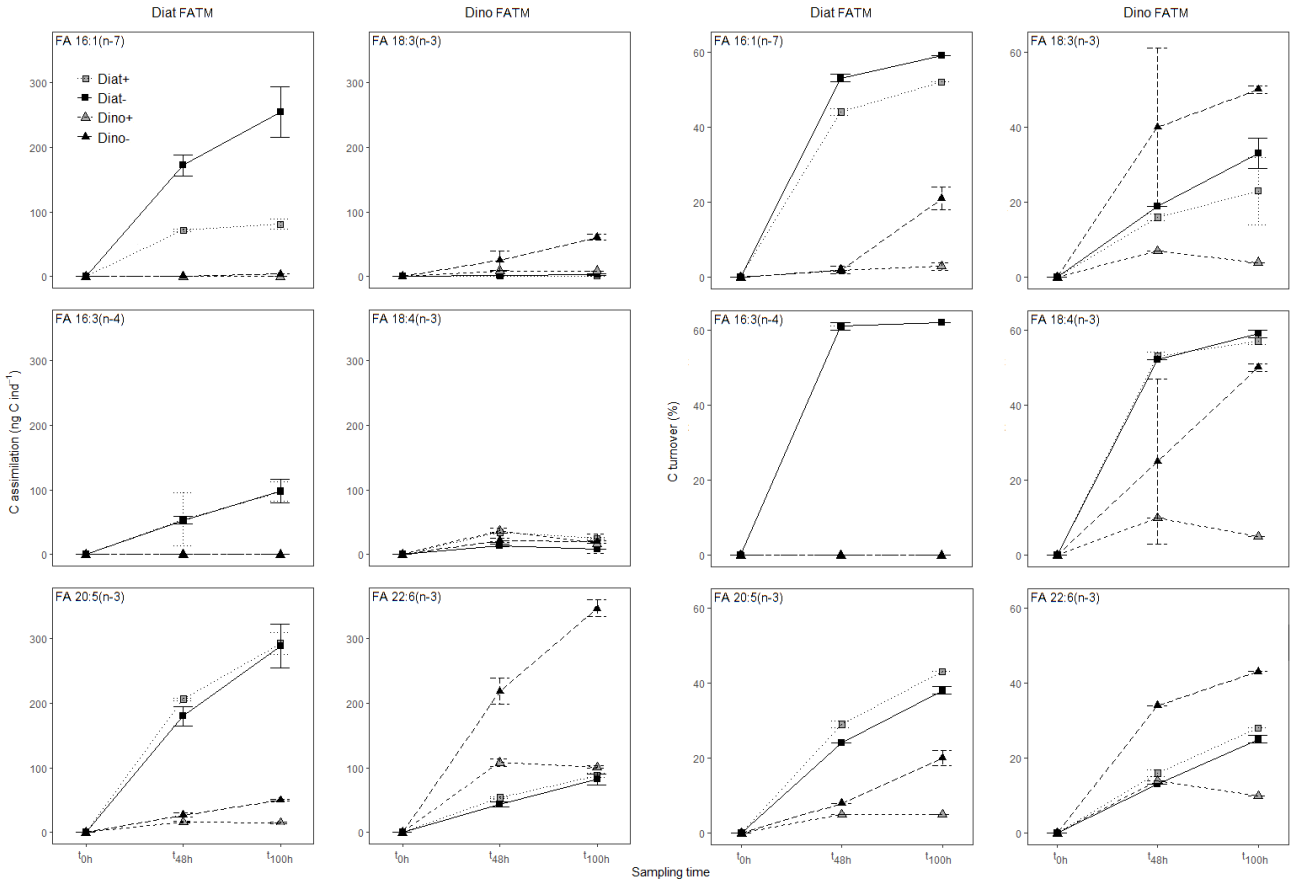

Figure 6: Carbon (C) assimilation (ng C ind^{-1}) and turnover rate (%) recorded for *Temora longicornis* at the beginning (t_{0h}), middle (t_{48h}), and end (t_{120h}) of the feeding experiments in relation to the diet treatments. Values are shown for diatom (16:1(n-7), 16:3(n-4), and 20:5(n-3)) and dinoflagellate (18:3(n-3), 18:4(n-3), and 22:6(n-3)) fatty acid trophic markers (FATM). Diet treatments consisted of monocultures of diatoms (*Conticribra weissflogii*) and dinoflagellates (*Oxyrrhis marina*) cultured in nutrient-replete (Diat+ and Dino+, respectively) and in nitrogen-depleted (Diat– and Dino–, respectively) conditions. Values are mean ± standard deviation of triplicate samples.

Lipic C turnover

Lipid C turnover rates, which ranged from 1 to 15% ind^{-1} day^{-1} (Table 6), were significantly higher during the first 2 days of experiment than for the total experiment duration (Supplementary Tables S1 and S2). No differences were found between the diatom and Dino– treatments within the same sampling day though. C turnover of SFA, MUFA and PUFA for these three treatments was also similar, though they were usually higher in copepods fed with Diat+ and Diat– (Fig. 5, Table 6). Overall, approximately 40-45% of all lipid C was replaced with labelled dietary lipid by the end of the experiment, except for copepods fed with the Dino+ diet, for which only approximately 10% of lipid C was substituted (Fig. 5). Approximately half (40-45%) of the internal SFA, MUFA, and

PUFA content was also replaced with dietary FAs by the end of the experiment for the diatom and Dino– diets and ~ 10% for Dino+ (Fig. 5). The diatom FATM 16:1(n-7) and 16:3(n-4) showed high C turnover (~ 50 to 60% at the end of the experiment) in diatom-fed copepods and low in dinoflagellate-fed copepods. Even though the diatom diets were relatively poor in the FAs 18:3(n-3) and 18:4(n-3) (< 1% and <5% TFA, respectively), which are dinoflagellate FATM, these FAs had high C turnover (~ 30% and 60% at the end of the experiment, respectively, Fig. 6, Table 6) in copepods fed with Diat+ and Diat–. High C turnover of these dinoflagellate FATMs were also recorded for copepods fed with Dino– (Fig. 6, Table 6). The replacement of internal by dietary EPA and DHA also followed the availability of each of these essential FA in the respective diet. Copepods fed with Diat+ and Diat– replaced 40-45% and 25-30% of their original EPA and DHA by the end of the experiment, respectively, whereas those fed with Dino– substituted 20% and 45% of their original EPA and DHA by the end of the experiment, respectively (Fig. 6). C turnover for copepods fed with Dino+ were usually well below 10%, with maximum rates recorded for DHA (~ 10% at the end of the experiment, Fig. 6, Table 6).

Table 6 Total (TFA) and fatty acid (FA)-specific lipid carbon (C) turnover rate (as % ind^{-1} day^{1}) between t_{0h}-t_{48h} and between t_{0h}-t_{120h} for *Temora longicornis* females. Diet treatments: single cultures of *Conticribra weissflogii* (Diat) and *Oxyrrhis marina* (Dino) in nutrient-replete (+) and N-depleted (–) conditions. SFA: saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; $PUFA_3$: set of FAs 18:3(n-3), 20:5(n-3), and 22:6(n-3). Values are mean ± standard deviation (n = 3 each). – : not detected.

	C turnover							
	Diat+		Diat–		Dino+		Dino–	
FA	t_{0h} - t_{48h}	t_{0h} - t_{120h}	t_{0h} - t_{48h}	t_{0h} - t_{120h}	t_{0h} - t_{48h}	t_{0h} - t_{120h}	t_{0h} - t_{48h}	t_{0h} - t_{120h}
TFA	15 ± 1	9 ± 0	15 ± 0	9 ± 0	5 ± 0	1 ± 1	14 ± 0	8 ± 0
14:0	15 ± 1	9 ± 0	14 ± 0	9 ± 0	3 ± 0	1 ± 0	10 ± 1	7 ± 0

15:0	21 ± 0	10 ± 0	24 ± 1	11 ± 0	7 ± 0	2 ± 0	10 ± 1	7 ± 0
16:0	16 ± 0	9 ± 0	17 ± 0	9 ± 0	5 ± 0	1 ± 0	16 ± 0	8 ± 0
16:1n5	26 ± 1	11 ± 0	22 ± 0	11 ± 0	-	-	-	-
16:1n7	22 ± 0	10 ± 0	27 ± 0	12 ± 0	1 ± 0	1 ± 0	1 ± 0	4 ± 1
16:2n4	29 ± 0	12 ± 0	27 ± 0	12 ± 0	-	-	-	-
16:3n4	31 ± 0	12 ± 0	30 ± 0	12 ± 0	-	-	-	-
16:4n1	8 ± 0	8 ± 0	4 ± 0	4 ± 1	-	-	-	-
17:0	2 ± 1	2 ± 0	8 ± 1	5 ± 0	6 ± 0	2 ± 0	10 ± 0	7 ± 0
18:0	10 ± 0	7 ± 1	9 ± 1	8 ± 0	2 ± 0	1 ± 0	8 ± 0	7 ± 0
18:1n7	13 ± 0	8 ± 1	14 ± 0	9 ± 0	4 ± 0	1 ± 0	14 ± 1	7 ± 0
18:1n9	4 ± 0	1 ± 1	13 ± 1	7 ± 0	3 ± 1	1 ± 0	21 ± 4	10 ± 0
18:2n6	15 ± 1	8 ± 0	12 ± 1	8 ± 0	5 ± 0	1 ± 0	25 ± 0	10 ± 0
18:3n3	8 ± 1	5 ± 2	9 ± 0	7 ± 1	4 ± 0	1 ± 0	20 ± 11	10 ± 0
18:3n6	24 ± 1	11 ± 0	19 ± 0	10 ± 0	4 ± 1	1 ± 0	16 ± 0	8 ± 0
18:4n3	27 ± 0	11 ± 0	26 ± 0	12 ± 0	5 ± 0	1 ± 0	13 ± 11	10 ± 0
20:1n7	6 ± 2	4 ± 2	6 ± 1	4 ± 1	3 ± 1	1 ± 1	0 ± 1	-
20:1n9	2 ± 0	2 ± 0	5 ± 0	4 ± 1	0 ± 1	1 ± 0	15 ± 1	9 ± 0
20:2n6	5 ± 2	5 ± 0	4 ± 1	3 ± 1	4 ± 1	1 ± 0	15 ± 3	9 ± 0
20:4n3	15 ± 2	8 ± 1	18 ± 1	10 ± 0	4 ± 0	1 ± 0	16 ± 2	9 ± 0
20:4n6	7 ± 1	5 ± 1	4 ± 0	4 ± 0	3 ± 1	1 ± 0	5 ± 2	6 ± 0
20:5n3	14 ± 0	9 ± 0	12 ± 0	8 ± 0	2 ± 0	1 ± 0	4 ± 0	4 ± 0
22:1n7	3 ± 3	4 ± 0	4 ± 1	3 ± 0	4 ± 1	1 ± 0	3 ± 1	2 ± 0
22:1n9	0 ± 1	1 ± 2	-2 ± 1	0 ± 1	-	-	-	-
22:5n3	6 ± 0	5 ± 0	8 ± 0	6 ± 0	4 ± 0	1 ± 0	8 ± 0	0 ± 0
22:6n3	8 ± 0	6 ± 0	6 ± 0	5 ± 0	7 ± 0	2 ± 0	17 ± 0	9 ± 0
ΣSFA	15 ± 0	9 ± 0	16 ± 0	9 ± 0	4 ± 0	1 ± 0	15 ± 0	8 ± 0
ΣMUFA	17 ± 1	9 ± 0	22 ± 1	11 ± 0	3 ± 0	1 ± 0	13 ± 1	8 ± 0
ΣPUFA	15 ± 1	9 ± 0	13 ± 0	8 ± 0	5 ± 0	2 ± 0	14 ± 0	8 ± 0

DISCUSSION

Lipid C assimilation

The results obtained in the present study indicate that feeding upon N-depleted food can influence the metabolism of *T. longicornis* females, as the copepods which were fed with the Diat–culture showed the highest lipid C content and assimilation. This is in agreement with results for other calanoid copepods such as *Acartia tonsa*, which accumulated more lipids when feeding on N-depleted in comparison to nutrient-replete *R. salina* (Burian, Grosse, Winder, and Boschker, 2018). However, when total C assimilation is calculated as the % dietary C that was ingested by copepods, there were no significant differences between the treatments. Although the quality of a food source affects the rate at which it is ingested by copepods (Franco-Santos et al., 2018), it would seem that it does not influence its assimilability. A possible explanation is provided by Mitra and Flynn (2005) who suggest that, where nutrient limitation significantly affects the ingestion of prey by zooplankton, it may be unimportant at the stage of food assimilation. Furthermore, it has been shown that, although phosphorus (P) assimilation efficiency differs for *Daphnia pulicaria* feeding on prey of different P (and C) contents, it does not affect its C assimilation efficiency (Urabe, Shimizu, and Yamaguchi, 2017), which could also explain the results obtained herein. Indeed, C was not the limiting element in the prey offered to copepods, so its assimilation from dietary material should not be affected by prey quality, as it was freely available for ingestion.

Copepod life history strategies

The differences in absolute C assimilation were most likely caused by varying copepod ingestion rates, as observed by Franco-Santos et al. (2018). If copepods ingested different amounts of dietary C but assimilated similar quantities, then it follows that they also had different C investment into reproduction. This assumption is confirmed when the C assimilation results of the present study are compared with the EPR reported by Franco-Santos et al. (2018), which were

higher for copepods fed with the Dino+ culture, followed by those fed with Dino–, Diat+, and Diat– (61, 41, 39, and 36 eggs ind^{-1} day^{-1}). As previously assumed, reproductive investment was inversely related to the C assimilation rates of the copepods, and both of these factors were affected by the elemental and biochemical compositions of the prey items.

All experimental diets allowed for lipid accumulation in *T. longicornis*, confirming both an active uptake of dietary FAs and that the quantity of food ingested was more than enough to cover for the metabolic costs of living, even though the latter are specific for each copepod-diet combination (Franco-Santos et al., 2018). The copepods fed with nutrient-replete diatoms did not shown an increase in lipid C between t_{48h} and t_{120h}, such that their metabolic cost during that time period was equal to lipid C turnover, 4% day^{-1}. It should be noted that the lipid C turnover rates decreased by ½ to ¼ from the first 2 days to the last 3 days of the experiments. This could indicate an opportunistic strategy of immediate accumulation of dietary energy when it is readily available, for which speed decreases as saturation is approached and depots are replenished. High efficiency in lipid assimilation is usually regarded as an adaptation of herbivorous copepods from higher latitudes to the strong (short) seasonal character of food supply (for a review on the topic see Hagen and Auel, 2001). Smaller species with a more flexible feeding mode which have high metabolic needs, on the other hand, generally do not depend on the accumulation of large lipid reserves for guaranteed survival and reproduction. The lipid C turnover rates reported by Boissonnot, Niehoff, Hagen, Søreide, and Graeve (2016) and Graeve, Albers, and Kattner (2005) for either of these life strategies (*Calanus finmarchicus*, 2.7% day^{-1}; *C. glacialis*, 3.0 % day^{-1}; *Pseudocalanus minutus*, 2.6% day^{-1}; *Oithona similis*, 0.5% day^{-1}) are lower than the ones obtained in the present study. This is true even for our lowest recorded rates, 4-6% day^{-1}, during the last three days of the experiments. This shows how well adapted *T. longicornis* is to feeding on these diatom and dinoflagellate species. *Temora longicornis* is believed to actively grow and reproduce during the winter in the North Sea (Wesche et al., 2007), and stored dietary FAs could be mobilized for demanding metabolic processes or during events of food scarcity, thus enabling copepods to cope with

environmental changes in feeding conditions and to invest in reproductive output year round (Boissonnot, Niehoff, Hagen, Søreide, and Graeve, 2016; Gentsch, Kreibich, Hagen, and Niehoff, 2009). This is of utmost importance for the survival of *T. longicornis* inhabiting the North Sea, an area where environmental changes (a decrease in nutrient loading coupled to an increase in light penetration) in the past three decades have affected prey quality (C:nutrient ratios) and drastically reduced the densities of calanoid copepod populations (Boersma, Wiltshire, Kong, Greve, and Renz, 2015).

Relating prey biochemical composition and egg production in copepods

Several studies have suggested the existence of (multiple) correlations between various biochemicals (in special the level of (n-3) PUFAs) and different indices of copepod reproduction (Anderson and Pond, 2000; Jónasdóttir, Fields, and Pantoja, 1995; Jónasdóttir, Visser, and Jespersen, 2009; Peters, Dutz, and Hagen, 2007; Sterner and Schulz, 1998). EPA is a precursor to several molecules involved in the reproductive processes, although the pathway for its use in egg production is not fully understood in copepods; DHA is essential for neural function; and both have an important role in cellular physiology regulating membrane fluidity (Brett & Müller-Navarra, 1997; Jónasdóttir, Visser, and Jespersen, 2009; Sterner and Schulz, 1998). In agreement with the literature, the absolute content of certain dietary PUFAs seemed to positively affect EPR. Dino+ provided copepods with only half the amount of EPA delivered by diatom diets, but it supplied per volume of culture from 100-150x more 18:3(n-3) and 10x more DHA than the latter. It also contained 2-3x more of these PUFAs than its N-limited counterpart. Dino– also showed higher 18:3(n-3) and DHA and lower EPA than the diatom diets, and yielded the second highest EPR. The combination of the results from this study and from Franco-Santos et al. (2018) suggests that the FAs 18:3(n-3) and DHA are more important than EPA for egg production in *T. longicornis* females. This is contrary to the findings by Jónasdóttir, Visser, and Jespersen (2009), which highlight the importance of EPA and total C for EPR and relate 18:3(n-3) and DHA to egg hatching success in *T.*

longicornis. There is contradictory information as to whether EPR can also be influenced by maternal contribution of energy reserves or not (Anderson and Pond, 2000; Graeve, Albers, and Kattner, 2005; Jónasdóttir, Visser, and Jespersen, 2009; Peters, Dutz, and Hagen, 2007), but it is possible that dinoflagellate-fed copepods used field-accumulated EPA reserves to fuel higher EPR.

Methodological constrains of results

The low level of labelling transfer from *R. salina* to *O. marina* in the Dino+ cultures made it difficult to follow the transfer of labelled FAs from the latter into the copepods they were fed to. It is not possible to affirm with certainty whether the low APE and C assimilation and turnover values obtained for these copepods were due to methodological problems, biological processes, or both. There is indication, however, that it was a methodology problem. The C assimilation of these copepods accounted for only 15-26% of the recorded increase in their total lipid C, suggesting that C assimilation was underestimated. Henceforth we refer to the increase in total lipid C between t_{0h}-t_{48h} and t_{0h}-t_{120h} as a proxy or minimum value for C assimilation (adjusted to 0.4 ± 0.1 and 0.3 ± 0.0 µg C ind^{-1} day^{-1}, respectively, and to 3 ± 1 % food ingested) and turnover (adjusted to 19 ± 4 and 10 ± 1 % day^{-1}, respectively) in the copepods fed with the Dino+ diet. FA-specific data on C assimilation and turnover for these copepods, however, are not included in the discussion unless in a qualitative manner.

CONCLUSIONS

Three major results can be described from the data reported herein and are discussed in the following sections. Absolute C assimilation in copepods was inversely proportional to their C investment in reproductive output, and these were influenced by the chemical and elemental composition (nutritional quality) of the prey copepods were fed with. Furthermore, relative C assimilation (as % food ingested) was similar between copepods fed with different diets, suggesting

that differences in reproductive output were related to copepod C ingestion rates. The high C turnover rates indicate an opportunistic feeding strategy for *T. longicornis*, which would accumulate dietary C as soon as it is readily available with a speed that decreases as saturation is approached and its lipid storage is replenished. This strategy would enable them to cope with environmental changes in feeding conditions and to invest in reproduction year round. Egg production rates were directly proportional to the copepod content of the FAs 18:3(n-3) and DHA. These results are an important addition to a small, yet growing, body of literature which estimates the transfer of lipid C between prey and predator, and to the field of copepod feeding ecology.

AUTHOR CONTRIBUTIONS

All authors contributed to conceiving the ideas and to design the methodology;

RMFS collected and analyzed the data and led the writing of the manuscript;

All authors contributed critically to the drafts and gave final approval for publication.

SUPPLEMENTARY DATA

Supplementary tables are available at Functional Ecology online.

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DATA ARCHIVING

The laboratory experimental data and metadata for this study are available through the PANGEA repository under the doi number (to be included once submission to PANGEA is completed).

INTEGRITY OF RESEARCH AND REPORTING

Ethical standards

The experiments described in the present study comply with the current laws of the country in which they were performed.

Conflict of interest

The authors declare that they have no conflict of interest.

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766 **SUPPLEMENTARY MATERIAL**
767
768 **Supplementary table S1** Post hoc test results. Tukey HSD results (*p*-value) for differences in (1)
769 prey culture: carbon (C), nitrogen (N), and total lipid contents, molar C:N ratios, and ^{13}C labelling
770 (atom%); and (2) *Temora longicornis* females: C, N, and total lipid contents, molar C:N ratios, ^{13}C
771 enrichment (APE), C assimilation rate (C_{assim}, as µg C ind^{-1} day^{-1}), and C turnover rate (C_T, % day^{-1}). Similarity percentages (SIMPER) results (% average dissimilarity) for differences in FA
773 composition of prey cultures. Sampling days: t_{0h} (*in situ*), t_{48h} (after 48h of experiment), t_{120h} (at the
774 end of the experiment). Diet treatments: single cultures in nutrient-replete and N-depleted
775 conditions of *Conticribra weissflogii* (Diat+ and Diat–, respectively) and *Oxyrrhis marina* (Dino+
776 and Dino–, respectively). Significant values: * ($p < 0.05$), ** ($p < 0.01$); *** ($p < 0.001$). *p*-values
777 for tests with an adjusted *p*-value of 0.0167 are given in number format.
778

	Effect	Factor	Variable	Treatment pair	Result
Prey cultures			C content	Dino+/Diat–	**
				Dino+/Diat+; Dino+/Dino–	***
			N content	Diat–/Diat+; Diat–/Dino+; Diat–/Dino–; Dino+/Diat+; Dino+/Dino–	***
			C:N	Diat–/Diat+; Diat–/Dino+; Diat–/Dino–	***
			Lipid content	Dino+/Dino–	**
				Dino+/Diat+; Dino+/Diat–	***
			atom%	Dino+/Diat+; Dino+/Diat–; Dino+/Dino–; Dino–/Diat+; Dino–/ Diat–	***
	Day	Diat+	atom%	Day 1/Day 5	**
		Diat+		Day 4/Day 1; Day 4/Day 3; Day 4/Day 5	***
		Diat–		Day 5/Day 1; Day 5/Day 4	**
		Diat–		Day 5/Day 2	***
		Dino+		Day 3/Day 5	*
		Dino+		Day 1/Day 3; Day 1/Day 4; Day 1/Day 5; Day 2/Day 3; Day 2/Day 4; Day 2/Day 5	***
		Dino–		Day 4/Day 5	**
		Dino–		Day 5/Day 1; Day 5/Day 3	***
			SIMPER	Diat+/Diat–	21%
				Diat+/Dino+	65%
				Diat+/Dino–	68%
				Diat–/Dino+	58%
				Diat–/Dino–	60%
				Dino+/Dino–	25%
Copepod			APE	Dino+/Diat+; Dino+/Diat–; Dino+/Dino–; Dino–/Diat+; Dino–/Diat–	***
	Diet		C_{assim}	Diat+/Diat–	*
				Diat+/Dino–; Diat–/Dino–	**
				Dino+/Diat–; Dino+/Dino–	***
			C_T	Diat–/Dino–	**
				Dino+/Diat+; Dino+/Diat–; Dino+/Dino–	***
	Day		APE	t_{48h}/t_{120h}	***
			C_{assim}	t_{48h}/t_{120h}	***
			C_T	t_{48h}/t_{120h}	***
	Diet: Day	Diat+	C content	t_{0h}/t_{48h}	0.009
				t_{0h}/t_{120h}	0.014
		Dino–		t_{0h}/t_{48h}	0.008
				t_{0h}/t_{120h}	0.009
		Dino–	N content	t_{0h}/t_{48h}	0.013
		Diat+		t_{0h}/t_{120h}	0.0163
		Diat–	C:N	t_{0h}/t_{48h}	0.011
		Dino+		t_{0h}/t_{120h}	0.002
		Dino–		t_{0h}/t_{120h}	0.004

Diat+	Lipid content	t_{0h}/t_{48h}	0.007
Diat–			0.014
Dino+			0.002
Diat+		t_{0h}/t_{120h}	0.008
Dino+			0.0003
Dino–			0.006
Dino–			0.009
t_{48h}	C content	Diat+/Diat–; Diat+/Dino–	*
t_{48h}	N content	Diat–/Diat+; Diat–/Dino+; Diat–/Dino–	***
t_{48h}	C:N	Diat–/Diat+; Diat+/Dino–	**
		Diat–/Dino+; Diat–/Dino–	***
t_{120h}		Dino–/Diat–; Dino–/Dino+	*
		Diat+/Diat–; Diat+/Dino+	**
t_{48h}	Lipid content	Diat–/Dino+	*
		Diat–/Dino–	**
t_{120h}		Diat–/Diat+; Diat–/Dino–	*
t_{48h}	APE	Dino–/Diat+;Dino–/Diat–	***
t_{120h}		Diat+/Diat–	*
		Dino–/Diat+;Dino–/Diat–	***
t_{48h}	C_{assim}	Diat+/Dino–	*
		Diat–/Dino–	**
t_{120h}		Diat–/Diat+; Diat–/ Dino–	*
t_{48h}	C_{assim} SFA	Diat–/Diat+; Diat–/Dino–	*
t_{120h}		Diat+/Diat–	*
t_{48h}	C_{assim} MUFA	Diat+/Diat–; Diat+/Dino–; Diat–/Dino–	***
		Diat+/Dino–	**
t_{120h}		Diat–/Diat+; Diat–/Dino–	***
t_{48h}	C_{assim} PUFA	Diat+/Dino–	*
t_{48h}	C_T SFA	Diat–/Dino–	*
		Diat–/Diat+	*
t_{120h}		Diat–/Dino–	**
t_{48h}	C_T MUFA	Diat+/Diat–; Diat+/Dino–; Diat–/Dino–	***
		Diat+/Dino–	*
t_{120h}		Diat–/Diat+; Diat–/Dino–	***
t_{48h}	C_T PUFA	Diat+/Dino–	*
		Diat+/Diat–	**
t_{120h}		Diat+/Diat–	*
		Diat+/Dino–	**

Supplementary table S2 One-way Analysis of Variance (ANOVA) and one- and two-way repeated measures ANOVA (RM ANOVA) results (F-statistics, degrees of freedom (df), and *p*-values) for statistical analyses of (1) prey culture: carbon (C), nitrogen (N), and total lipid contents, molar C:N ratios, and ^{13}C labelling of fatty acids (atom%); and (2) *Temora longicornis* females: C, N, and lipid content, molar C:N ratios, ^{13}C enrichment (APE), total and FA-specific C assimilation rate (C_{assim}, as µg C ind^{-1} day^{-1}), and total and FA-specific C turnover rate (C_T % day^{-1}). T-test results (t-statistics, df, *p*-values) for within treatment comparisons of copepod APE, C_{assim} and C_T are also given. Non-significant values are omitted. Significant values: * ($p < 0.05$), ** ($p < 0.01$); *** ($p < 0.001$). FA denominations: saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

		Test	Factor	Response variable	F-statistics	df	*p*-value
Prey cultures		1-way ANOVA		C content	19.99	3, 16	***
				N content	121.1	3, 16	***
				molar C:N ratio	74.31	3, 16	***
				Lipid content	22.73	3, 14	***
				atom%	362.3	3, 14	***
			Diat+	atom%	21.79	3, 71	***
			Diat–		5.824	4, 89	***
			Dino+		30.32	4, 76	***
			Dino–		9.901	3, 52	***
Copepods	Diet treatments	2-way RM ANOVA	All	C content	11.38	3	**
				N content	17.76	3	***
				molar C:N ratio	36.52	3	***
				Total lipid content	14.26	3	**
		1-way RM ANOVA	Diat+	C content	20.69	2,4	**
			Dino+		12.64	2,4	*
			Dino–		23.93	2,4	**
			Diat+	N content	14.61	2,4	*
			Dino+		8.888	2,4	*
			Dino–		15.01	2,4	*
			Diat–	molar C:N ratio	15.61	2,4	*
			Dino+		35.67	2,4	**
			Dino–		28.05	2,4	**
			Diat+	Total lipid content	25.46	2,4	**
			Diat–		15.54	2,4	*
			Dino+		116.4	2,4	***
			Dino–		21.77	2,4	**
		t-test	Diat+	APE	-11.977	2	**
			Diat–		-17.397	2	**
			Dino+		4.5826	2	*

			Dino–		-9.2307	2	*
			Diat+	C_{assim}	8.1848	2	*
			Dino–		4.3235	2	*
			Diat+	C_{assim} SFA	22.258	2	**
			Dino–		4.7035	2	*
			Diat+	C_{assim} MUFA	20.524	2	**
			Diat+	C_{assim} PUFA	5.5807	2	*
			Dino–		4.5783	2	*
			Diat+	C_T	14.637	2	**
			Diat–		113.28	2	***
			Dino+		501.43	2	***
			Dino–		35.43	2	***
			Diat+	C_T SFA	22.942	2	**
			Diat–		35.203	2	***
			Dino–		31.262	2	**
			Diat+	C_T MUFA	11.75	2	**
			Diat–		43.64	2	***
			Dino–		11.426	2	**
			Diat+	C_T PUFA	7.1813	2	*
			Diat–		54.049	2	***
			Dino–		43.25	2	***
	Days sampled	2-way RM ANOVA	All	C content	43.448	2	***
				N content	23.345	2	***
				molar C:N ratio	42.315	2	***
				Total lipid content	94.937	2	***
		1-way ANOVA	t_{48h}	C content	7.265	3,8	*
			t_{48h}	N content	29.43	3,8	***
			t_{48h}	molar C:N ratio	45.52	3,8	***
			t_{100h}		11.74	3,8	**
			t_{48h}	Total lipid content	8	3,8	**
			t_{100h}		6.101	3,8	*
			t_{48h}	APE	60.7	2,6	***
			t_{100h}		124.6	2,6	***
			t_{48h}	C_{ass}	15.88	2,6	**
			t_{100h}		9.81	2,6	*
			t_{48h}	C_{assim} SFA	11.09	2,6	**
			t_{100h}		9.391	2,6	*
			t_{48h}	C_{assim} MUFA	237.5	2,6	***
			t_{100h}		130.8	2,6	***
			t_{48h}	C_{assim} PUFA	5.34	2,6	*
			t_{48h}	C_T SFA	6.333	2,6	*
			t_{100h}		21.44	2,6	**
			t_{48h}	C_T MUFA	162.1	2,6	***
			t_{100h}		135.1	2,6	***
			t_{48h}	C_T PUFA	16.78	2,6	**
			t_{100h}		16.8	2,6	**
	Diet treatment : Day sampled		All	molar C:N ratio	9.693	6	***

Supplementary table S3 Permutational Multivariate Analysis of Variance (PERMANOVA) results (pseudo F-statistics or t-statistics, *p*-values for standard permutations (p(perm)) and for Monte Carlo simulations (p(MC)), unique number of permutations (UNP)) for *Temora longicornis* fatty acid (FA) profiles (>1% total FAs). Diet treatments: nutrient-replete and N-depleted *Conticribra weissflogii* (Diat+ and Diat–, respectively) and *Oxyrrhis marina* (Dino+ and Dino–, respectively). Non-significant values are omitted. Significant values: * ($p < 0.05$), ** ($p < 0.01$); *** ($p < 0.001$).

Main effects		F-statistics	p(perm)	p(MC)	UNP
Treatment		968.73	***	***	971
Day sampled		360.93	***	***	997
Treatment : Day sampled		120.01	***	***	997
Pairwise comparisons		**t-statistics**	**p(perm)**	**p(MC)**	**UNP**
Diat+ x Diat–		8.7585		***	10
Diat+ x Dino+		32.515		***	10
Diat+ x Dino–		30.096		***	10
Diat– x Dino+		52.824		***	10
Diat– x Dino–		45.824		***	10
Dino+ x Dino–		8.8395		***	10
t_{0h} x t_{48h}		16.796	***	***	998
t_{0h} x t_{120h}		26.109	***	***	998
t_{48h} x t_{120h}		7.7516	***	***	998
Diat+	t_{0h} x t_{48h}	5.2527		*	38
	t_{0h} x t_{120h}	8.8608		**	38
	t_{48h} x t_{120h}	1.7474			38
Diat–	t_{0h} x t_{48h}	10.718		**	38
	t_{0h} x t_{120h}	14.355		**	38
	t_{48h} x t_{120h}	5.6138		*	38
Dino+	t_{0h} x t_{48h}	22.247		***	38
	t_{0h} x t_{120h}	28.433		**	38
	t_{48h} x t_{120h}	Negative			38
Dino–	t_{0h} x t_{48h}	15.496		**	38
	t_{0h} x t_{120h}	24.23		***	38
	t_{48h} x t_{120h}	13.578		**	38
t_{48h}	Diat+ x Diat–	4.4046		**	10
	Diat+ x Dino+	18.338		***	10
	Diat+ x Dino–	16.359		***	10
	Diat– x Dino+	35.394		***	10
	Diat– x Dino–	26.979		***	10
	Dino+ x Dino–	8.9636		***	10
t_{120h}	Diat+ x Diat–	7.4701		***	10
	Diat+ x Dino+	30.698		***	10
	Diat+ x Dino–	28.165		***	10
	Diat– x Dino+	57.689		***	10
	Diat– x Dino–	45.74		***	10
	Dino+ x Dino–	12.907		***	10

Supplementary table S4 Total (TFA) and fatty acid-specific ^{13}C enrichment (APE) between t_{0h}-t_{48h} and between t_{0h}-t_{120h} for *Temora longicornis* females. Diet treatments: single cultures of *Conticribra weissflogii* (Diat) and *Oxyrrhis marina* (Dino) in nutrient-replete (+) and N-depleted (–) conditions. Values are mean ± standard deviation (n = 3 each). – : not detected.

	APE							
	Diat+		Diat–		Dino+		Dino–	
FA	t_{48h}	t_{120h}	t_{48h}	t_{120h}	t_{48h}	t_{120h}	t_{48h}	t_{120h}
TFA	0.7 ± 0.0	0.9 ± 0.0	0.7 ± 0.0	1.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.0	0.7 ± 0.0
14:0	0.8 ± 0.0	1.2 ± 0.0	0.8 ± 0.0	1.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.7 ± 0.1
i-15:0	0.4 ± 0.1	0.6 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.9 ± 0.0	0.9 ± 0.0
15:0	1.2 ± 0.0	1.4 ± 0.0	1.4 ± 0.1	1.6 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.7 ± 0.0
16:0	0.9 ± 0.0	1.2 ± 0.0	1.0 ± 0.0	1.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.0	0.8 ± 0.0
16:1n5	1.5 ± 0.0	1.5 ± 0.0	1.3 ± 0.0	1.6 ± 0.0	–	–	–	–
16:1n7	1.3 ± 0.0	1.4 ± 0.0	1.5 ± 0.0	1.7 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.4 ± 0.1
16:2n4	1.7 ± 0.0	1.6 ± 0.0	1.5 ± 0.0	1.7 ± 0.0	–	–	–	–
16:3n4	1.7 ± 0.0	1.7 ± 0.0	1.8 ± 0.0	1.8 ± 0.0	–	–	–	–
16:4n1	0.5 ± 0.0	1.1 ± 0.0	0.2 ± 0.0	0.6 ± 0.1	–	–	–	–
17:0	0.1 ± 0.0	0.3 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.7 ± 0.0
18:0	0.3 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.7 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
18:1n7	0.8 ± 0.0	1.1 ± 0.1	0.8 ± 0.0	1.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.1	0.7 ± 0.0
18:1n9	0.2 ± 0.0	0.2 ± 0.1	0.7 ± 0.1	1.0 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.9 ± 0.2	1.0 ± 0.0
18:2n6	0.9 ± 0.0	1.1 ± 0.0	0.7 ± 0.0	1.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	1.1 ± 0.0	1.0 ± 0.0
18:3n3	0.5 ± 0.0	0.6 ± 0.3	0.5 ± 0.0	0.9 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.0
18:3n6	1.3 ± 0.1	1.5 ± 0.0	1.1 ± 0.0	1.5 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.0	0.8 ± 0.0
18:4n3	1.5 ± 0.0	1.6 ± 0.0	1.5 ± 0.0	1.7 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.8 ± 0.0	1.0 ± 0.0
20:1n7	0.4 ± 0.1	0.6 ± 0.3	0.4 ± 0.1	0.6 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	–
20:1n9	0.1 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.6 ± 0.0	0.9 ± 0.0
20:2n6	0.3 ± 0.1	0.7 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.1	0.9 ± 0.0
20:4n3	0.8 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.1	0.9 ± 0.0
20:4n6	0.4 ± 0.1	0.7 ± 0.2	0.3 ± 0.0	0.6 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.6 ± 0.0

20:5n3	0.8 ± 0.0	1.2 ± 0.0	0.7 ± 0.0	1.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
22:1n7	0.2 ± 0.1	0.6 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
22:1n9	–	0.1 ± 0.2	-0.1 ± 0.1	0.0 ± 0.1	–	–	0.1 ± 0.0	–
22:5n3	0.4 ± 0.0	0.8 ± 0.0	0.4 ± 0.0	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.0 ± 0.0
22:6n3	0.5 ± 0.0	0.8 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.7 ± 0.0	0.9 ± 0.0

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CHAPTER III

Fatty acid bioconversion and homeostasis in the larvae of the sand mason worm *Lanice conchilega*

Franco-Santos RM, Auel H, Boersma M, De Troch M, Graeve M, Meunier CL, Niehoff B

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You are not always what you eat - Fatty acid bioconversion and lipid homeostasis in the larvae of the sand mason worm Lanice conchilega

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Corresponding Author:	Rita Melo Franco-Santos, M.Sc. Alfred-Wegener-Institut Helmholtz-Zentrum fur Polar- und Meeresforschung Bremerhaven, GERMANY
Keywords:	assimilation; bioconversion; compound-specific stable isotope analysis; fatty acids; larvae; polychaete
Abstract:	The meroplanktonic larvae of benthic adults are an important seasonal component of the zooplankton in temperate coastal waters. The larvae of the reef-building polychaete Lanice conchilega can make up to 15% of the summer zooplankton biomass in the North Sea. Despite their importance for reef maintenance (which positively affects the benthic community), little is known of the trophic ecology of these meroplanktonic larvae. Qualitative and quantitative estimates of carbon (C) transfer between trophic levels and of FA-specific assimilation, biosynthesis, and bioconversion can be obtained by compound-specific stable isotope analysis of fatty acids (FA). The present work tested the hypothesis that the concept of fatty acid trophic markers (FATM), widely used for studies on holoplankton with intermediate to high lipid contents, is also applicable to lipid-poor organisms such as meroplanktonic larvae. The incorporation of isotopically-enriched dietary C by L. conchilega larvae was traced, and lipid assimilation did not follow FA-specific relative availabilities in the diet. Furthermore, FAs that were unavailable in the diet, such as 22:5(n-3), were recorded in L. conchilega, suggesting their bioconversion by the larvae. The results indicate that L. conchilega larvae preferentially assimilate certain FAs and regulate their FA composition (lipid homeostasis) independently of that of their diet. Their quasi-homeostatic response to dietary FA availability could imply that the concept of FATM has limited application in lipid-poor organisms such as L. conchilega larvae.
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Dear Editor-in-Chief Dr. Joerg Heber, October 30th, 2018

Enclosed you will find the manuscript entitled "**You are not always what you eat - Fatty acid bioconversion and lipid homeostasis in the larvae of the sand mason worm *Lanice conchilega***", that I wish to submit for publication in PLoS ONE. Given that the topic of this manuscript encompasses zooplankton ecology, I would like to suggest that it be handled by Dr. Jian-Shiou Hwang. This is the first submission of this manuscript, and albeit we do not oppose any reviewers, we prefer reviewers with ecological backgrounds.

This manuscript is a research article with two main goals. The first was to provide data on the feeding ecology of *L. conchilega* larvae, which is scarcely found in the literature. The second was to investigate whether the concept of fatty acid trophic markers (FATM), commonly applied in tracer studies with holoplankton of intermediate to high total lipid contents, can be applied in feeding studies with lipid-poor meroplanktonic larvae. In order to do so, we followed the assimilation of ^{13}C-enriched dietary lipid into the larvae.

To the best of our knowledge, this study is the first to present data on carbon assimilation and turnover and on fatty acid (FA) bioconversion in any meroplanktonic organism. The results show that *L. conchilega* larvae do not assimilate dietary FAs in an unmodified manner, which is one of the assumptions for FATMs. Instead they regulate (maintain) their lipid composition (regardless of that of their prey) via preferential assimilation and bioconversion of dietary FAs. This behavior limits the application of the FATM concept in feeding studies with *L. conchilega* larvae and, probably, with other lipid-poor meroplanktonic larvae. The combination of our results with published work reporting on the total lipid and wax ester contents of several zooplankton species led to our suggestion of the hypothesis that 3 different patterns in lipid homeostasis (regulation) are found among meroplanktonic and holoplanktonic organisms in relation to their lipid content.

I look forward to hearing from you at your earliest convenience regarding the submission of this manuscript.

Yours sincerely,

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You are not always what you eat - Fatty acid bioconversion and lipid homeostasis in the larvae of the sand mason worm *Lanice conchilega*

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Abstract

The meroplanktonic larvae of benthic adults are an important seasonal component of the zooplankton in temperate coastal waters. The larvae of the reef-building polychaete *Lanice conchilega* can make up to 15% of the summer zooplankton biomass in the North Sea. Despite their importance for reef maintenance (which positively affects the benthic community), little is known of the trophic ecology of these meroplanktonic larvae. Qualitative and quantitative estimates of carbon (C) transfer between trophic levels and of FA-specific assimilation, biosynthesis, and bioconversion can be obtained by compound-specific stable isotope analysis of fatty acids (FA). The present work tested the hypothesis that the concept of fatty acid trophic markers (FATM), widely used for studies on holoplankton with intermediate to high lipid contents, is also applicable to lipid-poor organisms such as meroplanktonic larvae. The incorporation of isotopically-enriched dietary C by *L. conchilega* larvae was traced, and lipid assimilation did not follow FA-specific relative availabilities in the diet. Furthermore, FAs that were unavailable in the diet, such as 22:5(n-3), were recorded in *L. conchilega*, suggesting their bioconversion by the larvae. The results indicate that *L. conchilega* larvae preferentially assimilate certain FAs and regulate their FA composition (lipid homeostasis) independently of that of their diet. Their quasi-homeostatic

response to dietary FA availability could imply that the concept of FATM has limited application in lipid-poor organisms such as *L. conchilega* larvae.

Keywords: assimilation, bioconversion, compound-specific stable isotope analysis, fatty acids, larvae, polychaete

INTRODUCTION

The meroplanktonic larvae of macrobenthic organisms are an important seasonal component of the zooplankton in temperate coastal waters [1]. In the North Sea, the larvae of the sand mason worm *Lanice conchilega* (Pallas, 1766) can make up to 15% of the zooplankton biomass between July and September [2], when water temperatures are above 13ºC. The reproductive peak of *L. conchilega* in the southern North Sea occurs in spring, and is followed by smaller peaks during the summer and autumn [3], though larval supply can vary between years [4]. The larvae of this polychaete evolve from a short planktonic to a benthic phase and, after a few days, to a second planktonic stage, the aulophore larvae [5]. At this stage larvae already present a (transparent) tube, and display morphological features of a juvenile [6]. They are able to feed in the water column, where they can remain up to 60 days before succeeding to a benthic stage [7].

The benthic, tube-dwelling adults of *L. conchilega* are non-selective suspension-deposit feeders [8, 9, 10], which derive their diet from the organic matter available in the water column and in the sediment. Common food items include diatoms, bacteria and microphytobenthos, and the species is able to switch between food sources [8, 10, 11]. *Lanice. conchilega* forms reefs capable of structuring the surrounding habitat and creating a complex and heterogeneous environment [12, 13]. The reefs provide refuge against predation and physical and chemical stresses, attract a variety of other organisms, and serve as nursery and feeding grounds to several species, including fish and birds [14, 15, 16, 17, 18, 19]. *L. conchilega* is an ecosystem engineer whose presence favors species richness and faunal abundance and positively affects the benthic community [12, 13, 19, 20]. In comparison with adult organisms, few studies have been conducted on the ecology of *L. conchilega*

larvae, addressing topics such as juvenile settlement and buoyancy [4, 7, 21, 22]. The survival and development of these meroplanktonic larvae and, thus, the maintenance of *L. conchilega* reefs, depend, a.o., on their feeding success. To the best of our knowledge there are no studies published on the feeding ecology of the larvae, a gap which needs to be addressed if the larval stage of this species is to be better understood.

Information on the feeding ecology of an organism on short and long time scales and on the pathways of energy flow between prey and predator can be obtained from the analysis of fatty acid trophic markers (FATM) [23] in combination with the use of stable isotopes. FATMs are usually assimilated in a conservative manner by consumers [24, 25]. The FAs 16:1(n-7), 16:2(n-4), 16:3(n-4), and 20:5(n-3) (EPA) are characteristic of diatoms, whereas 18:4(n-3) and 22:6(n-3) (DHA) are considered dinoflagellate markers [8, 23, 26, 27]. Compound-specific stable isotope analysis (CSIA) is a powerful tool for tracing organic matter origin and fate and to investigate biochemical processes and patterns in individuals and ecosystems [e.g., 28, 29, 30]. When FA determination is coupled to measurements of isotopic composition, quantitative estimates can be obtained for the transfer of dietary carbon (C) from prey to predator and for FA-specific assimilation and potential biosynthesis and bioconversion [26, 27, 31, 32].

The present work had two objectives. The first was to start filling the gap of knowledge on the feeding ecology of *L. conchilega* larvae. Experiments investigating FA-related C assimilation have been conducted mostly for holoplanktonic organisms with intermediate to high lipid contents, whose FA profile tends to reflect that of the food items it ingests. Little is known, however, about how meroplanktonic organisms, which are usually lipid-poor, incorporate dietary FAs. It is possible that the lipid content and, thus, requirement, of an organism can dictate whether it will assimilate dietary FAs in an unmodified manner or not. We assume that the larvae of *L. conchilega* are lipid-poor, so our second objective was to test the hypothesis that the concept of FATM can also be applied in a feeding study with lipid-poor meroplanktonic larvae. In order to do so, the polychaete larvae were fed with a diatom culture previously labelled with ^{13}C and the incorporation of

isotopically enriched FA-specific lipid C into the consumer was followed. This allowed for the recording of C assimilation and turnover in the organism, and also for the investigation of possible bioconversion pathways.

METHOD

Field sampling

Zooplankton samples for the feeding experiment were collected on June 6th 2016 with a 500 µm mesh-size CalCOFI net, which was towed horizontally for 15 minutes at 5 m depth off the German island of Helgoland (54º11'N, 07º54'E), in the southern North Sea. Samples were taken to the laboratory and intact and active aulophore larvae of the polychaete *L. conchilega* were immediately sorted under an Olympus SZX16 stereoscopic microscope. A total of 390 larvae were sorted, 30 for determination of *in situ* body C and nitrogen (N) content, 150 for analyzing the *in situ* FA content and composition, and 210 for a feeding experiment. The planktonic diatom *Conticribra weissflogii* (Grunow) (Stachura-Suchoples and Williams, 2009) was used as prey in the feeding experiment with *L. conchilega* larvae.

Algae culture

Batch cultures were created daily for *C. weissflogii* for five consecutive days by diluting a stock solution with fresh f/2 medium [after 33]. Diatoms were labelled with ^{13}C by adding ^{13}C-enriched sodium bicarbonate ($NaH^{13}CO_3$) to the medium at a concentration of 4 mg L^{-1}. Cultures were kept in constant light inside a temperature-controlled room at 18ºC, and stirred twice a day in order to keep cells suspended. Algae were grown for five days and then used as food suspension (exponential growth phase) for the polychaete larvae during the experiment. *C. weissflogii* cultures were sampled daily during the experiment for determination of cell C, N, and FA content and of FA-specific ^{13}C isotopic enrichment. This was done by filtering subsamples of known cell

concentrations through pre-combusted (500°C for 24 h) Whatman GF/F filters (0.7 µm pore size, 25 mm diameter). Filters for determination of prey C and N content were dried at 60°C for 48 h, folded inside aluminum foil, and stored in a desiccator until analysis. Samples for FA analyses were placed into pre-combusted lipid vials and stored at -80°C. The remaining volume of the cultures was then used to feed polychaete larvae. Cell densities were determined with a BD Accuri C6 Flow Cytometer.

Experimental design

Feeding experiments with *L. conchilega* were initiated immediately after sorting of the larvae. Individuals were kept for five days in triplicate 500 mL glass beakers fitted with a 300 µm meshed-bottom cylinder (140 ind L^{-1}). The diatom suspension was provided on a daily basis at a concentration of 8000 cells mL^{-1}. The polychaete larvae were kept in a dark temperature-controlled room at the temperature recorded when they were sampled in the field, i.e., 13.5 ± 0.3°C. The beakers were gently stirred three times a day for food resuspension in the water. A partial water exchange (66%) was performed daily and followed by the addition of new algae culture, with a final diatom concentration > 8000 cells mL^{-1}. At the end of the experiment the larvae were sampled from each replicate for body C and N content determination and for analysis of FA composition and FA-specific ^{13}C isotopic enrichment (10 and 50 individuals per sample, respectively). Individuals (inside their tubes) were gently washed in distilled water, placed into pre-weighed tin capsules (5*9 mm, IVA Analysentechnik) for body C and N content determination or into pre-combusted lipid vials for FA analyses, and stored at -80°C until further analysis. Tin capsules with larvae were dried at 60°C for 48 h, weighed with an ultra micro-balance (detection limit: 0.1 µg; XP6U Ultra Micro Balance, Mettler Toledo, Germany) and stored in a desiccator until analysis.

Carbon, nitrogen and fatty acid content analyses

The C and N contents of all larvae and algae samples were measured with an elemental analyzer (detection limit: 2 µg C / 0.5 µg N; maximum error: ± 3%, Euro EA 3000, EuroVector S.P.A., Milan, Italy) using acetanilide as a standard.

Lipids were extracted and FAs identified as described by [26]. Samples were homogenized in a dichloromethane:methanol (2:1, v:v) solution, from which total lipids were extracted. A known amount of an internal standard, the tricosanoic acid methyl ester (23:0), was added to each sample. Potassium chloride (0.88% solution) was added to create a biphasic system and aid in extraction. Lipid extracts were transesterified by heating samples with 3% sulfuric acid in methanol at 80ºC under nitrogen atmosphere for 4 hours. Fatty acid methyl esters (FAMEs) were then extracted with cyclohexane, and determined and quantified with a gas chromatograph (HP 6890 N, Agilent Technologies Deutschland GmbH & Co. KG) equipped with a 60m × 0.25mm i.d. wall-coated open tubular capillary column (film thickness: 0.25 µm; liquid phase: DB-FFAP), a split/splitless injector (250°C) and a flame ionization detector (280°C). The chromatograms generated were evaluated with the ChemStation software from Agilent. The A:B(n-X) shorthand notation was used to refer to FAs, where A is the number of carbon atoms, B is the number of double bonds, and (n-X) gives the position of the double bond closest to the terminal methyl group. Total lipid contents of larvae and algae cultures were calculated by adding the mass of all FAs.

Compound specific stable isotope analysis (CSIA)

The FA-specific stable isotope composition of carbon in FAMEs extracted was obtained according to [26] with a Thermo gas chromatography combustion-isotope ratio mass spectrometry (GC-c-IRMS) system, equipped with a Trace GC Ultra gas chromatograph, a GC Isolink, and a Delta V Plus isotope ratio mass spectrometer connected via a Conflo IV interface (Thermo Scientific Corporation, Bremen, Germany). The chromatograms containing peak areas and C isotope ratios were analyzed with the IRMS software Isodat 3.0. The 14:0 and 18:0 FAME reference standards (Iowa University) with known δ-values were used for further calculations.

The equations used by [26] to calculate carbon assimilation were also applied in the present study, and were:

Isotopic ratios of FAs

$$\delta^{13}\mathrm{C}\ (‰) = \left[\left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}}\right) - 1\right] * 1000, \quad (1)$$

where R_{sample} and R_{standard} are the ratio of $^{13}C/^{12}C$ in the sample and reference standard, respectively;

Atom percent (atom%), which are converted δ-values and express isotope data in terms of isotope concentrations to inform on the ^{13}C enrichment in each FA

$$\mathrm{atom\%} = \left(\frac{R_{\mathrm{sample}}}{(R_{\mathrm{sample}}+1)}\right) * 100, \quad (2)$$

where R is the ratio $^{13}C/^{12}C$ in the sample;

Atom percent excess (APE), which considers the isotopic enrichment in experimental larvae (in comparison to field individuals) due to the assimilation of algae enriched in ^{13}C

$$\mathrm{APE} = \mathrm{atom\%}_{\text{experimental polychaetes}} - \mathrm{atom\%}_{\text{field polychaetes}} \quad (3)$$

Carbon mass of each FA (C_{mass}, in µg C ind^{-1}), which divides the FA mass by the FAME mass

$$\mathrm{C_{mass}} = \frac{\{(\mathrm{atom\%} / 100 * \mathrm{A_{13C}}) + [(100 - \mathrm{atom\%}) / 100 * \mathrm{A_{12C}}]\} * \mathrm{C_{FA}} * \mathrm{FA_{mass}}}{\{[(\mathrm{atom\%} / 100 * \mathrm{A_{13C}}) + ((100 - \mathrm{atom\%}) / 100 * \mathrm{A_{12C}})] * \mathrm{C_{FA}}\} + \mathrm{A_{12C}} + (\mathrm{H_{FAME}} * \mathrm{A_H}) + (\mathrm{O_{FAME}} * \mathrm{A_O})}, \quad (4)$$

where A_{12C}, A_{13C}, A_H, and A_O are the atomic masses of ^{12}C, ^{13}C, H and O, respectively, C_{FA} is the number of carbon atoms in the FA, H_{FAME} and O_{FAME} are the number of hydrogen and oxygen atoms in the FAME, and FA_{mass} is the mass (in µg ind^{-1}) of the FA;

189 Proportion of carbon assimilated (PA)

190 $$PA = \frac{APE}{L}, \quad (5)$$

191 where L is the average labeling (in atom%) in all algae FAs but 18:0 (L = 3.62%). The averaging is
192 to account for the elongation and/or desaturation of small amounts of dietary FAs when they are
193 assimilated by the larvae [23], and the exclusion of 18:0 is due to its poor labelling in comparison to
194 other FAs in prey cultures [26, 34]. When calculating the PA in the larvae FA 18:0, L was the
195 average of labeling in algae FA 18:0 ($L_{FA\ 18:0}$ = 1.87%);

196

197 Carbon assimilation (C_{assim}, in µg C ind^{-1}), calculated for each FA

198 $$C_{assim} = C_{mass} * PA \quad (6)$$

199 Total C assimilation was obtained by summing the C_{assim} values for all FAs. Assimilation rates were
200 obtained by dividing C_{assim} by the number of experimental days when sample was collected;

201

202 Carbon turnover rates (C_T in % day^{-1})

203 $$C_T = \frac{\frac{C_{assim(t)}}{C_{mass(t)}}}{\Delta t}, \quad (7)$$

204 where $_{(t)}$ indicates the specific sampling time for which the values of C_{assim} and C_{mass} should be used
205 and Δt the number of experimental days when sample was collected.

206

207 **Statistical analysis**

208 Differences in atom% for the different batches of the diatom culture were investigated with
209 one-way analysis of variance (ANOVA), and the origin of differences was identified by applying
210 the Tukey HSD (Honestly Significant Difference) post-hoc test with a 95% confidence limit. Prior
211 to the ANOVAs, the data were tested for normality and homogeneity of variances with Shapiro-
212 Wilk and Bartlett tests, respectively. Differences between *in situ* and laboratory-fed polychaete

larvae in dry mass (DM), C, N, and total lipid contents and C:N molar ratio were investigated with t-tests. All univariate analyses were performed using R ver. 3.4.4 [35].

Multivariate analysis of FA composition for *L. conchilega* individuals was performed with a dendrogram, which clustered individuals with similar FA profiles (based on their group average linkage clustering). The dendrogram was generated from a Bray-Curtis similarity matrix obtained from absolute and percentage (with logit transformation, as suggested by 36) data on FAs with relative content > 1% total fatty acids (TFA). All multivariate analyses were performed with PRIMER 7.0 software [37].

RESULTS

Algal composition and labelling

The average C and N contents of the *C. weissflogii* cultures were 580 and 120 µg L^{-1}, respectively, resulting in an average molar C:N ratio of 5.8 (Table 1). The average lipid content was 120 µg L^{-1} (Table 1). The FA profile of the diatom (Table 1, Fig. 1) was dominated by polyunsaturated fatty acids (PUFAs), which comprised 61% of total fatty acids (TFA). Saturated (SFA) and monounsaturated (MUFA) fatty acids were present in similar amounts, 22 and 17% TFA, respectively. The most important FAs in the *C. weissflogii* batches were 20:5(n-3) (eicosapentaenoic acid, EPA), 16:3(n-4), 16:1(n-7), and 16:0 (22, 19, 15 and 13% TFA, respectively). The FA 22:6(n-3) (docosahexaenoic acid, DHA) was present in a smaller amount, i.e., 5% of TFA.

Fig 1. Fatty acid absolute and relative concentrations in diatoms (*Conticribra weissflogii*) and in *Lanice conchilega* larvae.

Absolute and relative (concentrations are given in ng C ind^{-1} and in % total FA (%TFA), respectively. Data for larvae are differentiated between organisms sampled from the field and sampled after 5 days of feeding on the diatom.

Table 1 Dry mass (DM), carbon (C), nitrogen (N) and total lipid contents, molar C:N ratio, and FA-specific absolute and relative composition of *Lanice conchilega* larvae and its diet, the diatom *Conticribra weissflogii*. Total lipid C content is given by the ΣFAs term. A distinction is made between the internal (non-labelled, from *in situ* origin) and the internal + dietary (labelled, assimilated from the diet) FA contents in *L. conchilega* at the end of the feeding experiment. DM is reported in µg ind^{-1}. C, N, and lipid contents are reported in ng ind^{-1} for larvae samples and in µg L^{-1} and in pg $cell^{-1}$ (in parenthesis) for algae samples. Absolute concentration of FAs are reported in ng C ind^{-1} and in ng C L^{-1} for larvae and algae samples, respectively; and relative concentration of FAs are shown in % total FA (TFA, in parenthesis) for all samples. Only FAs > 1% TFA are indicated. Values are means ± standard deviation from samples and from the sum of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (n = 3 or 4). – : not detected.

	L. conchilega			*C. weissflogii*
	in situ	Experiment		
		Internal and dietary C	Internal C	
DM	55 ± 6	52 ± 13		
C content	7668 ± 2858	8511 ± 966		577 ± 44 (72 ± 6)
N content	1929 ± 628	2170 ± 270		117 ± 11 (15 ± 1)
Molar C:N ratio	4.6 ± 0.3	4.6 ± 0.1		5.8 ± 0.5
Total lipid content	632 ± 116	845 ± 11		116 ± 13 (15 ± 2)
ΣFAs	465 ± 85	622 ± 8	423 ± 26	84920 ± 9311
14:0	28 ± 11 (6 ± 1)	21 ± 1 (4 ± 0)	16 ± 2	5214 ± 1086 (6 ± 1)
16:0	91 ± 18 (20 ± 0)	132 ± 4 (22 ± 1)	88 ± 8	11166 ±1591 (13 ± 0)
16:1n7	20 ± 5 (4 ± 0)	29 ± 2 (5 ± 0)	16 ± 0	12155 ± 2206 (15 ± 1)
16:1n5	–	–	-	930 ± 149 (1 ± 0)
16:2n4	–	8 ± 1 (1.3 ± 0)	4 ± 0	6860 ± 736 (8 ± 0)
16:3n4	–	4 ± 1 (0.7 ± 0)	2 ± 0	16110 ± 1781 (19 ± 2)
18:0	49 ± 4 (11 ± 1)	57 ± 3 (9 ± 1)	37 ± 6	475 ± 85 (1 ± 0)
18:1n11	18 ± 3 (4 ± 0)	31 ± 2 (5 ± 0)	26 ± 3	–

18:1n9	6 ± 3 (1.2 ± 0)	–	1 ± 2	348 ± 46 (0.4 ± 0)
18:1n7	26 ± 5 (6 ± 0)	33 ± 1 (5 ± 0)	24 ± 1	474 ± 81 (1 ± 0)
18:2n6	–	–	-	862 ± 52 (1 ± 0)
18:4n3	–	3 ± 0 (0.5 ± 0)	1 ± 1	3833 ± 995 (4 ± 1)
20:1n11	11 ± 2 (2 ± 0)	17 ± 1 (3 ± 0)	15 ± 2	–
20:5n3	103 ± 12 (22 ± 1)	119 ± 2 (19 ± 0)	77 ± 3	19363 ± 1661 (22 ± 1)
22:5n3	73 ± 8 (15 ± 1)	99 ± 5 (15 ± 1)	74 ± 6	–
22:6n3	31 ± 4 (6 ± 0)	53 ± 1 (8 ± 0)	32 ± 1	4839 ± 216 (5 ± 1)
ΣSFA	173 ± 34 (38 ± 1)	219 ± 8 (36 ± 1)	148 ± 15	18140 ± 2743 (22 ± 1)
ΣMUFA	83 ± 22 (18 ± 1)	112 ± 1 (18 ± 0)	81 ± 5	13907 ± 2329 (17 ± 1)
ΣPUFA	209 ± 29 (44 ± 2)	291 ± 8 (45 ± 1)	194 ± 8	52873 ± 4641 (61 ± 2)

The ^{13}C isotopic enrichment in all FAs (but 18:0) varied from 2.88 ± 0.21 atom% to 4.00 ± 0.19 atom% between the different batches, averaging 3.62 ± 0.50 atom% for all days (Table 2, Fig. 2). For the FA 18:0, isotopic enrichment averaged 1.87 ± 0.30 atom% between the different batches (Table 2, Fig. 2). Labelling was significantly different between batches (ANOVA, F = 21.66, df = 3,67, *p* = ***), being higher on days 1 and 5 than on day 4 and on day 2 (Tukey, *p* = * and *p* = ***, respectively), and higher on day 4 than on day 2 (Tukey, *p* = ***). The major FAs cited above (or PUFAs in general) showed the highest labelling values within the FAs (Table 2).

Fig 2: ^{13}C isotopic enrichment of *Conticribra weissflogii* batch cultures fed to polychaete larvae during the experiment.

^{13}C isotopic enrichment is presented in atom%. Straight lines represent atom% values for the fatty acid (FA) 18:0, whereas boxplots and open circles represent average atom% values from all the other FAs.

Table 2 Total fatty acid (TFA) and FA-specific (> 1% TFA) ^{13}C isotopic labelling (APE) and carbon assimilation (C_{assim}, as ng C ind^{-1} and as % total C assimilated, in parenthesis) and turnover rate (C_{turn}, as % day^{-1}) for *Lanice conchilega*. ^{13}C isotopic labelling is also shown for *Conticribra weissflogii* (atom% - the atom% of TFA does not include values for the FA 18:0). SFA:

saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA. Values are mean ± standard deviation (n = 3 or 4 each). – : not detected.

	C. weissflogii	*L. conchilega*		
FA	atom%	APE	C_{assim}	C_{turn}
TFA	3.62 ± 0.50	1.16 ± 0.48	199 ± 21	6 ± 1
14:0	3.72 ± 0.53	0.93 ± 0.13	6 ± 1 (3 ± 0)	5 ± 1
16:0	3.62 ± 0.52	1.21 ± 0.14	44 ± 4 (22 ± 0)	7 ± 1
16:1n7	3.72 ± 0.54	1.67 ± 0.09	14 ± 1 (7 ± 0)	9 ± 0
16:1n5	3.48 ± 0.52	-	-	-
16:2n4	3.73 ± 0.52	1.94 ± 0.02	4 ± 0 (2 ± 0)	11 ± 0
16:3n4	3.73 ± 0.52	2.01 ± 0.06	2 ± 1 (1 ± 0)	11 ± 0
18:0	1.87 ± 0.30	0.66 ± 0.11	20 ± 2 (10 ± 0)	7 ± 1
18:1n11	-	0.67 ± 0.10	6 ± 1 (3 ± 0)	4 ± 1
18:1n9	3.19 ± 0.55	-	-	-
18:1n7	3.26 ± 0.45	0.97 ± 0.11	9 ± 1 (4 ± 0)	5 ± 1
18:2n6	3.68 ± 0.51	-	-	-
18:4n3	3.76 ± 0.54	1.91 ± 0.09	2 ± 0 (1 ± 0)	11 ± 0
20:1n11	-	0.49 ± 0.08	2 ± 0 (1 ± 0)	3 ± 0
20:5n3	3.74 ± 0.53	1.26 ± 0.12	41 ± 4 (21 ± 0)	7 ± 1
22:5n3	-	0.94 ± 0.11	25 ± 2 (13 ± 0)	5 ± 1
22:6n3	3.76 ± 0.53	1.42 ± 0.10	21 ± 2 (11 ± 0)	8 ± 1
ΣSFA	3.30 ± 0.90	0.95 ± 0.24	72 ± 7 (36 ± 0)	7 ± 1
ΣMUFA	3.41 ± 0.51	0.90 ± 0.49	31 ± 3 (15 ± 0)	5 ± 1
ΣPUFA	3.70 ± 0.45	1.46 ± 0.44	97 ± 11 (49 ± 0)	7 ± 1

Larval composition and labelling

Approximately 85% of the *L. conchilega* larvae survived at the end of the experiment. The average *in situ* DM and C and N contents of larvae were 55 µg ind^{-1} and 7668 and 1929 ng ind^{-1}, respectively, and the average values at end of the experiment were 52 µg ind^{-1} and 8511 and 2170

ng ind^{-1}, respectively (Table 1). Changes in DM and C, N, and lipid contents between larvae sampled from the field and after the experiment were not significant, indicating that the organisms did not lose or gain weight during the five-day incubation. The molar C:N ratio of larvae remained the same throughout the experiment at an average of 4.6. The average lipid content was similar between field (632 ng ind^{-1}) and experimental (845 ng ind^{-1}) individuals (Table 1). The lipid C content accounted for approximately 7% of the total C content of larvae sampled from the field and at the end of the experiment. Total lipid content comprised 1.2 ± 0.3% DM for *in situ* larvae and 1.7 ± 0.4% DM for larvae at the end of the experiment.

The major FAs in *L. conchilega* larvae were EPA, 16:0 and 22:5n3, which corresponded to 22, 20, and 15% TFA at the beginning and to 19, 22, and 15% TFA at the end of the experiment, respectively (Table 1). About a quarter of the FAs identified were only present in small amounts (<1% TFA, Fig. 1). The relative FA composition of *L. conchilega* larvae fed with diatoms remained similar to that of the individuals collected *in situ* (Table 1, Fig. 1), as conveyed by the 95.5% similarity between samples shown in the Bray-Curtis dendrogram (Fig. 3a). A similar pattern was observed for the absolute FA composition of the larvae (Table 1, Fig. 1), except that one of the *in situ* replicates was grouped with the experimental ones at 91% similarity, and these were found to be 82% similar to the other *in situ* replicates (Fig. 3b). These results further reinforce the above mentioned statistically non-significant differences in lipid C between *in situ* larvae and those fed during the experiment. In general, changes (i.e., increase or decrease) in the absolute concentration of a FA matched the change in its relative concentration (Fig. 1). The only exception to this pattern were the FAs 18:0, 18:1(n-7) and EPA, whose relative concentrations in the larvae decreased despite an increase in their absolute concentrations (Fig. 1).

Fig 3: Dendrograms generated with Bray-Curtis similarity matrices comparing fatty acid profiles of *Lanice conchilega* larvae.

Similarity matrices present data on the FA profile of *Lanice conchilega* larvae sampled from the field and after 5 days of feeding on the diatom *Conticribra weissflogii*. Only FAs with relative

content > 1% TFA were used to generate the similarity matrices. a) logit transformed relative values for FA composition (% TFA). b) absolute (ng C ind^{-1}) values for FA composition.

The average ^{13}C enrichment of *L. conchilega* FAs was of 1.16 ± 0.48 APE, ranging from 0.49 APE in FA 20:1(n-11) to 2.01 APE for FA 16:3(n-4) (Table 2). The FAs 18:1(n-11), 20:1(n-11), and 22:5(n-3) had the lowest labelling values (together with 18:0) in *L. conchilega*, whereas the FAs 16:2(n-4), 16:3(n-4), and 16:1(n-7) (diatom fatty acid trophic markers, FATM) had the highest APE (together with 18:4(n-3)).

Origin of FAs in the larvae

The labelling of FAs with ^{13}C enabled for the discrimination of the source of each FA-specific lipid C content: internal (Table 1), for FAs already present in the *in situ* larvae, and dietary (Table 2), for FAs assimilated via feeding on the labelled diatoms. The absolute content of internal FAs at the end of the experiment remained the same (~98%) as recorded from *in situ* larvae for two of the three replicates, and on average represented 92% of the *in situ* internal FA content (Table 1). Given the similarity of C content of internal FA between the beginning and end of the experiment the FA-specific differences are not considerable. The internal FA which showed the greatest decrease in terms of C content during the experiment was EPA, which fell from 22% TFA in *in situ* larvae to 18% of the total internal FA in diatom-fed larvae (Table 1). SFAs also showed slight decreases in C content between the beginning and end of the experiment.

Lipid C assimilation and turnover in larvae

Total assimilation of C into lipids in polychaete larvae at the end of the experiment was 200 ng lipid C ind^{-1} (Table 2). The relative assimilation of lipid C into FAs (Table 2) followed the relative composition of internal FAs (Table 1). Approximately half of the lipid C was assimilated into PUFAs, a third into SFAs, and only a sixth into MUFAs (Table 2, Fig. 4). The majority of the total lipid C was assimilated into 16:0 and EPA (22 and 21%, respectively), followed by 22:5(n-3),

DHA, and 18:0 (13, 11 and 10%, respectively). It is worth noting that the incorporation of dietary C into specific FAs did not follow their relative availability in the diet. The absolute concentration of the FAs 17:0, 18:1(n-11), 20:1(n-11), and 22:5(n-3) increased in *L. conchilega*, even though they were absent from the diatom cultures fed to the larvae (Table 2). The absolute concentration of the FAs 18:0 and DHA also increased in the diatom-fed larvae (Table 2), even though they were available in low amounts in the diet (1 and 5% TFA in *C. weissflogii*, respectively). Furthermore, the FAs i-15:0, 16:1(n-5), 18:2(n-6), 18:3(n-6), and 20:4(n-3) were only identified in *C. weissflogii* (Fig. 1) and were either not assimilated or bioconverted by the polychaete larvae. C assimilation in FATM was low in comparison to that into other FAs, but amounted to the highest C turnover rates (Table 2).

Fig 4: Carbon assimilation and turnover in *Lanice conchilega* larvae after the feeding experiment.

Carbon assimilation and turnover are given in ng C ind^{-1} and in %, respectively. Values are shown for the sum of all fatty acids (TFA), saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids; and for specific FAs.

The average lipid C turnover rate was of 6% day^{-1}, but varied between 3 and 11% day^{-1} for different FAs (Table 2). C turnover of SFA and PUFA was similar, 7% day^{-1}, and slightly higher than that of MUFAs, 5% day^{-1}. Overall, 32% of all lipid C was replaced with labelled dietary lipid C by the end of the experiment (Fig. 4).

DISCUSSION

The present study is the first to our knowledge to present data on C assimilation and turnover and on FA bioconversion in the larvae of the polychaete *L. conchilega*, and in any other meroplanktonic larvae for that matter. Although these were recorded from a short-term feeding experiment (5 days), they indicate how quickly polychaete larvae can assimilate dietary material.

These lipid-poor larvae seem to be able to regulate their lipid composition (homeostasis) by preferentially assimilating certain FAs and through bioconversion of dietary and internal FAs.

Experimental conditions

The active uptake of dietary C during the experiment was sufficient to compensate for the larval metabolic costs of living during the 5 days. This indicates that diatoms are a nutritious source of food for *L. conchilega* larvae, which readily accept this alga and utilize it as metabolically necessary. The lack of literature on feeding studies with meroplanktonic larvae prompted us to look for guidelines on food concentrations from laboratory experiments with small planktonic copepods [e.g., 38], so it is possible that individuals would have shown higher growth if they had received more food.

A common value of 40% DM is usually attributed for C content in marine organisms [e.g., 39]. The values obtained in the present study were almost 3 times lower, with larval C content at approximately 15% DM. As previously stated in the methodology, larvae were sampled while still inside their tubes. An attempt was made to remove the larvae, but we observed that it was not possible to do so without damaging the individuals or without the application of a sedative. The larval tube is composed of a thin organic layer, to which sand and shell fragments are attached at later stages [21]. This may have influenced the sample C and N contents and weight, and resulted on low relative values for C content in relation to DM.

Lipid composition, homeostasis, and bioconversion in *L. conchilega* larvae

The low total lipid content recorded for *in situ* larvae in the present study (1.2% DM) is similar to that reported for field and starving crab zoeae and bivalve larvae [40, 41, 42]. *Lanice conchilega* larvae thus appear to be lipid-poor organisms.

The relative concentration of single FAs and of SFAs, MUFAs, and PUFAs was preserved in the larvae during the feeding period. This occurred despite the provision of, and successful

feeding upon, a diet with a different FA composition. These results indicate that *L. conchilega* larvae can selectively accumulate FAs and/or control its FA metabolism and, thus, regulate lipid composition. A quasi-homeostatic response to variation in FA availability in the food source has also been shown for other zooplankton (daphnids) by [43]. The preferential retention of a FA over another can be easily identified if their relative proportions in the diet are similar, but their assimilation by the larvae differs. In the present study this is the case for the pairs of FAs 16:0 + 16:1(n-7), EPA + 16:3(n-4), and DHA + 18:4(n-3), for which the first FA of each pair was assimilated by *L. conchilega* in quantities that were 3-20x higher than those of the second FA, indicating their preferential accumulation. EPA and DHA have an important function for marine consumers as they fuel reproductive processes and neural function and regulate cell membrane fluidity [44, 45, 46]. It has been observed that adult *L. conchilega* accumulate (n-3) FAs to store energy during gametogenesis [8], but larvae probably use EPA and DHA for growth, as tissue hormones (eicosanoids) are produced from PUFA [47]. The FA 16:0, on the other hand, can be elongated and desaturated into other necessary FAs.

There are three different metabolic sources from which the FAs composing the lipid reserves of a predator can be derived. FAs can be (1) assimilated unmodified from the food (dietary origin), (2) synthesized *de novo* (dietary or internal origin), or (3) bioconverted (synthesized via chain elongation and desaturation) from another FA of dietary or internal origin [48]. FA bioconversion has more often been reported for crustaceans [23, 31] than for polychaetes [49]. In the present study we report the bioconversion of the FAs 17:0, 18:1(n-11), 20:1(n-11), and 22:5(n-3). It should be noted that the synthesis of odd-chained FAs is uncommon in consumers, and that the FA 17:0 is usually considered a bacterial FATM. The presence of this FA in the lipid profile of *L. conchilega* larvae indicates that either the organisms are able to synthesize odd-chained FAs or that bacteria were present in small amounts in the experimental units and were ingested by the larvae. Bioconversion occurred for both endogenously-derived FAs, which increased in absolute content during the experiment, and for dietary-derived FAs, which were enriched in ^{13}C. FA elongation and

405 desaturation involve reactions catalyzed by enzymes and are subject to isotopic fractionation [50],
406 which could explain why the ^{13}C enrichment was lower in the bioconverted FAs. The FA 22:5(n-3)
407 was bioconverted at high quantities, and made up 13% of all C assimilated by the larvae. Although
408 marine invertebrates are not able to biosynthesize *de novo* the FAs 18:2(n-6) and 18:3(n-3), they
409 have a limited ability to convert them into PUFAs via chain elongation and desaturation [23, 51]. In
410 the present study neither the diet nor the larvae contained 18:3(n-3), so 22:5(n-3) must have been
411 bioconverted from EPA, as has been suggested for brittle stars [52].
412

413 **Lipid homeostasis in lipid-poor and lipid-rich marine planktonic organisms**

414 The ability to regulate lipid composition does not come without an energetic cost. FA
415 homeostasis can be easy to maintain when an organism feeds upon a prey of similar lipid
416 composition, but will require that energy be allocated to bioconversion if prey have a different
417 biochemical make-up. Furthermore, the way in which an individual utilizes its assimilated FAs is
418 life-stage specific [23]. Based on our results and on the available literature on lipid content and
419 assimilation of mero- and holoplanktonic marine species [26, 27, 53, 54, other references shown in
420 Table 3, Franco-Santos et al., unpublished data], we put forward the hypothesis that 3 different
421 patterns in lipid homeostasis can be found among lipid-poor and lipid-rich planktonic organisms.

422 Table 3 Total lipid content (TLC, in % dry mass) and wax ester content (WE, in % total
423 lipid content) of several meroplanktonic and holoplanktonic species.

SPECIES	LOCATION	TLC	WE	REFERENCE
Meroplankton (lipid-poor)				
Bivalvia				
Crassostrea gigas larvae	Bay of Archacon, France	2-8	-	[42]
Teredo navalis larvae (lab-reared)	Great Harbor, MA, USA	2-4	-	[54]
Bankia gouldi larvae (lab-reared)	Pivers Island, NC, USA	3-8	-	[54]
Crassadoma gigantean larvae (lab-spawned)	?	4-8	-	[55]
Bryozoa				
Celleporella hyalina larvae (lab-spawned)	Menai Strait, UK	6-9	-	[56]
Cirripedia				
Balanus balanoides	Menai Strait, UK	13-15	-	[57]
Cephalopoda				
Octopus vulgaris hatchlings	Ría de Vigo, Spain	< 15	< 2	[58]
Decapoda				
Campylonotus vagans zoeae (lab-reared)	Beagle Channel, Argentina	7-9	-	[59]

Carcinus maenas zoeae	Helgoland	1-9	-	[41]
Nephrops norvegicus larvae	Mediterranean and Irish Seas	6-8	-	[60]
Panulirus cygnus larvae	Western Australia	9-13	-	[61]
Vertebrata				
Pleuragramma antarcticum (larvae)	Antarctic Peninsula	12	4	[62]
Solea senegalensis (lab-spawned eggs/larvae)	Bay of Cadiz, Spain	11-12	-	[63]
Holoplankton (lipid-poor)				
Amphipoda				
Cyphocaris richardi	Antarctic Peninsula	21	11	[62]
Euprimno abyssalis	Bute Inlet, Canada	26	12	[64]
Hyperia galba	Bute Inlet, Canada	19	8	[64]
Eusirus propaperdentatus	Antarctic Peninsula	22	22	[62]
Parandania boecki	Antarctic Peninsula	20	33	[62]
Parathemisto gaudichaudii	Antarctic Peninsula	19	9	[62]
Annelida				
Tomapteris septentrionalis	Bute Inlet, Canada	22	< 0.5	[64]
Copepoda				
Calanus helgolandicus	?	12	37	[25]
Euchaeta marina	Andaman Sea, India	11	-	[65]
Metridia gerlachei	Antarctic Peninsula	21	52	[62]
Rhincalanus gigas	Antarctic Peninsula	8	< blank	[62]
Undinula vulgaris	Andaman Sea, India	9	-	[65]
Chaetognatha				
Eukrohnia hamata	Arctic	19	12	[66]
Sagitta enflata	Andaman Sea, India	8	-	[65]
Sagitta elegans	Bute Inlet, Canada	14	6	[64]
Sagitta gazellae	Antarctic Peninsula	17	3	[62]
Coelenterata				
Atolla wyvillei	Antarctic Peninsula	1	48	[62]
Beroe cucumis	Bute Inlet, Canada	13	12	[64]
Diphyes antarctica	Antarctic Peninsula	1	16	[62]
Pleurobrachia pileus	Bute Inlet, Canada	9	6	[64]
Decapoda				
Acanthepyra sanguinea	Andaman Sea, India	14	-	[65]
Alpheus sp.	Andaman Sea, India	13	-	[65]
Lucifer hanseni	Cochin estuary, India	10-16	-	[67]
Pasiphaea pacifica	Bute Inlet, Canada	21	4	[64]
Euphasiacea				
Euphausia diomedae	Andaman Sea, India	13	-	[65]
Euphausia pacifica	Bute Inlet, Canada	19	1	[64]
Mysidacea				
Siriella sp.	Andaman Sea, India	11	-	[65]
Ostracoda				
Conchoecla elegans	Bute Inlet, Canada	17	4	[64]
Cypridina dentata	Andaman Sea, India	11	-	[65]
Tunicata				
Salpa thompsoni	Antarctic Peninsula	24	2	[62]
Holoplankton (lipid-rich)				
Amphipoda				
Eurythenes gryllus	Antarctic Peninsula	55	19	[62]
Copepoda				
Calanoides acutus	Antarctic Peninsula	45	64	[62]
Calanus hyperboreous	Arctic	37-74	34-91	[68]

Calanus finmarchicus	Norway	31	71	[69]
Calanus glacialis	Svalbard, Norway	70	68	[70]
Calanus plumchrus	Bute Inlet, Canada	47	86	[64]
Heterorhadus tanneri	Bute Inlet, Canada	43	69	[64]
Metridia longa	Arctic	57	76	[66]
Paraeuchaeta glacialis	Arctic	43	72	[66]
Decapoda				
Hymenodora glacialis	Arctic	35-39	44-62	[66]
Euphasiacea				
Euphausia superba (subadults/adults)	Weddell and Lazarev Seas	7-40	-	[71]
Thysanoessa macrura	Antarctic Peninsula	60	50	[62]
Pteropoda				
Clione limacina	Bute Inlet, Canada and Arctic	19-31	4-12	[64, 66]

Lipid-poor meroplanktonic larvae from decapods, bryozoans, and vertebrates, a.o., generally display total lipid contents in the order of 5-15% DM (Table 3). According to the available literature, total lipid content is on the lower side of this range for bivalve larvae (2-8% DM) and on the higher part for barnacle larvae (13-15% DM) and octopus paralarvae (< 15% DM) (Table 3). Both lecithotrophic (which possess and feed on yolk reserves) and planktotrophic (which feed on plankton) larvae rely on obtaining enough energy to sustain metabolic functions and growth into juvenile stages, and cannot dispense of energy for storage purposes. Lipid classes which are destined for energy storage, such as wax esters and triacylglycerols, represent a small proportion of total lipid content in lipid-poor meroplankton (Table 3). These organisms have specific requirements of FAs to sustain their body functions, such as formation of biomembranes, and will thus display lipid homeostasis, even though this may require the allocation of further energy for FA bioconversion. This is the case of the meroplanktonic larvae of *L. conchilega*, as discussed in the previous section. It appears, however, that the larvae of hermatypic and soft coral are lipid-rich (total lipid content reported to range between 41-68% DM; 72, 73). These would be an exception within the lipid-poor meroplankton, and more information is necessary before we can infer on their ability to regulate lipid composition during the larval stage.

Holoplanktonic organisms with an intermediate total lipid content ranging from 15-25% DM include small copepods, tunicates, chaetognaths, and ostracods (Table 3). Generally speaking, the lower end of this range is observed in organisms found in tropical regions, whereas the higher end is

recorded for individuals from temperate and polar regions (Table 3). The wax ester content of organisms varies between different groups, and ranges from values as low as 1% total lipid content to as high as 52% total lipid content (Table 3). These holoplanktonic organisms generally have a small to modest ability for energy storage as lipid reserves, a strategy whose focus is likely the maximization of reproductive output. Such species will mostly assimilate dietary FAs in an unmodified manner (non-homeostatic).

Lipid-rich holoplanktonic species, on the other hand, will display total lipid contents > 30% DM (Table 3). The species for which data was available were mostly from polar regions, and represent amphipods (55% DM), large copepods (31-74% DM), krill (7-60% DM), decapods (35-39% DM), and pteropods (19-31% DM). These organisms need to store energy in the most dense and effective way to survive low feeding conditions during the winter and for buoyancy purposes, and will thus bioconvert dietary FAs into long chain MUFAs / wax esters (semi-homeostatic). This can be exemplified by the large proportion of wax esters within the total lipid content in the mentioned groups, which is approximately 50% in decapods and krill and 34-90% in copepods (Table 3). Only pteropods and amphipods have a lower proportion of wax esters, which is approximately 15-20% of the total lipid content (Table 3).

The applicability of the FATM concept

In theory, FATM are assimilated by consumers in a conservative manner [24, 25]. The applicability of the FATM concept has been broadly acknowledged to depend, a.o., on the qualitative and quantitative transfer of FAs between trophic levels and on their metabolic stability and non-selective incorporation into consumer tissues [23]. In the present study the diatom FATMs were transferred between the producer and consumer trophic levels in a qualitative manner. In quantitative terms, however, the dietary FAs 16:0, EPA, and DHA were preferentially assimilated by *L. conchilega* larvae. Furthermore, we have postulated that EPA, which is a diatom FATM, was likely bioconverted (via chain elongation, as indicated in Fig. 1 of 74) into 22:5(n-3) by the

polychaete larvae. Unlike the trends shown by lipid-rich or lipid-accumulating organisms, the results of this study indicate that the applicability of the concept of FATM is limited in the lipid-poor larvae of *L. conchilega*. We also suggest that this will be the case for other lipid-poor meroplanktonic larvae, as they probably also display preferential assimilation and bioconversion of FAs in order to sustain body functions.

CONCLUSIONS

The present study documented preferential assimilation of FAs by *L. conchilega* larvae, which seem to be able to regulate their FA composition regardless of the FA profile of their food source. Bioconversion was also recorded in these individuals, which synthesized the PUFA 22:5(n-3) from dietary FA precursors. Our results show that dietary FAs are not transferred in a conservative (quantitative) manner, indicating that the concept of FATM is of limited applicability in the trophic study of lipid-poor *L. conchilega* larvae. This is probably also the case for other lipid-poor meroplanktonic larvae. Based on the results of this study and on data available from the literature, we propose that lipid homeostasis depends upon the lipid content of zooplanktonic organisms. Lipid-poor meroplanktonic larvae should display lipid homeostasis in order to satisfy their energetic requirements; holoplanktonic organisms with intermediate lipid levels will usually assimilate dietary lipids in a conservative manner and invest assimilated energy into reproduction; whereas lipid-rich holoplanktonic organisms will be semi-homeostatic, converting stored energy (FAs) into wax esters in order to survive starvation conditions during winter and to increase buoyancy.

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Fig 1

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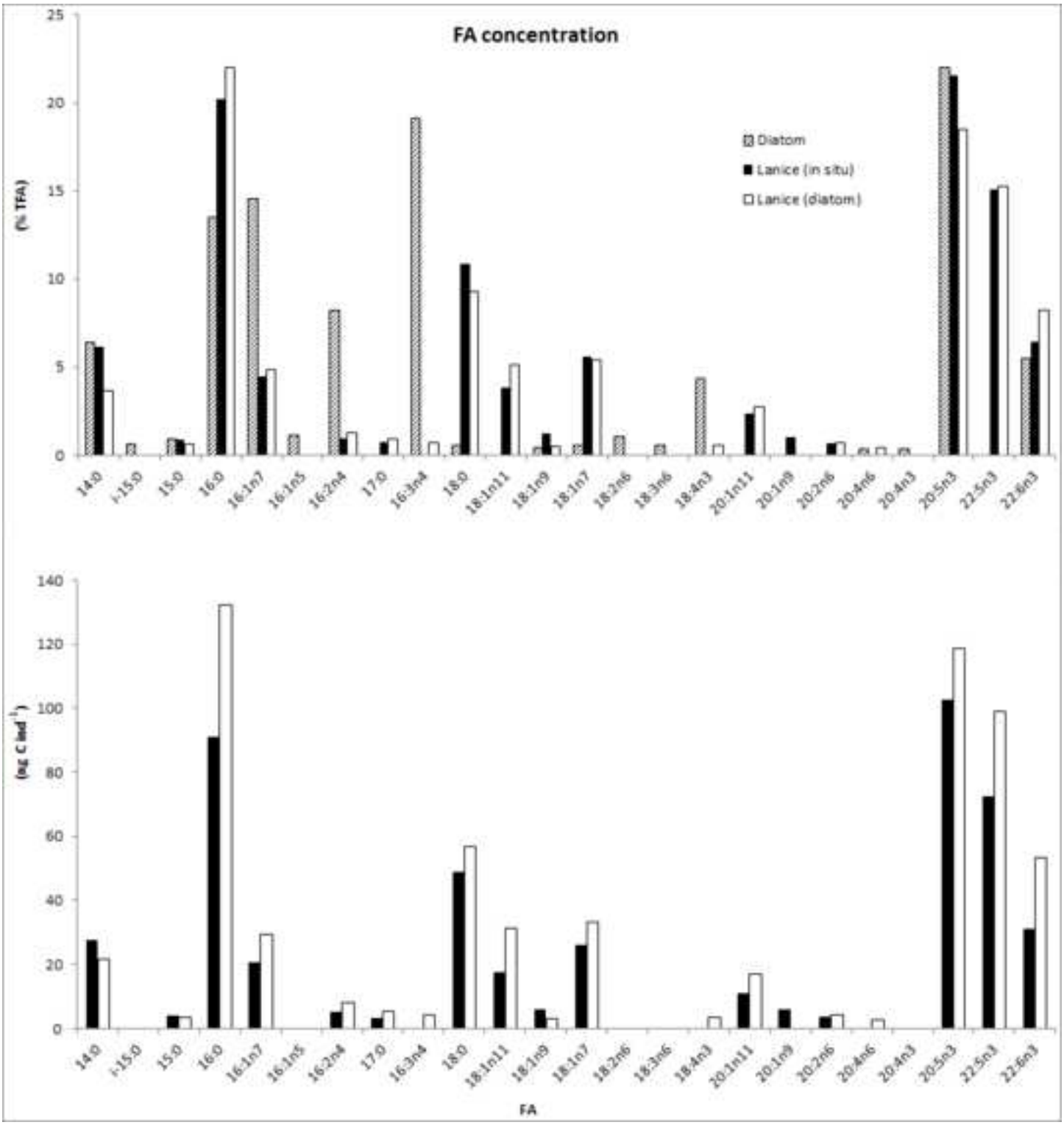

Fig 2

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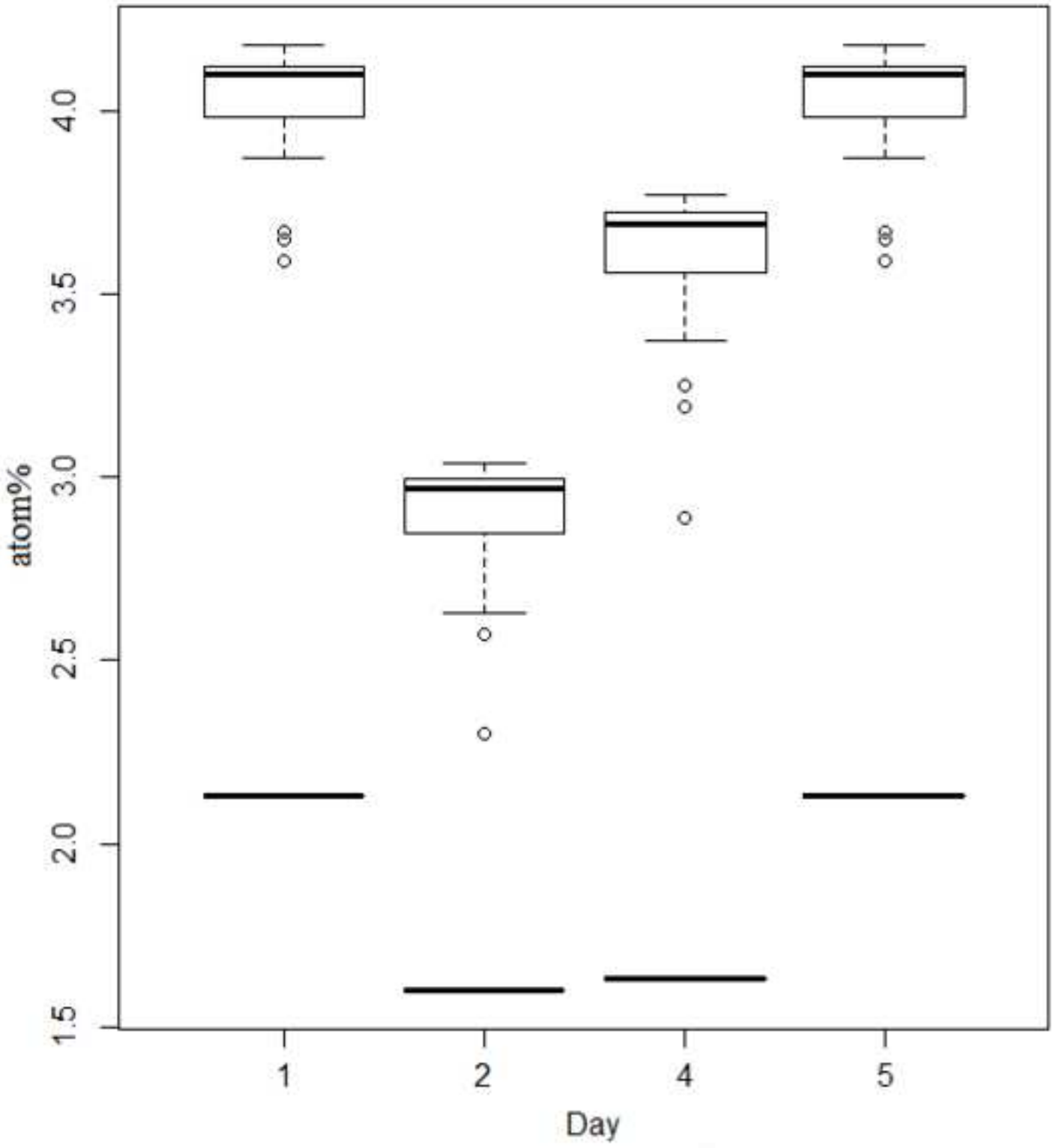

Fig 3

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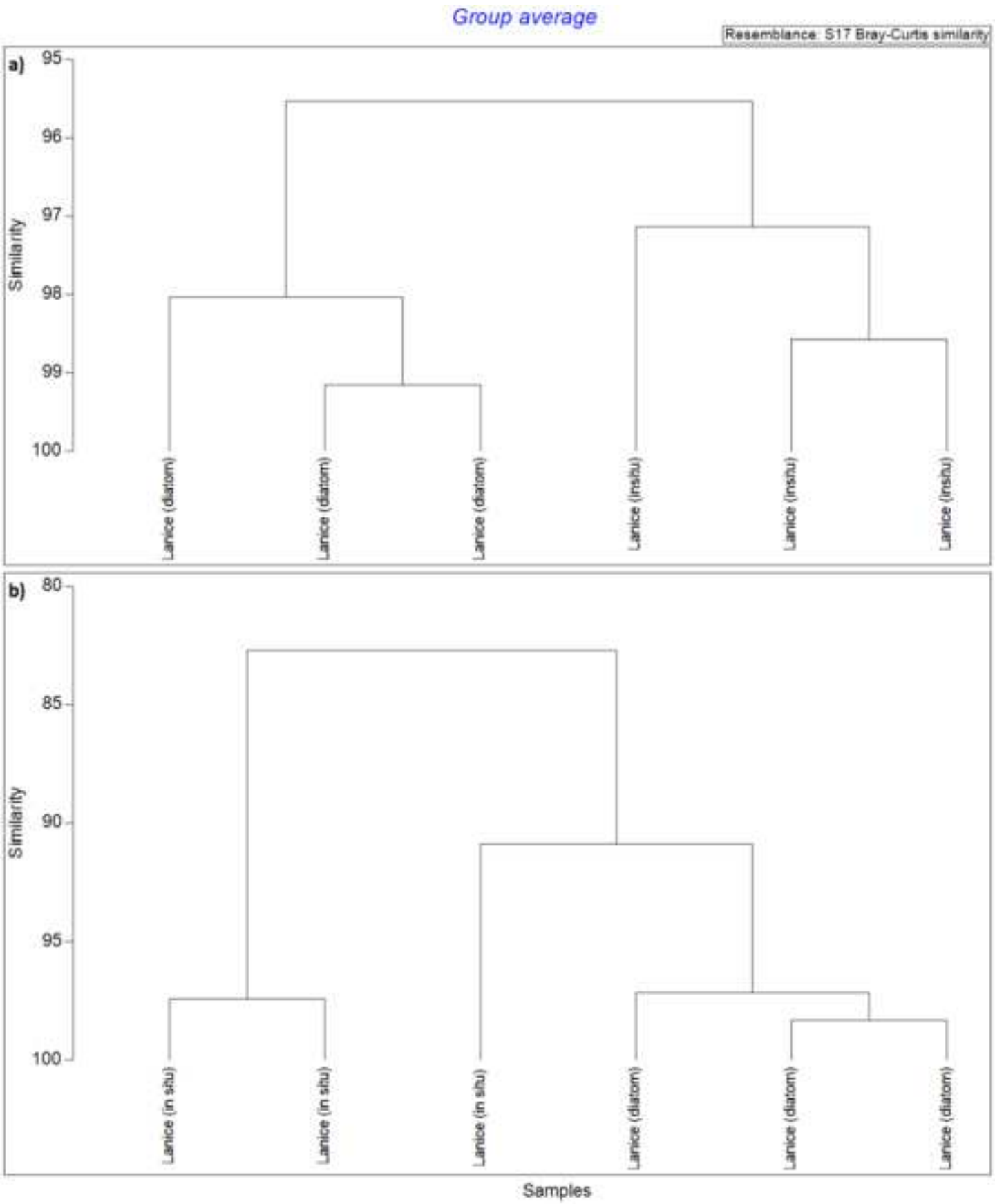
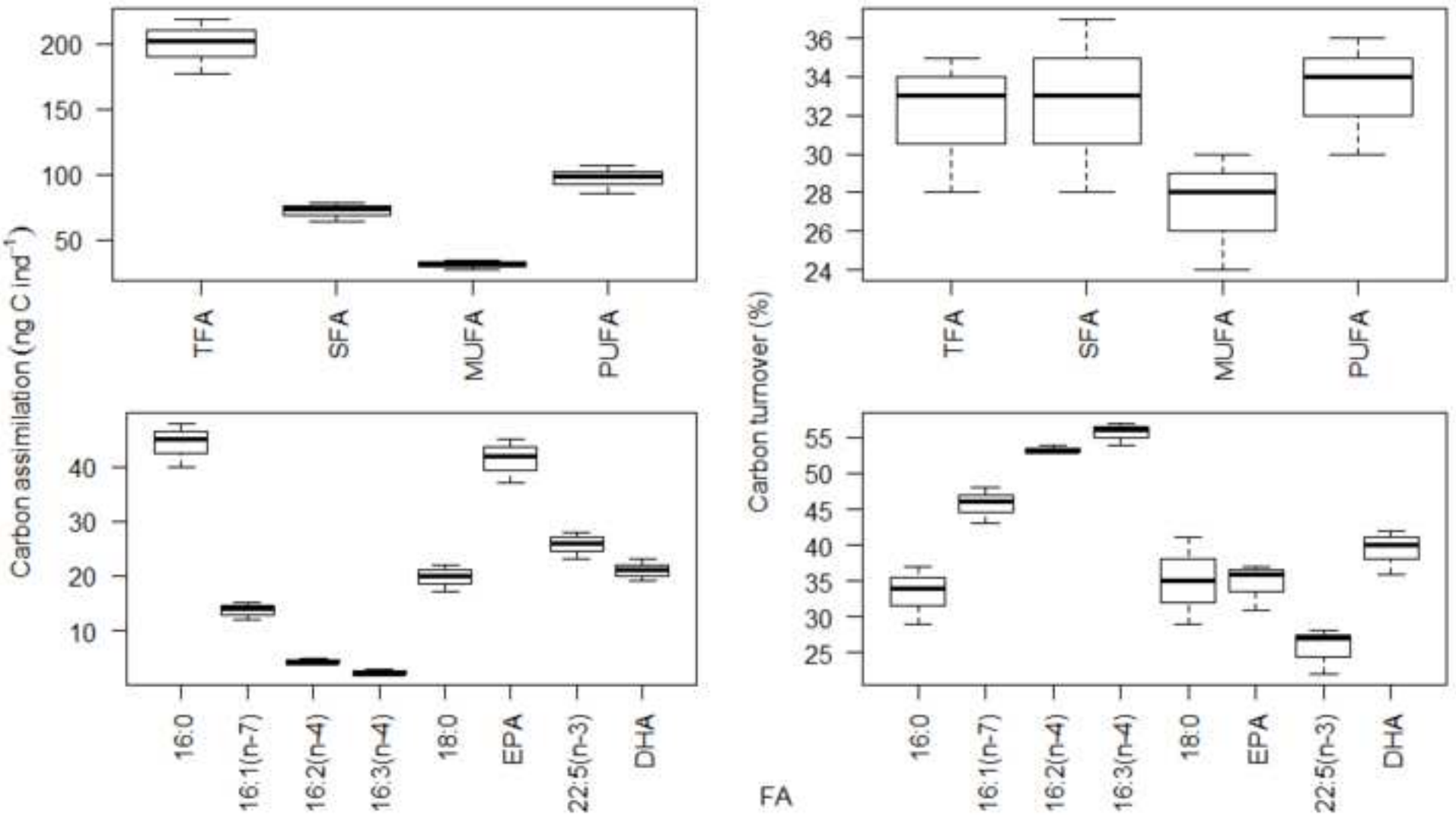


Fig 4



CHAPTER IV

Temperature driven changes in the diet preference of omnivorous copepods: no more meat when it's hot?

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LETTER

Temperature driven changes in the diet preference of omnivorous copepods: no more meat when it's hot?

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Abstract

Herbivory is more prevalent in the tropics than at higher latitudes. If differences in ambient temperature are the direct cause for this phenomenon, then the same pattern should be visible in a seasonal gradient, as well as in experiments manipulating temperature. Using ^{15}N stable isotope analyses of natural populations of the copepod *Temora longicornis* we indeed observed seasonal differences in the trophic level of the copepod and a decrease in trophic level with increasing temperature. In a grazing experiment, with a mixed diet of the cryptophyte *Rhodomonas salina* and the heterotrophic dinoflagellate *Oxyrrhis marina, T. longicornis* preferred the cryptophyte at higher temperatures, whereas at lower temperatures it preferred the non-autotrophic prey. We explain these results by the higher relative carbon content of primary producers compared to consumers, in combination with the higher demand for metabolic carbon at higher temperatures. Thus, currently increasing temperatures may cause changes in dietary preferences of many consumers.

Keywords

Global warming, grazing, homeostasis, metabolism, stoichiometry, zooplankton.

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INTRODUCTION

For fish in particular, a clear latitudinal gradient exists with respect to the prevalence of herbivory (Floeter *et al*. 2005; Behrens & Lafferty 2007; Gonzalez-Bergonzoni *et al*. 2012), with higher incidences of herbivory in the tropics, whereas carnivory is more important at higher latitudes. Several explanations for this phenomenon have been brought forward. These were well-developed by Clements *et al*. (2009) and Gonzalez-Bergonzoni *et al*. (2012), to which we refer readers for more details, but explanations range from evolutionary constraints (colder systems are younger so herbivory has not developed as yet), through availability (in colder systems there are no plants in winter), and quality (digestibility of tropical plants tissue is higher) to a suite of directly temperature-related explanations. Digestion of plant material is considered easier in higher temperatures, so herbivory should be more prevalent (Floeter *et al*. 2005). This temperature constraint hypothesis seems to be the most widely accepted explanation for the observed gradient, and essentially argues that with declining temperature feeding rates decrease more rapidly than metabolic rates. The critical temperature required for net positive energy balance is thought to be higher for herbivores than for carnivores because animal material is a high quality food and easier to assimilate than plant material (Floeter *et al*. 2005). In their review on herbivory in marine fish Clements *et al*. (2009) argue that it is essential to also consider the nutritional content of the food and take into account nutrient intake. They state that temperature can also affect the nutrient content of plant material, and with this, feeding rates of herbivores.

Especially the food content of nitrogen (N) and phosphorus (P) in their concentration relative to carbon (C) have received a lot of attention (Sterner & Elser 2002), and there is strong consensus in the aquatic literature that plants (algae) have higher (and more variable) carbon to nitrogen and phosphorus contents than animals (e.g. van de Waal *et al*. 2009). Hence, a consumer requiring relatively more C should select for plants, whereas a higher P or N requirement should lead to a selection for animal prey. Several studies have investigated the temperature sensitivity of nutritional demands with respect to C : N : P stoichiometry, very well summarised recently by Cross *et al*. (2015). Two competing predictions exist. The Growth Rate Hypothesis (Elser *et al*. 1996) links growth rate to phosphorus demand, and, as in poikilotherms growth rates increase with temperature, this hypothesis predicts a higher P-content and hence P-demand at higher temperatures (Dobberfuhl & Elser 2000; Persson *et al*. 2011). In a later study though, Elser *et al*. (2000) include evolutionary arguments in their reasoning, stating that as a result of shorter growing seasons, growth rates of arctic clones of the cladocerans *Daphnia pulex* are actually higher than those of temperate conspecifics, and hence they should have a higher P-demand (see also Daines *et al*. 2014 for a similar discussion in algae). This is of course a completely different line of reasoning, invoking evolutionary aspects rather than physiological ones. It is also argued that higher temperatures lead to an increase in protein denaturing and higher protein turn-over

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rates, thus increasing the demand for nitrogen at higher temperatures (Lemoine *et al.* 2013). In contrast, higher temperatures also imply higher metabolic rates. If there is a different temperature dependence of anabolic and catabolic processes (e.g. Karl & Fischer 2008), which has been suggested especially in relation to the wide spread phenomenon of temperature related changes in body size (Forster *et al.* 2011, 2012), and respiratory processes have a higher Q_{10} than growth (see also Larsson & Berglund 2005), then we would expect that at higher temperatures the relative demand for carbon (respiration) is higher than for N or P. Cross *et al.* (2015) make this argument, leading to a prediction of the temperature sensitivity to quality limitation which is concurrent with the observed latitudinal variability in herbivory. Lower temperatures trigger a higher necessity for nutrients relative to carbon, and thus a higher necessity for meat.

If the explanation of direct temperature-dependent differences in nutrient demands is correct, this implies that there should be a direct influence of temperature on feeding patterns and selectivity. This can be tested. First, we would expect the pattern of changing feeding modes also to be present within one species over different seasons of the year. Furthermore, in an experimental set-up, we would predict a direct dependence of the grade of herbivory on temperature, and a higher affinity for high carbon food at higher temperatures. To answer these questions, we sampled the calanoid copepod *Temora longicornis* over several seasonal cycles, and investigated the trophic level of this copepod, which can switch between herbivorous and carnivorous feeding modes (Gentsch *et al.* 2009; Boersma *et al.* 2014), using nitrogen stable isotope measurements. Furthermore, we directly investigated the selectivity of adult females of this copepod species cultured under a range of different temperatures when fed autotrophic and heterotrophic food, and investigated feeding rates of *T. longicornis* on high C : P and low C : P algae at two different temperatures.

MATERIALS AND METHODS

Sampling site

The samples for the present study were collected from the Helgoland Roads time series station in the German Bight of the North Sea. This sampling station is located between the island of Helgoland and the adjacent Düne island at the Kabeltonne site (54° 11.3′ N, 7° 54.0′ E) (Wiltshire & Manly 2004).

Sample collection and analysis

The samples were collected over a period of 4 years from 2004 to 2008. The copepod *Temora longicornis* was sampled using a 500 μm CalCOFI net with an aperture of 100 cm and net length 400 cm (Greve *et al.* 2004). The collected *T. longicornis* were transferred to the laboratory and immediately sorted alive using a stereomicroscope. Then the copepods were individually washed in distilled water to remove particles and salt. Three groups of 10–20 individuals were transferred into tin cartridges, and dried at 60 °C for up to 24 h. Similarly, seawater surface samples were collected and filtered, using pre-combusted (6 h, 420 °C) glass fibre filters (Whatman, GF/C, 25 mm diameter). Depending on the density of algae 200 mL–1 L was filtered. All filters were also heat dried at 60 °C for 12 h and transferred to tin capsules for further analysis. To estimate the trophic position of the *T. longicornis* females, we analysed the $\delta^{15}N$ content of both the animals and the seston. The samples were analysed by ARGROISOLAB (Jülich/Germany) or at the Stable Isotope Facility at University of California, Davis using an Elementar Vario EL Cube and Micro Cube elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany) which was interfaced to PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The difference between the $\delta^{15}N$ value of animals and seston was used to calculate the trophic level (TL) position of the *Temora longicornis* females, using a trophic fraction constant of 3.4 per trophic level (Minagawa & Wada 1984; Vander Zanden & Rasmussen 2001). In order not to artificially inflate the number of samples, we averaged the values of the three replicates per sampling day, before entering the analysis. We then related the computed trophic level of the copepods with ambient temperatures.

Copepod grazing experiments

For the grazing experiment on the two different prey the protocols of Löder *et al.* (2011) were followed. *T. longicornis* was collected from the field, and five adult female *T. longicornis* were sorted and transferred to 800 mL glass beakers containing 500 mL of filtered seawater. Five replicate beakers per temperature were subjected to temperatures from 10 to 28 °C (with a total of 10 different temperatures) for 24 h to precondition the animals for experiments. To determine grazing selectivity of *T. longicornis* a mixed diet of the cryptophyte *Rhodomonas salina* and the heterotrophic dinoflagellate *Oxyrrhis marina* was used. *R. salina* was cultured in F/2 culture medium, whereas *O. marina* was cultured in the same medium but pre-fed *R. salina*. Feeding conditions for the dinoflagellate before the experimental incubations were such that the food algae in the *O. marina* cultures had virtually been depleted at the time of the start of the experiment. For culture conditions and experimental protocol see Meunier *et al.* (2011). The C : N : P stoichiometry of *R. salina* and *O. marina* grown under these conditions are 320 : 38 : 1 and 175 : 25 : 1, respectively (see also Hantzsche & Boersma 2010). This demonstrates the premise of higher relative nutrient content in heterotrophs.

The experimental containers with *T. longicornis* were inoculated with *R. salina* and *O. marina* in cell densities of 8000 and 1000 cells mL^{-1}, respectively, to provide similar concentrations of prey carbon for *T. longicornis*. The cell number of the algal cultures was determined using a CASY particle counter (Schärfe Systems, Reutlingen, Germany) (Meunier *et al.* 2013), manual counts were carried out to determine the density of *O. marina*. The experiment was conducted using a temperature table (Thomas *et al.* 1963) with a temperature gradient that ranged from 10 to 28 °C. Each of the ten temperatures had five replicates. Since the dinoflagellate *O. marina* also feeds on *R. salina,* the grazing experiment needed

appropriate controls. The controls included one that contained *R. salina* only to estimate growth rates of the alga, and another one containing a mix of *R. salina* and *O. marina* at the same densities as in the treatment with *T. longicornis*. This allows the separate estimation of growth and consumption rates of *O. marina* on *R. salina*. The experiment ran for 24 h with 15 h of light and 9 h of darkness. At the end of the experiment, the number of surviving *T. longicornis* were counted, and Lugol's iodine solution was used to arrest the growth of the algae and to preserve the *R. salina* and *O. marina* for further analysis: *O. marina* was counted using a Sedgewick Rafter Cell Counter under the microscope and *R. salina* using the CASY particle counter. Typically, in an experiment such as this one, grazing and ingestion rates are computed using the equations as presented by Frost (1972), Heinbokel (1978), and Nejstgaard *et al.* (2001) (summarised by Löder *et al.* 2011). Despite the fact that this set of equations is much used in the literature, the way these equations are set up creates an interdependence of estimates of the grazing rates of the different prey. An increased estimate of the grazing impact of *T. longicornis* on *O. marina* leads to a decrease in the calculated grazing pressure of *O. marina* on the alga and, to balance numbers, automatically also to an increase in the estimate of the grazing impact of the copepod on the alga. Hence, since we were interested in the temperature dependent patterns, rather than the exact numbers, we decided to simplify the approach, and also compared the slopes with temperature of the average numbers of *R. salina* and *O. marina* in the treatments with and without predators, using ANCOVA analysis. We used the exact temperatures measured in each separate replicate for this analysis rather than the averages of each of the rows in the temperature table. Based on the computed grazing rates, using the appropriate equations (see above), we computed the ingestion on both prey types, and from this the amount of ingested carbon and phosphorus, and the C : P ratio of the ingested material.

The final step linking herbivory to potential nutritional demands is the investigation of feeding patterns on one food source of different nutritional content at different temperatures. If a higher temperature is linked to a higher C-demand, then one would expect higher relative grazing rates on nutrient limited (C-rich) algae at higher temperatures than at lower temperatures. Thus in two additional experiments, we collected *T. longicornis* from the field, and sorted 10 adult females per treatment. These were transferred to 100 mL plastic beakers containing 100 mL of filtered seawater, and placed in one of two temperature-controlled rooms at 10 and 20 °C for 72 h to pre-condition the animals for experiments. During the first 48 h the copepods were fed seston between 30 and 150 μm and were left without food for the last 24 h to reduce any potential pre-feeding effects. To determine grazing of *T. longicornis*, two cultures of the cryptophyte *R. salina* were used, one grown in F/2 medium while the other was grown in F/2 medium without P to induce a P-limitation (Meunier *et al.* 2011, 2015). The P content of P-replete and P-depleted *R. salina* were 0.30 (± 0.07) and 0.13 (± 0.01) pg P cell^{-1}, respectively, yielding C : P rates of 448 : 1 and 1100 : 1. Two experiments were carried out to investigate the robustness of the change in feeding. *T. longicornis* were caught from two different sites at two different occasions, with about 6 weeks between them. We incubated ten *T. longicornis* in 250 mL Falcon culture flasks with either P-replete or P-depleted *R. salina* in cell densities of 1000 cells mL^{-1}. The flasks were filled with artificial sea water at a salinity of 32 to prevent any change in *R. salina* nutritional quality. Another set of bottles were incubated without copepods to serve as control. At least three replicates per treatment were used and the flasks were placed on two plankton wheels (0.8 rpm) in temperature-controlled rooms at 10 and 20 °C. The experiment was run for 24 h and samples were taken and fixed with formalin (formaldehyde 20% buffered with hexamine). The samples were stored cool and dark before being counted with a BD ACURI C6 flow cytometer. For each sample, 500 μL of sample was processed by the flow cytometer to ensure that at least 200 *R. salina* cells were counted. Grazing rates were calculated as described above based on Frost (1972), and data were analysed with a three-way ANOVA using experiment, food type and temperature as factor and grazing rate as dependent variables.

RESULTS

Stable isotope analysis of natural populations

The δ^{15}N values from the natural populations of *T. longicornis* and seston showed considerable variation with time (Fig. 1a). The difference in δ^{15}N values between *T. longicornis* and seston was used to compute the trophic level of the copepods. A trophic level of two indicates that the only food source of the copepod is seston, and that *T. longicornis* is the primary consumer of this seston, whereas a trophic level of three indicates that there is at least one trophic level between the seston and the copepod, and hence that the copepod is in fact the secondary consumer (Fig. 1b). The trophic level position of *T. longicornis* ranged between 1.01 and 3.45, and it significantly decreased with increasing in temperature ($r^2 = 0.32$; $P < 0.0001$). This decrease in the trophic level position indicates that *T. longicornis* fed on a diet richer in phytoplankton at warmer temperatures, the slope of −0.064 °C indicates that with every 15–16 °C of temperature increase the trophic position of *T. longicornis* decreases by one level (Fig. 1c). This relationship still holds after collating all the data into temperature bins/ groups of 4 °C (Fig. 1c, inset), to avoid the problem of autocorrelations in the data. The regression with the means of the bins against temperature is still significant, despite an n of only five points ($r^2 = 0.85$; $P = 0.03$). Furthermore, if we compute the change in trophic level of *T. longicornis* between two adjacent measuring points from the data series, the change in trophic level (TL) averages −0.14 (SE = 0.10) with a temperature increase between two points, whereas the change in TL is positive (0.24; SE = 0.12) when the temperature decreases. This difference is significant (*t*-test; $P = 0.01$).

Temperature dependent copepod grazing on heterotrophic vs. autotrophic prey

The grazing experiment with *T. longicornis* was conducted using *R. salina* and *O. marina* as prey. All *T. longicornis* died

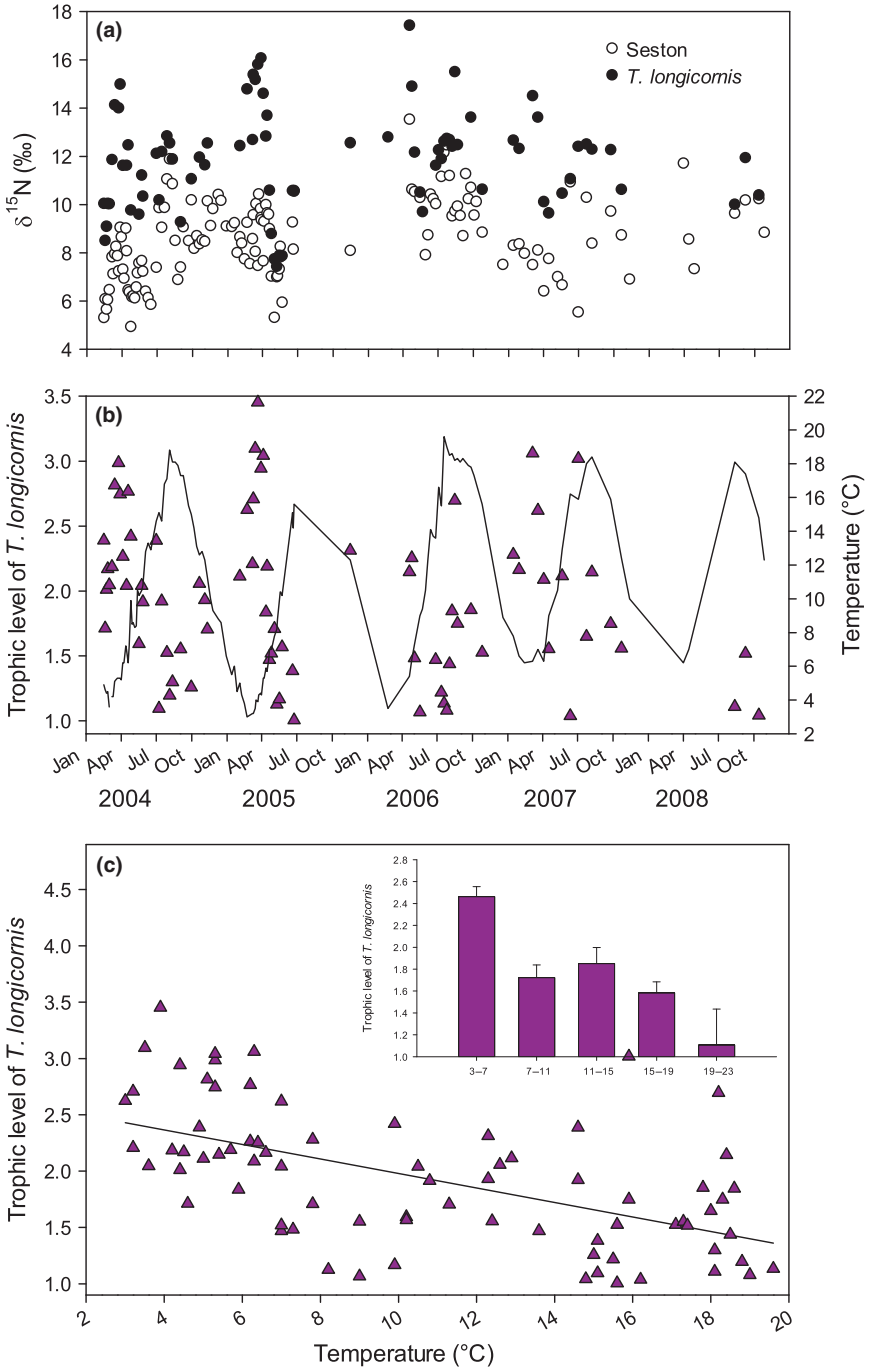

Figure 1 (a) δ^{15}N values of seston and the copepod *Temora longicornis* for samples collected from 2004 to 2008; (b) Trophic level position of *T. longicornis* (triangles) with water temperature at the moment of sampling (line); (c) Correlation of the trophic position of *T. longicornis* with temperature. The regression line (trophic level = 2.62 − 0.064 × Temp) explains 32% of the variance in the trophic level of *T. longicornis* ($P < 0.0001$; $n = 76$), and indicates a loss of one trophic level with every 15–16 °C. The inset shows the average trophic level of the *T. longicornis* in 4 °C bins. Also the linear regression with only the averages ($n = 5$) as dependent variables is significant ($P = 0.03$).

at 28 °C, survival was very good in the other temperatures. The growth rate of *R. salina* was affected by the temperature, as indicated by the temperature dependence of the average densities in the treatment with *R. salina* alone (Fig. 2a; $r^2 = 0.19$; $P < 0.005$). In the presence of only *O. marina* as a grazer, we observed no temperature dependence of the *R. salina* densities ($r^2 = 0.003$; $P = 0.75$), whereas with two predators present densities were again significantly correlated with temperature ($r^2 = 0.48$; $P < 0.001$), indicating higher consumption rates of *T. longicornis* on algae at higher temperatures. Homogeneity of slope analysis revealed that the slopes of the two significant regressions (*R. salina* alone, and *R. salina* with both predators) with temperature were not significantly different from each other (ANCOVA, $F_{1,1} = 1.03$;

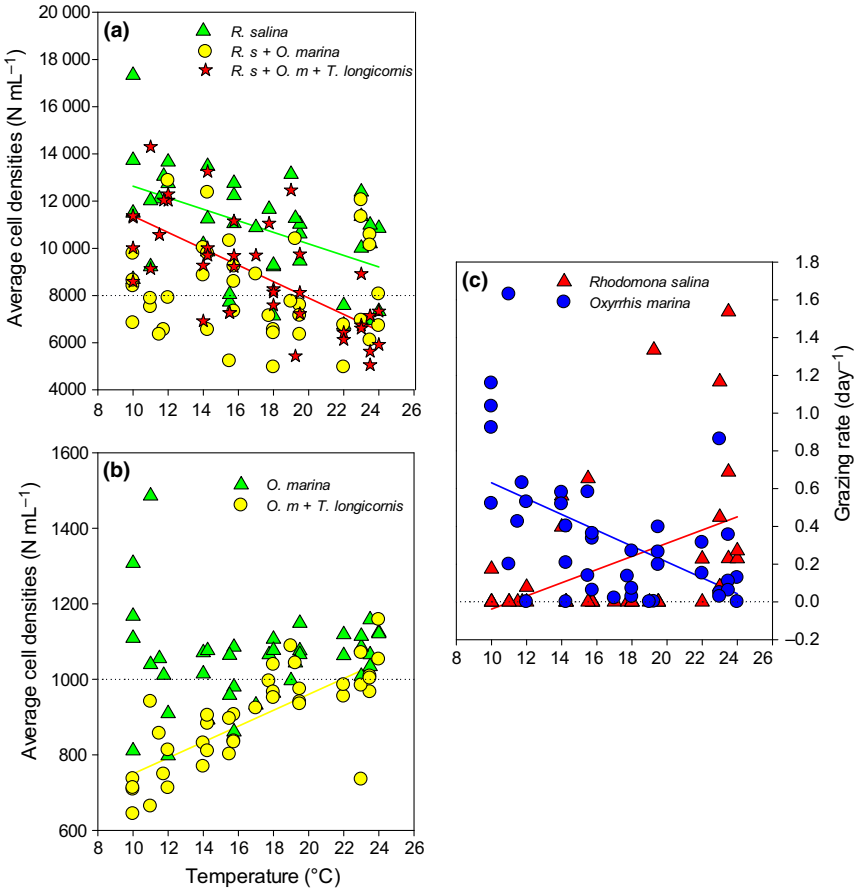

Figure 2 Densities of (a) *Rhodomonas salina,* and (b) *Oxyrrhis marina* in treatments with and without predators. Regression lines indicate significant relationships between temperature and cell numbers. Dotted lines are the initial densities at the beginning of the experiment; (a) *Rhodomonas salina* alone (green triangles; Density = 15 080 − 244 × Temp; $r^2 = 0.19$; $P < 0.005$), with one predator (yellow circles), and two predators (red stars; Density = 14 835 − 347 × Temp; $r^2 = 0.48$; $P < 0.001$); (b) *Oxyrrhis marina* densities alone (green triangles), and with predator (yellow circles; Density = 540 + 21 × Temp; $r^2 = 0.59$; $P < 0.001$); (c) Grazing rates of *Temora longicornis* on *R. salina* (red triangles; rate = −0.4 + 0.035 × Temp; $r^2 = 0.18$; $P < 0.007$) and *O. marina* (blue circles; rate = 1.05 − 0.04 × Temp; $r^2 = 0.28$; $P < 0.0005$) indicates that the grazing on *R. salina* increases with temperature, whereas the grazing on *O. marina* decreases. All computed negative feeding rates were set to zero.

$P = 0.313$) but both were different from the treatment with only *Oxyrrhis* as a grazer. When comparing the average number of *O. marina* in relation to the temperature (Fig. 2b) it becomes clear that whereas the growth rate of *O. marina* is not correlated with temperature (no significant correlation, $r^2 = 0.01$; $P = 0.56$), the average density in the presence of *T. longicornis* is affected ($r^2 = 0.59$; $p < 0.001$), resulting in a significant difference in the slopes of the regression lines with temperature (ANCOVA, interaction between temperature and treatment, $F_{1,1} = 12.90$; $P < 0.001$). This difference remained significant even after removing the data points for the lowest temperature for *O. marina* without the copepods, thus this result was not dependent on these points. With higher temperatures the average numbers of *O. marina* cells in the experimental units increased significantly, which indicates a lower grazing pressure of *T. longicornis* on *O. marina* with increasing temperature. The data are summarised in Fig. 2c, which shows the computed grazing rates of *T. longicornis* on both potential prey (with negative grazing rates set to zero, see also Nejstgaard *et al.* (2001)). The grazing rate of *T. longicornis* on the alga *R. salina* showed a significantly positive correlation with temperature ($r^2 = 0.18$; $P < 0.01$), whereas the grazing on the dinoflagellate *O. marina* was negatively correlated with temperature ($r^2 = 0.28$; $P < 0.0005$), thus indicating a switch in preference from 'animal' food at low temperatures to 'plant' food at higher temperatures. This result does not depend on our choice to set negative grazing rates to zero. Even with all values included (Table S1; Fig. S1a), or with negative values excluded from the analysis (Fig. S1c), the results are qualitatively the same. In fact, the slopes of the regression lines of the three different analyses are not significantly different from each other (Table S1). Based on the ingestion rates at different temperatures and the C : P ratios of both prey, we can compute the change in C : P ratio in the food taken up by the *T. longicornis* at the different temperatures. With a linear regression relating temperature to ingested C : P ($r^2 = 0.18$; $P < 0.001$) we estimated that at 10 °C the C : P ratio of the ingested food was 175 (essentially only taking up *O. marina*), and at 20 °C the C : P of the food taken up was 216. This means that at higher temperatures *Temora longicornis* take up ~ 25% more carbon relative to phosphorus.

Temperature dependent copepod grazing on high C : P vs. low C : P algae

In the two experiments with differently grown *R. salina* we observed a significant interaction between temperature and food type (Table 1; Fig. 3). Feeding on P-limited *R. salina* increased with temperature, whereas feeding on P-replete algae decreased, consistent with the predictions and observations from the previous experiments. This shows that P-limited food is a more attractive food source at higher temperatures.

DISCUSSION

In this study, we showed that the trophic level of the calanoid copepod *Temora longicornis* varied seasonally, leading to a significantly negative correlation between the estimated trophic level and temperature of the environment. This finding is consistent with the global patterns of the relationship between intensity of herbivory and latitude, and shows that this pattern can even be observed within one system on a seasonal scale. The experiment directly investigating the effect of temperature on dietary preference of *T. longicornis* corroborated these findings. At higher temperatures the copepod showed a higher preference for algae than for heterotrophic prey. Thus the demand for nutrients is higher at lower temperatures, and/or the demand for carbon is higher at higher temperatures. These are difficult to separate, but we will argue that the latter is the more logical explanation. Furthermore the third experiment revealed a significant interaction between temperature and C : P ratio of the food on the grazing rates of the copepods, stressing the importance of the C : P ratio in determining the grazing rates.

Table 1 ANOVA results of the analysis on the effects of experiment, temperature and food type on the grazing rate of *Temora longicornis*

Factor	Sum of squares	d.f.	Mean squares	*F*	*P*
Experiment (E)	0.116	1	0.116	16.55	< 0.001
Temperature (T)	0	1	0	0.01	0.925
Food type (F)	0.035	1	0.035	5.04	0.031
E × T	0.044	1	0.044	6.29	0.017
E × F	0.007	1	0.007	1.01	0.321
T × F	0.047	1	0.047	6.74	0.013
E × T × F	0.001	1	0.001	0.18	0.673

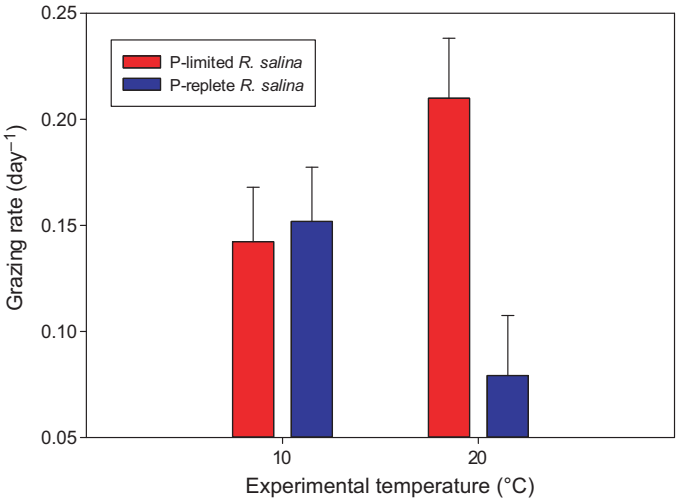

Figure 3 Grazing rates (SE) of *Temora longicornis* on P-limited (C : P = 1100 : 1) and P-replete (C : P = 448 : 1) *R. salina*. A significant interaction between temperature and food quality was observed (Table 1).

Obviously, the correlative approach of the field survey is not without caveats. The first one is in the nature of the analysis. Correlative analyses can never infer causality, and it could well be that temperature is not the factor driving the change in $\delta^{15}N$ signal, but other correlated factors such as the availability of nutrients (Aberle & Malzahn 2007). Unfortunately, there is no real way to resolve this. Moreover, trophic fractionation of stable nitrogen isotopes may be temperature dependent. The literature on the effect of temperature on stable isotope physiology gives conflicting information ranging from increased to decreased fractionation of stable nitrogen isotopes with temperature (Power *et al.* 2003; Barnes *et al.* 2007; Matthews & Mazumder 2008). For crustacean zooplankton we are aware of only two studies on the effects of temperature on the stable isotope physiology. Whereas Matthews & Mazumder (2008) reported no effects of temperature on the fractionation, Power *et al.* (2003) observed an increase of trophic fractionation of 0.1‰ per °C. The latter would result in a much steeper slope of the regression line in Fig. 2, and a much stronger dependency of the trophic level on the temperature. The third issue in studies like this one is the matter of finding the proper baseline. We do realise that filtering seston throughout a season does not give a clean algal (primary producer) baseline signal. Smaller heterotrophic organisms such as ciliates and flagellates are included in this fraction, but they are impossible to omit from the analysis as they are essentially the same size as the algae. To date, there is no good alternative for this, except the comparison of stable isotope signals of specific compounds, some of which are essential for herbivores, such as some amino acids, and within the herbivore tissue represent the food source signal, with those substances that undergo fractionation and represent the consumer signal (e.g. Chikaraishi *et al.* 2014). However, these techniques are still highly demanding and not suitable for routine samplings. Hence, the traditional way of filtering the seston fraction to obtain a base-line estimate is still very much in use (Hansson *et al.* 1997; Sommer & Sommer 2004; Gentsch *et al.* 2009; Guzzo *et al.* 2011; Kürten *et al.* 2013), and even if it does not resolve the absolute trophic position, the $\delta^{15}N$ value gives a clear indication of the relative position of a consumer in the food web. We also realise that the trophic level of the *Temora* individuals reported here is sometimes low. This is caused by our choice of trophic fractionation factor for ^{15}N of 3.4‰ per food level. In fact, reported values differ widely in the literature going as low as 0.1‰ per trophic level (D'Ambra *et al.* 2014). Obviously, this affects our estimates of the absolute trophic level, but using a different fraction factor would not have affected the pattern in the data at all. Of course, this trophic position does not say anything about what drives the change. If consumers were to

simply follow availability of food sources then they might be more herbivorous in summer when algae are more plentiful. In fact, we observed a significant negative correlation between total algal biomass and trophic level of *T. longicornis*, but the relationship of trophic level with temperature remained significant even after correcting for the effect of algal biomass.

Grazing experiments with more than one predator are notoriously difficult and usually show a lot of variation, potentially leading to several estimates of negative grazing rates (Löder *et al.* 2011). The corrections proposed by Nejstgaard *et al.* (2001) are useful in this respect, but they create an interdependence between the grazing rates on the different food sources, which is difficult to interpret. Moreover, they assume a constant grazing of the predator over the whole experimental period, which is not necessarily correct. Nevertheless, using these corrections we were able to show that the grazing rate of the copepod *T. longicornis* on the alga *R. salina* showed a significantly positive correlation with temperature, whereas the opposite was true for the grazing on *O. marina,* patterns that remain visible even when including all the data, or excluding all estimates of negative grazing rates (see Table S1 and Fig. S1). Especially the relationship of *T. longicornis* grazing on *O. marina* is very robust. From this we conclude that *T. longicornis* prefers 'meat' at lower temperatures and 'vegetables' at higher ones. This finding is consistent with the results from the field survey. Very few studies have experimentally investigated temperature related changes in feeding, and those that have do not yield a coherent picture. Whereas Sotka & Giddens (2009) reported an increase in feeding rates on low protein foods with temperature in at least a sub-set of the amphipods they investigated (with no change in the others), Lemoine *et al.* (2013), in their study on selective feeding in the beetle *Popillia japonica,* observed that at higher temperatures the beetles selected for plants with higher N-content. Unfortunately, in the latter study levels of allelopathy in the plants changed as well, so it is difficult to draw conclusions. Nevertheless, using the difference in nutrient content between different sources of prey, our study can provide a potential mechanistic explanation of the correlative evidence from the meta-analysis studies on latitudinal gradients of herbivory cited in the introduction.

Our results raise the question of whether the increase in feeding on lower quality plant material at higher temperatures is a luxury that generalist consumers can afford, thus broadening their food spectrum at higher temperatures, or a necessity to obtain the amount of energy necessary to pay for the increased metabolism. We suggest that is rather the latter. First, Castellani & Altunbaş (2014) reported that for the copepod *Temora longicornis* the respirational demand doubled from *c.* 5 to 10% of body carbon in the temperature range from 5 to 17.5 °C, whereas the change in egg production rate is much lower (Castellani & Altunbaş 2006). Under the assumption that respiration only requires carbon, and egg production both carbon and phosphorus this implies a higher carbon demand at higher temperatures, which can be met with the 25% higher uptake of carbon relative to phosphorus, something we indeed see in our last experiment. Moreover, Cross *et al.* (2015) argued that the threshold elemental ratio (TER, Urabe & Watanabe 1992) should increase with temperature, even though they also state that there is a great need to measure the dependence of the TER on temperature. Traditionally, this TER indicates the ratio between carbon and a nutrient below which the nutrient N or P is no longer limiting, and potentially carbon becomes limiting (Anderson *et al.* 2005), and organisms need to excrete the nutrient. Growth should be maximal at the TER (Boersma & Elser 2006), thus animals at higher temperatures should eat more carbon relative to nutrients to maximise their growth rate.

Implications

Our present study has three major implications. First, a methodological one: in any study on dietary preference of consumers it is very important to indicate the temperature at which the experiments were carried out, as the outcome of the experiments may be temperature dependent. Second, current changes in temperatures in many areas in the world may lead to a change in feeding preference of those organisms that have the initial dietary breadth to do so. Based on our results, we predict an increase in the overall incidence of herbivory. This may also cause a shift in species composition if some species are competitively superior herbivores while being less capable to feed on heterotrophs. Third, many water bodies in the world are undergoing re-oligotrophication, with decreasing inputs of N and P, in concert with an increase in the CO_2 availability. Where this might influence primary production, and increase the carbon to nutrient ratios of many primary producers, the effects of this apparent decrease in food quality might be counteracted by the increasing temperature, thus potentially leading to a higher secondary production.

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AUTHORSHIP

MB, BN and KLS conceived the study and collected data, KAM carried out one experiment, CLM and RMFS the others, MB wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

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4 SYNOPTIC DISCUSSION

This thesis provides a characterization of the laboratory performance for two members of the zooplankton from Helgoland, females of the calanoid copepod *Temora longicornis* and meroplanktonic larvae of the polychaete *Lanice conchilega*, in relation to food quality. An extensive dataset on physiological parameters is provided for the copepods in Chapters I and II, and includes rates of ingestion, respiration, excretion, egestion, growth and reproduction. The efficiency with which these processes occur is also provided, and a budget with C and N values for the rates is constructed in order to evaluate copepod performance. In addition, the effects of food quality on carbon assimilation and turnover rates in the copepod are also evaluated (Chapter II). It was not possible to obtain such a qualitative and quantitative dataset for the polychaete larvae. Nevertheless its carbon assimilation and turnover rates while feeding on nutrient-replete diatom cultures are reported (Chapter III). The work on lipid C assimilation in the copepods and polychaete larvae, together with data available from the literature, was also used to propose the hypothesis that lipid homeostasis depends upon the total lipid content of zooplanktonic organisms (Chapter III).

The synoptic discussion is divided into three parts. Section 4.1 provides a thorough evaluation of the CSIA method used in the experiments conducted during this PhD, with a special focus on methodological problems which need to be resolved in future studies. Section 4.2 provides an evaluation of the Ecological Stoichiometry framework, which was used in this PhD to study the impacts of diet quality on the physiology of the zooplankton. It discusses what are the applications and limitations of this framework in relation to the results obtained with experiments conducted with mero- and holoplanktonic species. Section 4.3 presents a description of how communities have been impacted by climate changes and anthropogenic activities in diverse marine ecosystems across all oceans. It continues by describing how distinct geographic regions within the North Sea have been affected differently in the past decades. It further focuses on regime shifts in the area surrounding the German island of Helgoland, from where the organisms used in the experiments performed for this PhD were sampled. Experimental results are then used to infer about food quality effects on copepod performance, and on how this group might fare in future scenarios of changing food regimes.

4.1 ADVANTAGES AND CAVEATS OF USING COMPOUND-SPECIFIC STABLE ISOTOPE ANALYSIS

Trophic ecology has long sought to determine the relationships between prey and predator. Several techniques can be used to determine the diet of a consumer, with varying degrees of invasiveness and lethality. Visual observation was probably the first method employed, followed by analysis of blood, regurgitated dietary material, faecal matter, and of gut contents. All of these conventional methods, however, can only provide a snapshot of the diet of a consumer, the most recent items it fed upon (Michener and Kaufman, 2007; Newton, 2010). Stable isotope analysis (SIA), on the other hand, offers the possibility of diet integration over a longer period of time, while also providing information on the source of a food item and on consumer trophic level (Dalsgaard et al., 2003; Michener and Kaufman, 2007; Newton, 2010). Stable isotope ratios were first applied to studies in the field of earth sciences, but over the past 30 years ecologists have sought it to better understand interactions within species and between them and their environment (Newton, 2010). Their great usefulness for many areas arises from changes in the ratio of lighter and heavier isotopes in a compound during physical and chemical processes (Newton, 2010). Of particular use to ecological studies are the isotopes of the light elements H, C, N, O, and S. In this section I mainly discuss about the use of the $^{13}C/^{12}C$ ratio, as this was the ratio I analyzed in my experiments, but several pertinent questions in ecology can be answered by using ratios of the other elements.

The ^{13}C SIA of bulk (or aliquot) samples (BSIA) is commonly used to identify the possible food items (or foraging area) ingested by a consumer (e.g.: Chen et al., 2018; Cherel et al., 2006; Gorbatenko et al., 2014), based upon the knowledge that different photosynthetic pathways in (C3 and C4) plants result in differing ^{13}C signatures (first observed by Smith and Epstein, 1971). It is also possible to estimate tissue-specific turnover rates in organisms with BSIA, though this involves multiple sampling and the use of two or more isotopically disparate food sources in laboratory experiments (Newton, 2010). In the past, the determination of stable isotope ratios was limited to BSIA (Lichtfouse, 2000). The analysis of ratios for individual compounds within complex molecules (CSIA) was only possible with the commercial production of the gas chromatograph/combustion/isotope ratio mass spectrometer (GC/C/IRMS) in the early 90's (Evershed et al., 2007; Meier-Augenstein, 1999, 2002). There are several advantages to using

CSIA over BSIA, though these depend upon the goal of an investigation. In terms of tracing the use of resources by consumers in complex marine systems, for example, BSIA cannot provide information on the isotopic signature of biochemical components, whether they are different or identical but belong to different pools (Evershed et al., 2007). As these compounds may have different functions, they may also reflect different turnovers of dietary material, i.e., provide information on temporal changes in diet. In a study on the use of food resources for reproduction in butterflies, for example, it was shown that the nonessential AAs in the eggs were derived from nectar sugar (adult diet), whereas essential AAs were obtained solely from the larval diet (O'Brien et al., 2004). In the case of a key marine copepod, *Acartia* spp., the use of CSIA of AA ^{13}C allowed for the identification of a selective feeding behavior which varied seasonally (Nielsen and Winder, 2015). In general, and due to the nature of the analysis itself, CSIA will provide a greater wealth of information than BSIA (specified in Evershed et al., 2007), which may be helpful in disentangling ecological questions.

CSIA is a methodology that provides accurate and precise (up to 0.3‰ or 0.0003 atom percent excess, APE) measurements of the isotopic composition of compounds (Meier-Augenstein, 2002). It is a powerful tool for tracing the origin and fate of organic matter and to investigate biochemical processes and patterns in individuals and ecosystems (Evershed et al., 2007; Guilini et al., 2010; Meier-Augenstein, 2002; Rix et al., 2018; Wegener et al., 2012). The applications of CSIA are as broad as the fields that have used it and include, a. o., forensics, environmental science, archaeology, food science, and doping control (Benson et al., 2006; Fogel and Tuross, 2003; Lichtfouse, 2000; Ogrine et al., 2003; Schmidt et al., 2004; Simpson et al., 1999; Tobias and Brenna, 2018). There has been an increase in the use of CSIA in tracer studies, especially those aiming to quantify biochemical processes such as assimilation, turnover, and synthesis of important molecules (Boissonnot et al., 2016; Budge et al., 2008; De Troch et al., 2012; Graeve et al., 2005; Kluijver et al., 2013; Middelburg et al., 2000). This was also the case in this PhD work. Before the advent of CSIA, such metabolic studies were only possible, if at all, by using stable isotope tracers at high enrichment levels, which were analyzed with a simple GC-MS, or by using radioactive tracers (Meier-Augenstein, 1999).

Within the context of this PhD, BSIA would have certainly been advantageous time-wise, as the sample preparations prior to data acquisition runs are simpler and faster for this technique than

for CSIA. The remarkably smaller data output obtained from a single run/sample analyzed with BSIA would also have made the interpretation of results a much simpler process than that required for the multiple dataset returned from a single run/sample analyzed with CSIA. This PhD project was designed with the goal to better understand the performance of zooplanktonic organisms in relation to diet nutritional quality. One of the specific goals was to obtain quantitative data on the transfer of dietary C from prey to predator, but I was also interested in investigating FA-specific assimilation, biosynthesis and bioconversion due to the importance of certain FAs in the reproductive process in copepods (Jónasdóttir et al., 2009; Thor et al., 2007). This type of information could only be achieved with CSIA.

Methodological constrains of CSIA in this PhD

The use of CSIA in tracer studies is still at its early stages and variables such as the duration of exposure of target species to ^{13}C and the amount of label necessary to obtain robust results are still being fine-tuned. High levels of isotopic enrichment are not desirable, as they cannot be properly detected by the GC-c-IRMS (M Graeve, pers. obs.). Low concentrations of $NaH^{13}CO_3$ which still enable correct and reproducible estimations of C assimilation and turnover should thus be favored, as they also minimize research costs associated with the use of the bicarbonate powder. The concentration of $NaH^{13}CO_3$ used to enrich *C. weissflogii* cultures, for example, has decreased over the years from 200 mg L^{-1} (Graeve et al., 2005) to 15 mg L^{-1} (Boissonnot et al., 2016). For the experiments conducted during this PhD, I used, for the same diatom species, a concentration of 4 mg L^{-1}. Although the atom% values that I obtained for these cultures (and for the copepods that fed on them) were low, they were above the natural concentrations (1.08 atom% or -30‰ $^{13}\delta C$). This enabled me to reliably quantify the C assimilation and turnover rates in the copepods (Chapter II) and polychaetes (Chapter III) fed with the diatom diets. *Oxyrrhis marina*, the other prey item used to feed copepods and polychaetes, is a heterotrophic dinoflagellate, and had to be indirectly labelled via feeding on isotopically-enriched *R. salina*. The concentration of ^{13}C I used to enrich *R. salina*, 12 mg L^{-1}, did not seem to be enough for a transfer of adequate labelling to the next trophic level in one of the *O. marina* cultures. Future studies that attempt to use this species to indirectly label non-autotrophic organisms should run pre-experiment tests with enrichment of both trophic levels in order to determine the quantity of ^{13}C necessary to achieve their goals.

Labelling in the dinoflagellate cultures differed, as those fed with N-depleted (enriched) *R. salina* (Dino– cultures) showed isotopic composition above the natural concentrations and those fed with nutrient-replete *R. salina* had isotopic composition values at the edge of valid labelling. The data I gathered from the experiments cannot fully explain this result. It was not caused by poor labelling of nutrient-replete *R. salina*, as these cultures enabled the enrichment, though low, of the Dino+ culture and were also properly labelled in another experiment I conducted during the PhD (atom% = 1.24 ± 0.12, not mentioned in this thesis). Absence of feeding of *O. marina* on the nutrient-replete *R. salina* cultures was also not a problem, as suggested by a previous study (Hantzsche & Boersma, 2010) and confirmed by the flow cytometer readings (not shown in this thesis). It would be possible that Dino+ fed only partially on *R. salina* and complemented its nutrition via cannibalism (thus diluting its ^{13}C enrichment), though there is evidence that only a few cells within an *O. marina* culture are cannibalistic (Martel and Flynn, 2008). The variation in $\delta^{13}C$ and the atom% values were clearly higher in the Dino– cultures (339-1174‰ and > 1.47, respectively) than in the Dino+ (7-197‰ and > 1.09, respectively). This indicates that the difference in isotopic enrichment between the dinoflagellate cultures might have been caused by a differential labelling of *R. salina* cells when they were cultured under different nutrient conditions. Different growth and environmental conditions can trigger different pathways for C uptake by phytoplankton cells (Eek et al., 1999), which will result in varying isotopic composition of cells (Riebesell et al., 2000). Since nutrient supply can regulate phytoplankton growth under saturated light conditions, nutrient concentration should indirectly affect C isotopic fractionation. It would seem that when algae are deprived of N during growth, carbohydrates and lipids are accumulated at the expense of AAs, a shift in the flux of C which is accompanied by a lower isotopic fractionation and, thus, results in higher isotopic signal (Eek et al., 1999). The difference in labelling of *O. marina* cultures thus seems to have derived from a differential uptake of ^{13}C by *R. salina* cells in relation to nutrient availability during growth. The results I recorded for isotopic composition of both *C. weissflogii* and *R. salina* cells follow this pattern, with cells grown under N-depleted conditions showing higher isotopic signal than those grown under nutrient-replete conditions. Future tracer studies using phytoplankton cultures with different nutrient concentrations should adjust the amount of labelling added in order to account for fractionation differences.

The low level of labelling transfer from *R. salina* to *O. marina* in the Dino+ cultures made it difficult to follow the transfer of labelled FAs from the dinoflagellates into the copepods they were fed to. The APE of copepods fed with the diatom and Dino– cultures accounted for approximately 25-35% of the atom% of their respective diets, whereas the APE of those fed with the Dino+ cultures accounted for only 7-8%. Because the labelling of the Dino+ cultures was so low to begin with, it is not possible to affirm with certainty whether the low APE and C assimilation and turnover values obtained for copepods fed with this diet were due to methodological problems, biological processes, or both. There is, however, indication that it was due to the low labelling of the Dino+ cultures. The total C assimilation values recorded from copepods feeding on labelled diets should always be equal to or higher than the increase in total lipid C. These two values were similar in copepods fed with the diatom and Dino–, but the C assimilation of copepods fed with Dino+ accounted for only 15-26% of the recorded increase in total lipid C. There is no plausible explanation for an increase in lipid C that would not be recorded as C assimilated in animals feeding on isotopically-enriched food. During this PhD I have observed that *T. longicornis* not only feeds normally on nutrient-replete *O. marina*, but it also thrives on this diet (highest ingestion, growth and egg production rates; Chapter I). This lead me to conclude that, in the case of copepods fed with Dino+, the C assimilation values were highly underestimated due to methodological problems (i.e., with the isotopic enrichment of *R. salina*).

This methodological problem was the source of some academic frustration. As can be seen from Chapter I, the copepods fed on the Dino+ diet were regarded as displaying the best performance, and the FA-specific C assimilation and turnover data for this treatment could have offered many insights into why that was the case. Moreover, the inability to properly label *O. marina* also affected the experiments with the polychaete larvae. Originally, I would have been able to (a) verify whether the larvae can feed on dinoflagellates, for which no record exists, and (b) assuming it does, compare their performance on different diets. Due to the labelling problem, I was only able to investigate lipid assimilation and utilization in *L. conchilega* larvae while feeding on diatoms (Chapter III). Despite the problems, Chapters II and III are solid contributions to the field of trophic ecology, as there are few studies that have followed lipid transfer between copepods and their prey and no information in the literature regarding the feeding ecology of marine polychaete larvae. The results of this thesis also help to further

advance the establishment of guidelines for the experimental labelling of organisms in tracer studies in food webs.

Take-home messages for Section 4.1

- CSIA provides accurate measurements of the isotopic composition in specific compounds within molecules. Combined with the use of ^{13}C enrichment in laboratory experiments, it enables the quantification of biochemical processes such as lipid C transfer and FA assimilation, turnover, and synthesis in organisms.

- Species-specific protocols for using CSIA in tracer studies need to be fine-tuned, as variables such as the duration of exposure of target species to ^{13}C and the amount of label necessary to obtain robust results are still being tested.

- The uptake of C by algae and, thus, their enrichment with ^{13}C, is related to photosynthetic pathways and varies according to culture conditions. As the effects of the latter on ^{13}C labelling are not well described at present, it is recommended that the successful labelling of algae with ^{13}C be verified prior to their use in laboratory tracer experiments.

- Tracer studies which investigate lipid transfer between three or more trophic levels should make sure that the ^{13}C enrichment of the lowest trophic level is high enough to be transferred in measurable and differentiable quantities to the highest trophic level.

4.2 ECOLOGICAL STOICHIOMETRY AND HOMEOSTASIS AS TOOLS TO STUDY ZOOPLANKTON PHYSIOLOGY

Zooplankton physiology, and how it may be affected directly and indirectly by climate change, can be studied from the perspective of several nutritional frameworks, such as metabolic theory, ecological stoichiometry (ES), and nutritional geometry. ES has been presented as a useful tool for investigating environmental effects on the stoichiometry of organisms and, consequently, the flow of elements through different levels of biological organization and, ultimately, the cycling of nutrients and elements at the ecosystem level (Elser et al. 1988; Sterner & Elser 2002). For the work conducted during this thesis, the physiology of two members of the zooplankton was investigated under the framework of ES.

An important concept to ES is that of homeostasis - the ability of some organisms to maintain a constant body chemical composition despite changes in the chemical composition of their environment and, more specifically, their food (Kooijman, 1995). If the assimilation of nutrients by a consumer occurs in identical proportions to their relative content in the prey, then the consumer is nonhomeostatic – the concept of "you are what you eat" (Fig. 8a). Organisms which assimilate nutrients in constant proportions to their relative dietary abundance are also nonhomeostatic (Fig. 8a), as they do not adjust their elemental composition in response to that of their prey, but they no longer are what they eat. Homeostatic regulation occurs as a feedback (in the opposite direction to that of the disruption) between the composition of the consumer and that of its prey, such that in strict homeostasis there is no variation in the former in relation to the latter (Sterner and Elser, 2002; Fig. 8b). Strict homeostasis can also occur within certain physiological pools in the organism (structure vs. energy reserves) but the relative proportions of the pools may vary with time (Kooijman 1995; Sterner and Elser, 2002). Organisms will usually fall somewhere between nonhomeostatic and strictly homeostatic, with autotrophs being usually less homeostatic than heterotrophs (Sterner and Elser, 2002). The degree of homeostasis will vary in relation to several parameters, among which the most important are likely to be the species of consumer, its life stage, the element/molecule composition of its food source, and the comprehensiveness of the dataset (Sterner and Elser, 2002).

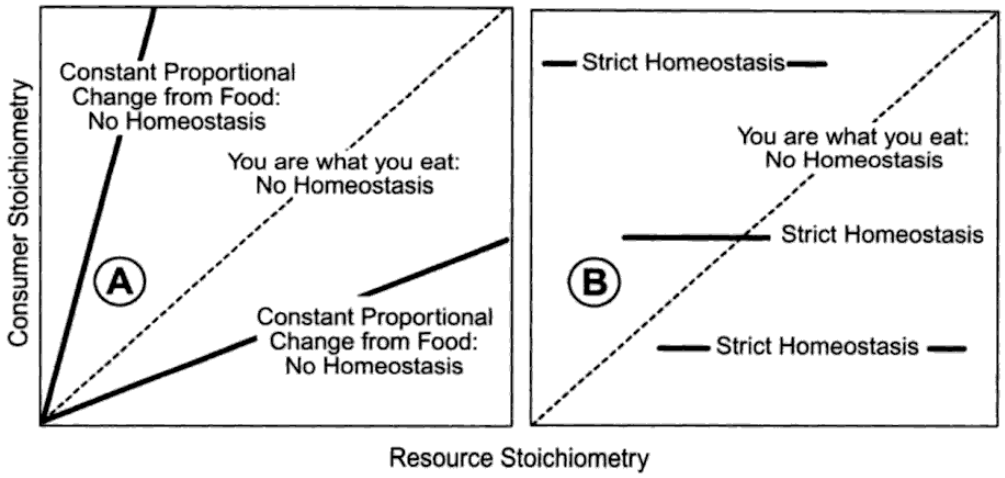

Figure 8: Stoichiometric patterns which relate the stoichiometry of consumers and resources. Horizontal and vertical axes can be any type of stoichiometric measure. If the stoichiometry of a consumer always matches that of the resources it feeds upon, then their relationship is indicated by the points in the line with slope 1 and intercept 0 ("you are what you eat: no homeostasis"). The solid diagonal lines in A) represent instances when the consumer performs constant differential nutrition retention (albeit nonhomeostatic). The solid horizontal lines (slope 0, intercept > 0) in B) characterize strictly homeostasis. Source: Sterner and Elser, 2002.

Consumers can react to the availability of prey items of differing elemental composition by changing feeding rates, food selection, production efficiency, and growth rate (Sterner and Elser, 2002). Homeostatic regulation of consumer stoichiometry can occur before and after the ingestion of nutritionally unbalanced food (i. e., prey with a different molar C:N ratio to that of the consumer) (Siuda and Dam, 2010; Sterner and Elser, 2002). Pre-ingestion regulation is related to consumer behavior, which can select higher quality prey (Jones and Flynn, 2005; Kiørboe et al., 1996) or increase ingestion of low-quality prey (compensatory feeding) (Augustin and Boersma, 2006; Plath and Boersma, 2001; Siuda and Dam, 2010). Post-ingestion regulation can be achieved through physiological processes. For individuals feeding on a nutrient-limited diet, ES predicts that consumer stoichiometry will be regulated by one or more of the following processes: (a) increase in assimilation of the limiting nutrient; (b) increase in waste production from the excess nutrient; (c) conservation of the limiting nutrient (i.e., decrease in nutrient loss) through altered biochemical pathways (Sterner and Elser, 2002). Furthermore, ES also stipulates that stoichiometric imbalance between prey and predator will negatively affect consumer growth, and that the more strictly homeostatic an organism is, the greater the effects of a nutritionally unbalanced diet on its elemental gross growth efficiency (GGE) (Sterner and Elser, 2002).

Over the years several studies have employed concepts from ES to assess performance in various copepod species, some obtaining results which agree with the predictions from ES and some with contrasting findings. When studying the effects of N-limitation on the calanoid copepod *Acartia tonsa*, Jones et al. (2002) verified that the GGE of N was greater than that of C, recording a more efficient use for the limiting element. The authors also recorded higher N use efficiency (relative to C use efficiency) for copepods fed with N-limited prey than for those preying upon N-replete food, and greater C waste associated with higher food molar C:N ratio, as would be expected from ES. Checkley and Entzeroth (1985) also reported a higher efficiency in the use of N than in the use of C for tissue production in copepods. On the other hand, higher N excretion rates were reported for carnivores in comparison to herbivores (despite the more nutrient-rich diet of the former in comparison to the latter), contradicting what would be expected from ES (Vanni and McIntyre, 2016). In general, a failure in the explanation of theoretical (model) or practical (laboratory) results by the assumptions of ES has related by the available literature to the possibility that parameters other than elemental composition might be playing a part in limiting consumer growth (Anderson and Hessen, 1995; Anderson and Pond, 2000; Jones and Flynn, 2005).

ES framework for the study of performance in adult females of T. longicornis

This PhD project was interested in relating the nutritional quality of prey items with the performance of *T. longicornis* by analyzing the effect of the former over each physiological process separately (Chapter I). Copepods were fed with four different diets, two of which consisting of diatoms and two of dinoflagellates, which were further divided into nutrient-replete and N-depleted growth conditions. Diet acclimation was conducted with copepods for 48h, after which they individuals were transferred to different incubation units for 24h to measure rates of ingestion, respiration, excretion, and egg and faecal pellet production. Respiration and excretion incubations were conducted without the addition of food to experimental units, whereas prey was available to copepods during grazing and EPR/FPR incubations.

The degree of homeostasis of an organism can be inferred from the plot in log scale between resource and consumer stoichiometry by calculating the inverse of the slope of the regression line. In Figure 8a, for example, it would be 1/1 for the nonhomeostatic organism. A degree of 7.7, for example, indicates strong homeostasis in a consumer (Sterner and Elser, 2002). The log

plot of resource vs. consumer stoichiometry obtained during this PhD for all *T. longicornis* samples and their respective diets (1/slope = 9.97) indicates that the adult females of this species are strictly homeostatic, at least for the range of prey molar C:N ratios investigated (Fig. 9b). This result further demands the analysis of the physiological measurements recorded during this PhD in relation to the assumptions of ES listed a few paragraphs above. This approach can indicate the appropriateness of using this framework to better understand copepod performance in relation to changing food regimes.

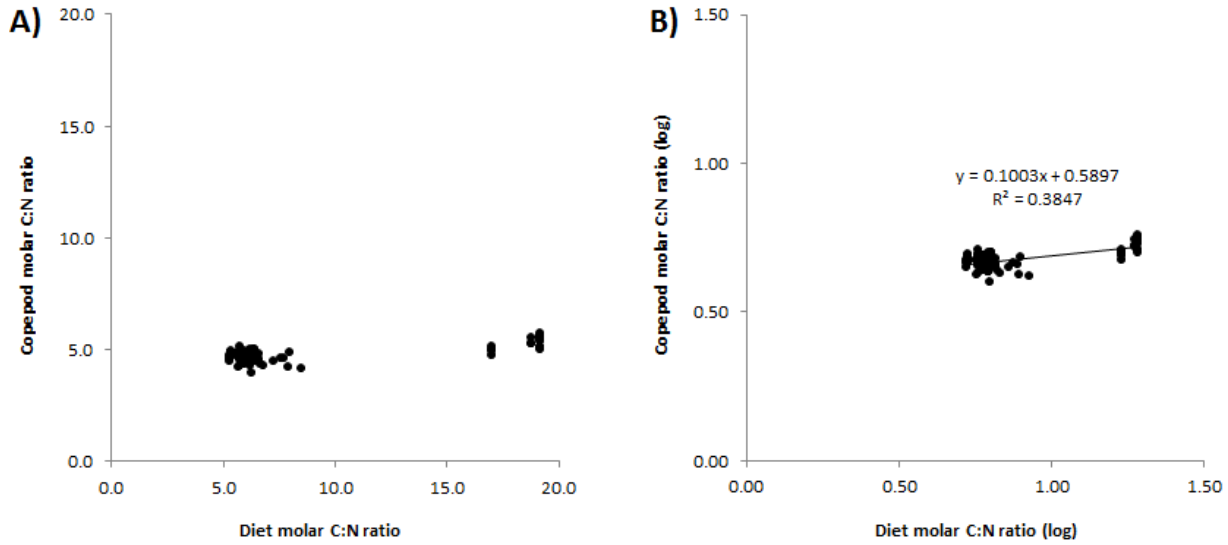

Figure 9: Resource vs. consumer stoichiometry plot obtained from samples collected during this PhD. Copepod values include adult male and female *T. longicornis*; diet values include *in situ* seston and the different diets used in the PhD experiments. A shows the molar C:N ratio as stoichiometric measure; B shows the same values under a logarithmic scale, as the slope of the regression line is used to calculate the level of homeostasis of the copepod.

Was it possible to observe an increase in the assimilation of the limiting nutrient, N, in the copepods? It is important to establish here the distinction between absorption and assimilation, which can be found in the literature with similar definitions despite their representing different processes. Absorption occurs after food ingestion and egestion by the individual and includes the energy which will be used for metabolism (in respiration and excretion) and growth (somatic and reproductive). Food assimilation only refers to the energy that is incorporated into growth. The absorption efficiency for N (AE_N, Chapter I) was fairly similar between copepods fed with different diets (60-73%). The N assimilation efficiency, which was not directly measured but can

be inferred from the sum of the net somatic growth ($NSGE_N$, Chapter I) and egg production ($NEPE_N$, Chapter I) efficiencies for N, was 2-3 times lower in the copepods fed with the N-limited diatoms (8-25%). These results contradict ES by reporting a decrease in the assimilation of N by copepods fed with a N-poor diet, and also suggest that most of the N absorbed by these copepods was used for maintenance and metabolism rather than for growth.

Did the waste production of C in copepods increase with the increase in the molar C:N ratio of the diets? If only the diatom diets are compared, then indeed the percentage of body C that was eliminated via FP production and via respiration was higher in copepods fed with N-limited (29% body C) than with nutrient-replete (20% body C) diatoms. The greater imbalance in molar C:N ratio between copepods and the N-limited diatoms did not result in higher waste production of C when rates are compared with those from copepods feeding on the dinoflagellate treatments (30-37% body C). It is interesting to note that, contrary to what is commonly assumed, the main pathway for eliminating excess C in the experiments conducted for this PhD was found to be FP production rather than respiration.

Were the copepods feeding on N-limited prey able to conserve their N reserves through altered biochemical pathways? It seems they were not. As a matter of fact, the lowest N excretion rates were recorded for copepods fed with nutrient-replete diets (5-8% body N), whereas the highest rate was measured for the copepods feeding on N-limited diatoms (27% body N). It has been suggested that the level of N excretion depends not only on the molar C:N ratios of prey and predator, but also on food intake, the energetic demands of respiration, and on the nature of substrates that were respired (Anderson, 1992). Given the high N excretion rates of the copepods fed with the Diat– diet, it would appear that the homeostatic regulation required to offset the nutritional imbalance of this diet had high energetic costs, and that nitrogenous substrates (amino acids and proteins) were the ones respired by copepods. The copepods fed with the Dino– diet also display high N excretion rates (15% body N) in relation to the nutrient-replete diets, despite the fairly similar molar C.N ratio between copepods and dinoflagellates in this diet. This indicates that N excretion and C respiration are not only linked to the availability of these elements in the diet, but to the biochemical molecules in which they are embeded.

And last but not least, was consumer growth negatively affected by stoichiometric imbalance between prey and predator? Growth can be divided into two components for adult female

copepods, somatic growth and reproductive growth. The results from this PhD indicate that both forms of growth were negatively affected (to various degrees) by the stoichiometric imbalance between copepods and the diet with the highest molar C:N ratio, Diat–, with the exception for the C net egg production efficiency ($NEPE_C$), which was similar between all treatments. The $NSGE_N$ seems to have been affected the most by prey-predator nutritional imbalance, and was irrelevant in copepods fed with the Diat– diet (-1% body N) and between 25-36% body N in copepods fed with the other diets. $NEPE_N$ in copepods fed with Diat– was approximately half that of copepods fed with nutrient-replete diets (48% body N), and the C net somatic growth efficiency ($NSGE_C$) in the former (20% body C) was approximately 65% of that recorded for copepods fed with the other diets.

From the ES assumptions mentioned above, only one was supported by the results from this PhD, that somatic and reproductive growth (both in terms of C and N) are negatively affected by increasing nutritional imbalance between prey and predator. The experimental results, however, mostly contradict ES assumptions, as the increase in nutritional imbalance between prey and predator did not lead to higher waste production from the excess element and resulted in a decrease in assimilation and in an increase in excretion of the limiting nutrient. Within the context of this PhD copepod performance was ultimately defined by its reproductive success. For this purpose, ES was a useful tool, as it was able to explain the differences observed between treatments in the context of varying food quality. It is evident however from the other results that ES may need a complimentary approach when other research goals are in play.

In their book about ecological stoichiometry, Sterner and Elser (2002) present the subject as a set of simple rules underlying ecological and biological complexity, capable of linking themes such as biochemical composition of subcellular structures, food web dynamics, and gene organization. The intention of being a unifying concept linking several scales of ecological patterns and processes has not been fully achieved by ES (Welti et al., 2017). Although it is necessary to understand prey-predator interactions in terms of the chemical nature of life, the authors themselves (and others) acknowledge the somewhat reductionist approach (Mitra and Flynn, 2005; Sperfeld et al., 2017; Sterner and Elser, 2002; Tang and Dam, 1999), as food substrates exist as compounds rather than as individual elements. It seems the ES approach would be better taken advantage of if used jointly with other approaches to nutritional ecology. Ecological

stoichiometry should also consider, for example, the importance of biochemical compounds, or integrate concepts from theories such as trait-based ecology and metabolic theory of ecology (as also suggested by Anderson and Pond, 2000; Anderson et al., 2004; Meunier et al., 2017; Moe et al., 2005; Vanni and McIntyre, 2016; Ventura, 2006; a.o.).

Biochemical (lipid) homeostasis patterns in zooplankton

Homeostasis has been said to be an ecological and evolutionary force (Sterner and Elser, 2002). From early to later prokaryotes, which were followed by unicellular and multicellular eukaryotes, respectively, homeostatic ability increased with the evolutionary development of ion pumps and the increase in cellular compartmentalization and biochemical complexity (Williams and Fraústo da Silva, 1996). But homeostatic regulation has an associated energetic cost and, as all activities which use up energy, will affect individual fitness (Sterner and Elser, 2002). What are the benefits of homeostatic regulation that offset this extra energy expenditure?

Chapter III provides one potential answer to this question. While studying the assimilation of dietary C into FAs of the meroplanktonic larvae of *L. conchilega* it was possible to observe that the FA profile of the individuals did not resemble that of the food items, as is common for organisms with intermediate total lipid contents, such as small copepods. On the contrary, *L. conchilega* larvae were able to selectively accumulate and/or metabolize certain FAs, thus regulating their lipid composition. FA homeostasis may be energetically simple when an organism feeds upon a prey of similar lipid composition. Just like elemental homeostasis, however, it will require that energy be allocated to bioconversion if prey items have a different biochemical make-up than that of the consumer. *Lanice conchilega* larvae, as most lecithotrophic and planktotrophic meroplanktonic larvae, are lipid-poor, as they spend most energy ingested to sustain metabolism and to grow into juveniles. These lipid-poor organisms likely have specific growth requirements (in terms of FAs) to sustain body function, so FA homeostasis is their solution to obtaining the nutrition they need regardless of the FA availability in the diet. One of the FAs *L. conchilega* was able to bioconvert, for example, was 22:5(n-3), an essential FA for consumers due to its role in membrane formation. This FA was not present in the diet with which the polychaete was fed, and had it not been able to bioconvert it from other precursor FAs its development and growth might have been physiologically compromised.

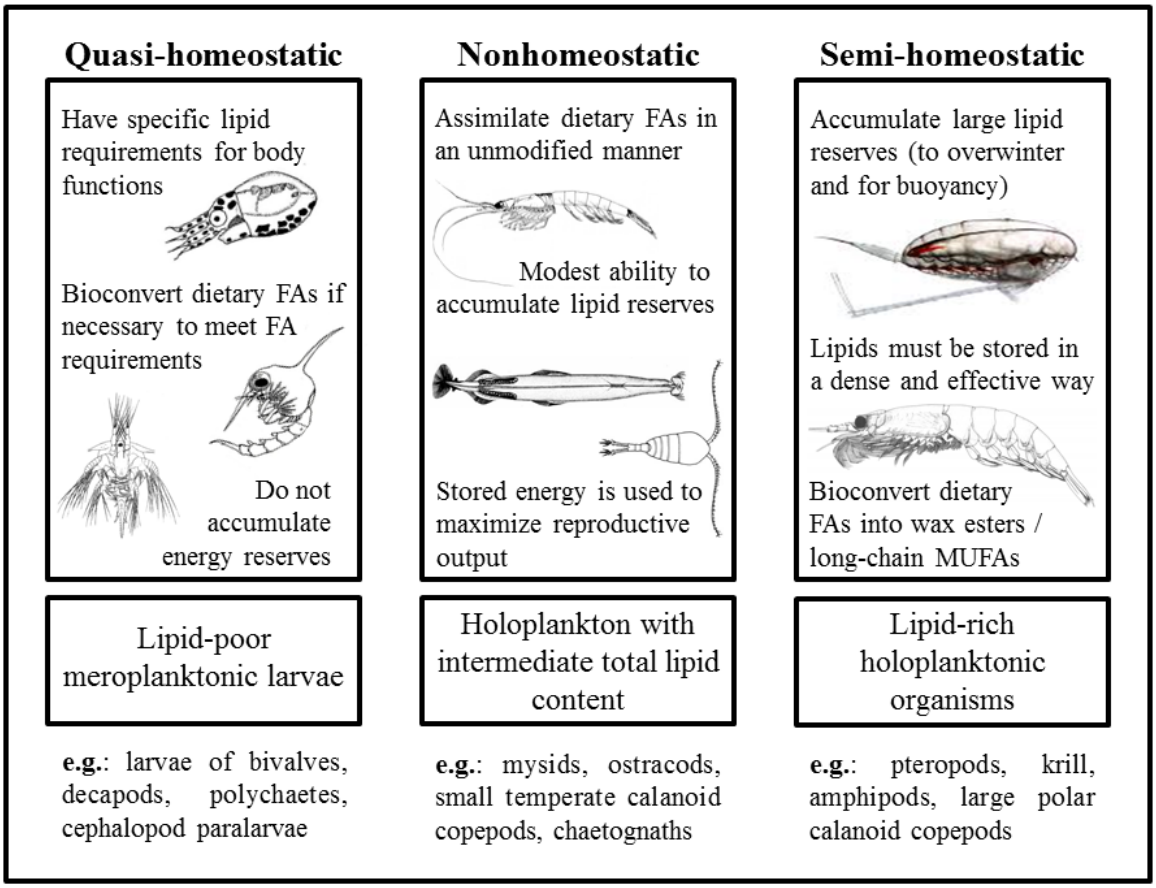

Figure 10: Lipid homeostasis patterns and associated total lipid content characteristics in zooplanktonic organisms. Sources of images: Jónasdóttir, 2017; Pérez-Losada et al., 2009; SERTC, 2018; Stübing, 2004; Vidal et al., 2010; WoRMS, 2018.

Based on these PhD results and on data available in the literature (Boissonnot et al., 2016; Graeve et al., 2005; Lee et al., 2006 and references therein; other references shown in Table 3 from Chapter III) it was possible to establish a hypothesis regarding patterns of lipid homeostasis in three different groups within the zooplankton (Fig. 10). Lipid-poor meroplanktonic larvae (with the exception of those from hermatypic and soft coral) have specific FA requirements to sustain body functions, and will thus display lipid homeostasis. *Lanice conchilega* larvae are an example of an organism with this strategy, as described above. Holoplanktonic organisms which have intermediate total lipid contents, such as small calanoid copepods like *T. longicornis*, have a modest ability to form lipid reserves in order to maximize reproductive output. These organisms are likely nonhomeostatic and assimilate dietary FAs in an unmodified manner. Lipid-rich holoplanktonic organisms, such as large polar calanoid copepods, need to store energy in the

most dense and effective way for overwintering and buoyancy purposes. These organisms are semi-homeostatic, as they will bioconvert dietary FAs into long chain MUFAs / wax esters.

It is also important to highlight that the majority of information on zooplankton feeding ecology in the North Sea (and in other areas) is available for copepods. Although they dominate the zooplankton in the region, countless other groups of organisms are being or will likely also be affected by environmental changes. In the North Sea, despite the decreasing abundances of holoplankton, data collected between 1958 and 1995 showed an increase in abundance of meroplanktonic organisms (Lindley and Batten, 2002). The meroplanktonic larvae of benthic echinoderm species, for example, now dominate numerically the summer plankton community in the North Sea (Kirby et al., 2007), and their peak occurrence is, on average, 47 days earlier in the season (Edwards and Richardson, 2004). If the entire ecosystem is to be better understood, especially under climate change scenarios, then other members of the zooplankton and their reaction to changing food regimes must also be characterized. In particular, the effects of regime shifts on the meroplankton should receive greater attention, as they will eventually propagate to the benthic populations. Furthermore, some species which have meroplanktonic larvae play a key role in their environment – *L. conchilega*, for example, is an ecosystem engineer whose presence is associated with higher benthic species richness and composition and faunal abundance (Callaway, 2006; Rabaut et al., 2007; Zühlke et al., 1998; Zühlke, 2001). It is important to better understand how these key species are affected by environmental changes, as any decrease in their populations may result in further shifts in ecosystem structure and reinforce regime shifts.

Take-home messages for Section 4.2

- ES explains the balance of energy and materials between prey and predator in terms of their elemental composition, and can be a useful tool for investigating environmental effects on the stoichiometry and, thus, physiology of organisms.

- Consumers can be characterized by their ability to handle nutrient-limited prey. ES assumes that the ingestion of nutritionally unbalanced diets by a consumer can lead to increasing assimilation of the limiting nutrient and waste production from the excess nutrient, decreasing (limiting) nutrient loss, and negative effects on consumer growth.

- ES was a useful tool to explain differences in copepod reproductive success in relation to diet nutritional quality. It failed, however, to explain other physiological results. This indicates a need for complimentary and combined approaches in nutritional ecology studies that also consider the biochemical composition of prey and predator.

- Patterns of lipid homeostasis in zooplankton can be related to the total lipid content of organisms. Lipid-poor meroplanktonic larvae, like those of *L. conchilega*, are quasi-homeostatic and can bioconvert dietary FAs to fulfill physiological/metabolic requirements. Holoplankton with intermediate lipid content, such as *T. longicornis* and other small temperate calanoids, are nonhomeostatic and assimilate dietary FAs in an unmodified manner, accumulating small lipid reserves for investment in reproduction. Lipid-rich holoplankton, such as large polar calanoid copepods, are semi-homeostatic and bioconvert dietary FAs into wax esters, forming large energy reserves for overwintering purposes.

- Studies on zooplankton feeding ecology must also address meroplanktonic organisms, which are understudied despite their ecological and economic importance.

4.3 CLIMATE CHANGE AND MARINE REGIME SHIFTS

Climate change in marine environments can be inferred from alterations in planktonic community structure, abundance, phenology and distribution (Hays et al., 2005). The plankton can, at times, be a more sensitive indicator of climate change than environmental variables themselves (Taylor et al., 2002), as biological patterns and ecological processes are profoundly influenced by variations in climate (Stenseth et al., 2004). On an individual level, changes such as increasing temperatures and decreasing nutrient availabilities can directly influence the physiology of an organism by increasing metabolic rates and through regulatory processes associated with altered food quality. Changes in temperature (i.e., global warming), both those which have already been observed and those which are still predicted for this century, are comparable in magnitude to the major global changes that the Earth experienced in the previous 65 million years (Diffenbaugh and Field, 2013). Combined land and ocean surface temperatures, for example, have risen globally by an average of $0.85 \pm 0.2^{o}C$ in the past 140 years (IPCC, 2014). Sea surface temperatures (SST) have increased by approximately $1^{o}C$ on the coast of Western Australia in the second half of the 20^{th} century (Hobday and Pecl, 2014) and by 0.2 to $0.6^{o}C$ $decade^{-1}$ on the North Atlantic since the late 1970's (Dulvy et al., 2008). As the climate changes, species which cannot tolerate the environmental alterations will have to either adapt, relocate, or face possible extinction. Responses to climate change are species-specific, and current interaction patterns between organisms can be disrupted and new ones can be formed (Alheit et al., 2005; Pecl et al., 2017).

Regime shifts

Regime shifts can be defined as abrupt, substantial, and persistent changes to the state (structure, dynamics, and feedback mechanisms) of a natural system (Reid et al., 2016), which will lead to its reorganization into a different set of mutually reinforcing structures and processes (Rocha et al., 2014). They usually encompass multiple and interacting variables and affect several trophic levels and key structural species (Lees et al., 2006; Möllmann et al., 2014). Regime shifts have been recorded for marine environments such as salt marshes, sea ice, coral reefs, mangroves, and seagrass beds across all oceans (Alheit et al., 2005; Anderson and Piatt, 1999; Auber et al., 2015; Hughes, 1994; Lees et al., 2006, and references therein; Llope et al., 2012; Rocha et al., 2014). An increase in SST and stronger westerly winds caused a shift to a warm regime in the Gulf of

Alaska in 1977, which resulted in an increase in groundfish recruitment and salmon catches, a decrease in forage fish populations, which negatively affected birds and mammals, and overall community reorganization over the next 2-20 years (Anderson and Piatt, 1999). According to the authors, the community in the Gulf of Alaska was expected to bounce back to a cold regime in the early 2000's, in accordance with previous ecosystem changes, but this was not observed in later studies, as temperatures continued rising between 2001-2005 (Litzow, 2006). In the eastern English Channel, the Atlantic Multidecadal Oscillation caused a shift from a cold to a warm water regime in the mid 1990's and resulted in an increase in large-sized, higher trophic level fish and a decrease of small-bodied, low trophic level fish, which migrated northward to escape the warmer waters (Auber et al., 2015). In the eastern Pacific, alternations between cold and warm water regimes, characterized by high abundances of anchovy and sardine, respectively, have been reported to occur every ~ 25 years since the beginning of the 20th century (Alheit and Niquen, 2004; Chavez et al., 2003), but future global warming might change this stable state shifting pattern to a more permanent regime shift.

Dramatic and possibly catastrophic ecological, economic and social consequences can arise from ecological regime shifts (Alheit and Hagen, 2002; Alheit and Niquen, 2004; Hughes, 1994; Scheffer et al., 2001), as they often result in collapsing ecosystems and loss of services and goods. This is the case, for example, when pelagic fish such as sardine, anchovy, and herring are involved, as they comprise about one quarter of the total annual world fisheries catch (Hunter and Alheit, 1995) and are an important food source for larger fish, birds, and mammals (Crawford et al., 1991; Trillmich and Ono, 1991). Furthermore, regime shifts may also affect climate conditions and influence biogeochemical cycles of several elements, including the biological C pump, via feedback mechanisms (Alheit, 2009; Boyd and Doney, 2003). It is, thus, vital that the drivers and mechanisms causing regime shifts be better understood (Fisher et al., 2015). In past studies, marine regime shifts were often associated to a specific cause (Conversi et al., 2015), but a recent review identified 54 drivers which were, individually or jointly, responsible for 13 different types of global marine regime shifts and resulted in impacts on ecosystem biodiversity, fisheries provision, and primary production, a.o. (Rocha et al., 2014). Global warming, nutrient inputs, and fishing have been suggested to be major drivers in marine regime shifts (Ichii et al., 2017; Jiao, 2009 and references therein; Lees et al., 2006 and

references therein; Rocha et al., 2014), with the former causing changes in key physiological, demographic, and community-scale processes (Bennett et al., 2015; Wernberg et al., 2010).

The dynamics of regime shifts can only be unraveled by better understanding predator-prey interactions (endogenous mechanisms) and spatial dynamics of climate influences (exogenous drivers) (Conversi et al., 2015; Fisher et al., 2015). The zooplankton is commonly regarded as a key group in marine ecosystems, given that it is the bridge between primary production and higher trophic levels. Any alteration in the predator-prey interaction at the base of the food web would, thus, impact energy transfer in the system as a whole, potentially affecting even commercial fisheries. A better understanding of the stability of the trophic interactions in the plankton could help improve the assessment of the effects of environmental changes on marine systems (Llope et al., 2012). It is also important to consider the spatial and temporal variability of the drivers affecting these trophic interactions, as they may affect contiguous ecosystems in different ways (Llope et al., 2012). The North Sea is a good example of how changes in climate will affect trophic interactions in different ways depending on the spatial scale considered.

The regime shifts in the southern North Sea

The establishment of monitoring programs such as the Continuous Plankton Recorder (CPR) and the Helgoland Roads time series likely resulted on the North Sea being one of the most intensively sampled marine systems in the world (~ 500,000 samples from CPR alone, McQuatters-Gollop et al., 2015). The analysis of data gathered by such time series has revealed the high variability characteristic of the North Sea (Llope et al., 2012). As previously mentioned in this thesis, at least 3 regime shifts have already been described for the North Sea, in the late 70's and 80's and early 2000's (Alheit, 2009; Alvarez-Fernandez et al., 2012; Beaugrand, 2004; Beaugrand et al., 2003; Becker and Pauly, 1996; Edwards et al., 2002; Kirby et al., 2008; Llope et al., 2012; Payne et al., 2009; Reid et al., 2003) with changes in all trophic levels, from phytoplankton to zooplankton, benthos, and nekton (Alheit et al., 2005). When this wealth of data is analyzed in relation to the different regions in the North Sea, it seems the effects of climate change were similar between the regions from 1958 and 1972, but varied according to spatial scale between 1972 and 2004 (Llope et al., 2012). In the second period top-down control mechanisms seem to prevail between phyto- and zooplankton on most areas in the North Sea,

especially off the Norwegian coast and the Skagerrak, but in the southern reach the control mechanisms are identified as bottom-up (Llope et al., 2012).

The work conducted during this PhD was performed in the area surrounding the small German island of Helgoland, in the southern North Sea, an ecosystem which has seen a considerable amount of changes in the past decades. One of the components of this thesis, the investigation into copepod performance in a changing ocean, was motivated by these major changes in environmental and biotic parameters. Between 1979 and 2011, water temperatures increased by 1.2^{o}C and loading of N and P decreased from 25.32 to 13.06 mol N L^{-1} and from 0.83 to 0.56 mol P L^{-1}, with molar N:P ratio going from 30.5 to 39.4 and down to 23.3 (Boersma et al., 2015). Light penetration and wind speeds have also been reported to increase in that period of time (Wiltshire and Boersma, 2016). In the phytoplankton compartment, the abundances of two important food items for zooplankton predators have also changed. Dinoflagellate abundances declined from 3.82 to 2.14 $*10^{9}$ μm^{3} L^{-1} (Boersma et al., 2015), whereas diatom abundances increased, especially that of large species (Wiltshire et al., 2010) which are difficult to ingest for organisms such as copepods (Roy et al., 1989). Members of the zooplankton were also affected, with temperature-related changes in phenology reported for copepods and gelatinous zooplankton (Boersma et al., 2015; Schlüter et al., 2010; Wiltshire and Boersma, 2016). Local calanoid copepod densities also drastically decreased (by approx. eightfold) since 1985, with the abundances of *T. longicornis* plummeting from 2000 ind m^{-3} then to < 250 ind m^{-3} in 2010 (Boersma et al., 2015). In order to better understand and predict how the Helgoland system will react in the present and near future to such changes, I designed experiments that attempted to reproduce environmental scenarios familiar to the region. This allowed me to look into the interaction between an important member of the zooplankton, the calanoid copepod *Temora longicornis*, and two of its important prey items, the diatom *Conticribra weissflogii* and the dinoflagellate *Oxyrrhis marina*, under two different nutrient availability scenarios.

Investigating zooplankton performance in relation to diet nutritional quality

Copepod performance was evaluated in two steps. First, their physiological responses to diet nutritional quality were quantified in terms of ingestion, respiration, excretion, egestion, growth, and reproduction rates. The efficiency with which these processes occurred, in terms of C and N, was also evaluated, and a budget was constructed with all of the rates in order to help understand

copepod responses to diet treatments. Ultimately, copepod performance was defined in terms of reproductive capacity, as this parameter defines the success and continuation of a population. The addition of ^{13}C-labelled sodium bicarbonate ($NaH^{13}CO_3$) to the diet cultures enabled the quantification of C transfer between prey and predator, such that it was possible to estimate C assimilation in relation to C ingestion rates. The analysis of ^{13}C-enriched copepod samples via CSIA also provided FA-specific information that helped me better explain and understand the physiological data gathered during the experiments. The next pages contain a summary of my major results, whose meaning I discuss on a population scale, in terms of copepod performance, and on an ecosystem scale, within the context of the changes recorded for the Helgoland region.

The quantity of energy ingested by copepods did not match, at times, the energy used by the individuals. Although the rate measurements were recorded in the same fashion for all replicates fed with all diet, some treatments had a nearly balanced budget (e.g. Fig. 3C, Chapter I), in which energy consumption matched its utilization, while others revealed unbalanced budgets (e.g. Fig. 3F, Chapter I). I hypothesized that this reflected an underestimation of ingestion rates due to copepods feeding on eggs and FPs, as these were not physically separated in the glass bottles used in the grazing incubation. These alternate food sources were unaccounted for when determining ingestion rates, and were likely ingested in quantities inversely proportional to the quality of the diet treatments provided to copepods. It would seem that, by feeding on their own eggs and FPs, copepods could have complemented nutritionally inadequate diets, though this response mechanism is probably not relevant for the species in its natural environment.

The interesting result regarding ingestion rates is obtained by combining them with the C assimilation rates. When C assimilation is calculated as the percentage of dietary C that was ingested by copepods, no significant difference is found between the different treatments. This implied that food quality did not affect the assimilability of food, only the rate at which it was ingested. Methodological problems unfortunately prevented the verification of this pattern in the Dino+ treatment, which returned the highest EPR and was, thus, considered to provide the highest nutritional quality among the diets tested. Nevertheless the finding about diet quality affecting ingestion but not C assimilation rates is still relevant to the evaluation of copepod performance while feeding on the other treatments. For immature copepods, this result would have meant different time and energy investments for a similar C assimilation in relation to the

food available in the environment. As these copepods were females, we can explain the ingestion of more or less C with investment in reproductive output. The superiority of dinoflagellates as quality food for reproductive purposes in copepods, even when the former are nutritionally unbalanced, was further evidenced by the second highest EPR values, recorded for individuals who had the lowest ingestion rates while feeding on N-limited *O. marina*.

The caveat of the EPR results is that egg production, albeit the first step, is only one part of the process of producing viable offspring which survive into adulthood. The lack of information on how egg viability and juvenile survival are affected by diet quality limits my ability to claim with full certainty that *T. longicornis* fares better when feeding on dinoflagellates than on diatoms. I was able to record, however, different levels of investment of body N into reproduction between the treatments, which resulted in eggs with differing molar C:N ratios. This result indicates that copepods feeding on N-limited diatoms will likely produce eggs with a low N:C content. Studies on the hatching success of copepod eggs and nauplii survival in relation to egg molar C:N ratio are not available for *T. longicornis*, but can be found for the calanoid, *Calanus helgolandicus*, albeit with contradicting results. Guisande and Harris (1995) found that increasing egg sizes, which are controlled by food quantity and positively correlated to egg protein, carbohydrate, and lipid contents, lead to increases in egg hatching success and in nauplii survival. Pond et al. (1996), on the other hand, reported that the C and N contents of eggs are inversely correlated with egg viability, and not at all correlated with nauplii survival. The same trend is visible if one converts the C and N content data from the latter into molar C:N ratio. It remains to be seen whether and how egg molar C:N ratio will affect the success of the reproductive process in *T. longicornis*. In light of the aspects discussed, I believe the results obtained during this PhD are a very good indication that *T. longicornis* females perform better when feeding on dinoflagellates than on diatoms, even if N levels in the environment are limiting.

The availability of nutrients for algal growth in the North Sea is expected to become even more dependent on the influx from the North Atlantic in the future (Boersma et al., 2015). Dinoflagellates are poor competitors for nitrate whereas large diatoms, which possess a N "storage-adapted" strategy, have the advantage in the competition for this nutrient (Litchman et al., 2007). The availability of silicate and nitrate in the water is, thus, a major determinant of the ratio between diatoms and flagellates (Black et al., 2016). Changes in diatom and dinoflagellate

abundances and nutritional quality have the potential to greatly impact their zooplanktonic predators, as indicated by the work conducted during this PhD with *T. longicornis* females, and to affect the entire system in the near future (Wiltshire and Manly, 2004; Wiltshire et al., 2008). As a matter of fact, the decrease in calanoid copepod abundance in Helgoland in the past decades, although correlated with increases in seawater temperature, was indirectly related to the decrease in N and P input in the region (Boersma et al., 2015; Wiltshire and Boersma, 2016), and a similar trend has been suggested for other regions in the North Sea (Llope et al., 2012).

If the trophic interactions between calanoid copepods and their food items are only analyzed in relation to decreasing nutrient input, the logical conclusion is that their populations will continue to be negatively affected around Helgoland, as the availability of the nutritionally best prey (dinoflagellates) decreases and that of the worst quality (N-limited diatoms) increases. It has been suggested, however, that temperature is the key physical variable impacting phytoplankton, zooplankton, and fish species in the North Sea (Alheit et al., 2005). Increasing temperatures in the region (Wiltshire et al., 2010) will likely result in higher metabolic (respiration) costs for copepods (Castellani and Altunbas, 2014). These can only be met by increasing the demand (ingestion) for C. The effects of feeding on prey with higher C:nutrient ratios (due to the decrease in N and P loading) would be dampened by the ingestion of a higher relative C content, which would offset increased metabolic demand (Boersma et al., 2016; Malzahn et al., 2016). In such a scenario, the suitability of N-limited diatoms as "quality" food for copepods changes, but the end result for copepod performance and population success remains grim. And that is if further changes in copepod and diatom phenologies, which have shifted (by different processes) to a month earlier in the season during the last 50 years (Wiltshire and Boersma, 2016), do not lead to a mismatch between prey and predator, which it might in future (Wiltshire and Boersma, 2016). Alternatively, copepods feeding on high C:N diets in a warming scenario will offset the higher demand for C with increased ingestion of food and from the excess C in it, remaining limited by N and favoring the selection of food with lower C:N ratios (Anderson et al., 2017).

The full scale of the effects and feedback mechanisms of climate change in Helgoland are yet to be clarified and merit further investigation. Concomitant changes in all of these variables make it difficult to evaluate how copepods will react to future prey composition changes in the area. Although the work for this thesis refers to organisms from a very specific location, a small island

in the German Bight, the ubiquitousness of the species and of the environmental variable (N availability) studied enable the comparison of my results with those from several marine ecosystems. I believe my work represents a robust contribution towards a better understanding of marine systems and how they are affected by regime shifts.

Take-home messages for Section 4.3

- Regime shifts are abrupt, substantial, and persistent changes to the state of an ecosystem. They encompass multiple and interacting variables and affect several trophic levels and key species. It is thus necessary to understand how key organisms, such as copepods, will react to changing food regimes in their ecosystems.

- Major ecosystem changes have been recorded around Helgoland in the past 5 decades: increasing water temperatures, light penetration, wind speeds, and diatom abundance; decreasing nutrient loading, dinoflagellate abundance, and calanoid copepod densities; and temperature-related changes in copepod and zooplankton phenologies.

- Copepod performance in relation to food nutritional quality was ultimately evaluated by their egg production rates. Dinoflagellates were a food source of superior nutritional quality than diatoms, but further studies need to assess the effect of these diets on egg viability and juvenile survival. Concomitant changes in more than one environmental variable make it difficult to fully evaluate and predict how copepods will react to future prey composition changes in Helgoland.

- The ubiquitousness of the species and environmental variable studied enable the comparison of these results with those from other calanoids and marine ecosystems.

5 PERSPECTIVES

Climate change and anthropogenic activities can affect ecosystems at all different levels of biological organization. In order for society to better understand the full scale of these (often synergistic) effects, a combined scientific effort must be adopted and make full use of approaches involving monitoring, modelling, and laboratory and field experiments. The long-term biological data sets obtained from time series such as Helgoland Roads provide an important temporal (and sometimes spatial) record of the patterns in ecosystem change (Alheit and Niquen, 2004; Pecl et al., 2017). Although laboratory and field experiments are unable to address all parameters at play in the investigation of ecosystem change, they can provide valuable insights into the feedback mechanisms generated from alterations. It is necessary to join monitoring data and experimental results in ecosystem modelling exercises in order to empirically test various mechanisms of regime shifts, and proposed courses of actions (Fisher et al., 2015). Only then will we be able to predict, prevent and/or respond to regime shifts.

I would recommend that more comprehensive experimental approaches be chosen for future scientific investigations into the effects of regime shifts on the zooplankton. Regarding the Helgoland region, I think it is important to investigate the combined effects of temperature and nutrient input on the planktonic community, though other parameters should also receive scientific attention. Temperature affects the ingestion of food, metabolism, and growth of individuals, and has a complex and poorly-understood impact on their nutritional requirements (Anderson et al., 2017). Given the fact that many zooplanktonic invertebrates are limited in nutrients (Hessen, 1992), it becomes clear the importance of investigating the synergistic effect of temperature and nutrient input on the zooplankton. These should be tested in terms of organism performance through all life stages, both in controlled conditions in the laboratory and in field mesocosm experiments, with results between both approaches compared. Nutrient limitation triggers changes at the molecular level which affect organism homeostasis (Sperfeld et al., 2017), and it would be interesting that the data gathered from experiments be analyzed under the framework of ES and another approach to nutritional ecology. I suggest that this should be the dynamic energy budget theory, which models energy uptake and usage for all vital physiological rates (as investigated during this thesis) at all life stages (Kooijman, 1995).

Last but not least, I also strongly encourage the procedural refinement for using labelled bicarbonate in tracing studies with organisms. In order to obtain accurate and pertinent results, it is important to understand how the phytoplankton incorporates ^{13}C during photosynthesis and how this process is affected by culture conditions, such as light intensity, photoperiod, nutrient availability, and so on. This is a methodology with great potential to resolve unanswered biochemical questions and it should be used accordingly. In order to answer these questions of environmental and societal relevance, we must first perform the repetitive studies required to define the quantities of $NaH^{13}CO_3$ that should be used for labelling organisms so that energy flow can be traced between trophic levels.

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ERKLÄRUNG

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel:

Zooplankton performance in a changing ocean: Adaptive capacities to a shifting food regime in the North Sea

selbstständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

Rita Melo Franco Santos

(Unterschrift)