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Food quality regulates the metabolism and reproduction of *Temora longicornis*

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Abstract

A laboratory study was undertaken to determine the effect of food quality on feeding, respiration, reproduction and the resulting carbon budget of *Temora longicornis.* The stoichiometric ratios N : P, C : N and C : P of *Rhodomonas salina* were used as indicators of food quality. *R. salina* was grown in media with different inorganic nutrient concentrations to produce food for *T. longicornis* with particulate organic N : P ratios ranging from 10 : 1 to 23 : 1. Feeding rate was not affected by food quality. Maximum respiration (R), egg production rate (EPR), assimilation efficiency (AE), gross growth efficiency (GGE) and metabolic increment (MI) occurred when *T. longicornis* was fed on phytoplankton with a food quality of 16N : 1P. EPR, GGE and AE also decreased with decreasing C : N ratio and the energy required to produce eggs (CoE) decreased with decreasing N : P ratio, indicative of nitrogen-dependent production.

These data suggest that an algal composition of 16N : 1P defines the Threshold Elemental Ratio (TER) and is the optimum diet for *T. longicornis*. The variations in metabolic rates and the resulting carbon budget are proportional to the quality of food ingested. GGE was negatively affected at dietary ratios above and below 16N : 1P, which in the natural environment could lead to a decline in species biomass with detrimental consequences for fisheries and carbon export. Field data show that phytoplankton organic N : P ratios can change on decadal timescales, and that an increase in the food N : P ratio can co-occur with a shift to smaller sized zooplankton and a change in species abundance. Further research is required to assess how much of the change in zooplankton community structure and activity can be attributed to changes in food quality, rather than to changes in temperature and food quantity.

1 Introduction

The physiology of copepods is affected by seasonal and latitudinal changes in environmental conditions (Mauchline, 1998). Alongside body mass and temperature variation,

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food availability and quality play important roles in regulating copepod physiology (Ambler, 1986; Jonasdottir et al., 1995; Guisande et al., 2000). Food quality in particular can become a significant factor limiting copepod production by reducing efficient nutrient uptake and energy availability (Hessen and Anderson, 2008 and references therein).

Nutritious food is defined by the presence of a range of nutrients and organic compounds, which occur at optimal concentrations and proportions required to provide a balanced diet. Stoichiometric determination of the organic nutrients present in the copepod's diet is therefore a reliable indicator of food quality (Sterner and Elser, 2002). Although elemental analyses are known to lack information on the nature of the different carbon-containing compounds (Tang and Dam, 1999), their ease of measurement can provide a more practical approach to the determination of food quality, and allow an important link to nutrient biogeochemical cycles.

The global average elemental ratio of particulate matter of 106C : 16N : 1P, known as the Redfield ratio (Sterner and Elser, 2002), is considered to provide a balanced diet for copepods, with nutrient limitation occurring above and below this ratio. However, the N : P ratio of marine particulate matter can vary from ∼ 5 : 1 to ∼ 34 : 1 due to temporal and spatial changes in inorganic nutrients and phytoplankton community structure (Falkowski, 2000; Geider and LaRoche, 2002).

The C : N ratio of phytoplankton has been typically used to quantify food quality for copepods in the marine environment whereas C : P and N : P ratios are not routinely measured (Touratier et al., 1999; Jones et al., 2002; Mayor et al., 2009). However, although phosphorous constitutes less than 1 % of the total dry biomass of copepods, it plays a major role in the synthesis of cellular ATP and RNA. As a consequence, this macronutrient is essential for protein synthesis and thus copepod production and growth (Sterner and Elser, 2002). Hence, using N : P as a food quality indicator alongside the C : N and C : P ratios, should provide a more complete measure of the nutritional value of the food (Vrede et al., 2004).

Copepod growth and egg production rate are influenced by food quality in terms of protein, amino acid and fatty acid composition (e.g. Jonasdottir, 1994; Kleppel and

Burkart, 1995; Koski et al., 1998; Guisande et al., 2000). However little is known of the effects of food quality on metabolism. Copepod metabolism, in terms of oxygen uptake, increases during feeding, in relation to a high protein diet, and decreases to basal rates when starved (e.g. Vidal, 1980; Kiorboe et al. 1985; Thor, 2000); but, to the best of our
5 knowledge, no information is available on the effects of different organic nutrient ratios on the respiration rate of copepods.

This study will investigate the effects of food quality on copepod physiology. To assess the effects of variable food stoichiometry on the neritic copepod *T. longicornis*, we measured feeding, respiration, and egg production rates at a range of phytoplankton
10 N : P, C : N and C : P nutrient ratios. We test the hypothesis that maximum physiological rates occur when *T. longicornis* is fed on phytoplankton considered to be of optimum food quality i.e. 16N : 1P. We assess the ecological implications of the different food environments on ecosystem productivity by calculating copepod carbon budgets. Finally, we apply our laboratory findings to field data in an effort to understand how current and
15 future changes in the interaction between primary producers and grazers could impact oceanic biogeochemical dynamics and the trophic transfer of energy.

2 Methods

2.1 Algal cultures

The cryptophyte *Rhodomonas salina*, equivalent spherical diameter ~ 7 µm (CCMP
20 1319) was grown in 1.5 L chemostats in ESAW medium (Berges et al., 2001) without silicate, supplied at 540 mL d^{-1} at 13 ± 1 °C in continuous light with an irradiance of 300 µmol photons m^{-2} s^{-1}. Three cultures were established under different nutrient conditions: balanced growth, N-limited and P-limited. The steady state of the cultures was monitored by cell counts using a Beckman multisizer 3 coulter counter and their
25 state of health was monitored using a Walz PhytoPAM phytoplankton analyser every three days.

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R. salina cultures were diluted with 0.2 µm filtered seawater (FSW) obtained from the North Sea at the same time as the copepods, and this was used as the medium for the incubations.

2.2 Elemental composition of the diet

Samples for particulate organic carbon (POC), nitrogen (PON), and phosphorus (POP) were collected on pre-combusted (450 °C for 4 h) 25 mm GF/F filters under < 5 mmHg vacuum. POC and PON filters were immediately dried at 60 °C for 24 h and stored until analysis on an Exeter 440 Elemental Analyzer. Particulate P (POP) filters were dried at 60 °C for 24 h, and stored at −20 °C prior to analysis. The samples were processed following the Potassium Persulfate ($K_2S_2O_8$) digestion method of Suzumura (2008) and analysed with the colorimetric method of Strickland and Parsons (1972) using a PerkinElmer Lambda 25 UV/Vis Spectrometer equipped with a PerkinElmer S10 autosampler and a 4 cm^3 cell.

The C, N and P concentrations and C : N, N : P and C : P mol mol^{-1} ratios of *R. salina* are reported as the average of the measurements made at the beginning and end of the incubations ± SD. The SD of the ratio was propagated using Eq. (1).

$$\sigma_{AB} = AB\sqrt{\left(\frac{\sigma_A}{A}\right)^2 + \left(\frac{\sigma_B}{B}\right)^2} \quad (1)$$

σ = standard deviation; A = ratio at time 0; B = ratio after 24 h.

2.3 *T. longicornis* sampling and maintenance

Individuals of *T. longicornis*, were collected from the North Sea off the coast of Great Yarmouth (UK) (52° 41.9′ N, 1° 48.5′ E) from June to December 2011 using a 120-µm plankton net towed horizontally for ~ 20 min. The sample was brought back to the laboratory and kept at 13 ± 1 °C in salinity 34 0.2 µm FSW in a darkened 30 L bucket for

up to 2 weeks. The animals were fed three times a week with a mixture of the cryptophyte *Rhodomonas salina*, the dinoflagellate *Oxyrrhis marina* (fed twice a week with the chlorophyte *Dunaliella marina*) and the diatom *Thalassiosira weissflogii*.

2.4 Physiological rates

Adult females of *T. longicornis* were isolated and acclimated for 48 h to the experimental conditions (13 °C in the dark) in 4 L 0.2 µm FSW at the food quality and quantity to be used during the incubations. The females were transferred daily to new medium and fed at a phytoplankton cell concentration corresponding to a biovolume of $5 \pm 0.5 \times 10^6$ µm^3 mL^{-1} to mimic bloom conditions (Jonasdottir and Kiorboe, 1996; O'Connors et al., 1980). Feeding, egg production and respiration rates were measured for each of the *R. salina* N : P ratios at 13 ± 1 °C. Copepods found dead or moribund at the end of the incubations were not included in the analysis.

2.4.1 Feeding rates

Up to 9 × 500 mL amber glass bottles were filled with *R. salina* in 0.2 µm FSW. A number of female *T. longicornis* (8–12) were placed in 1 to 3 of these bottles and 6 without copepods were used as controls. The bottles were sealed with parafilm (to avoid bubbles), capped and mounted on a slowly rotating plankton wheel (1 rpm). Three control bottles were sampled at the beginning and three at the end of the incubation for cell abundance and elemental composition of *R. salina*. At the end of the incubation *T. longicornis* clearance and ingestion rates were calculated according to Frost (1972).

2.4.2 Respiration rates

Copepod respiration rates were derived from the consumption of oxygen during an in vitro incubation in 100 % oxygen saturated 0.2 µm FSW. A preliminary experiment showed that there was no significant difference (Student t-test $P = 0.55$, $n = 16$)

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between the respiration rate of copepods incubated over 18 and the more usual 24 h. Therefore we measured copepod respiration over the shorter incubation time of 18 h.

Single adult *T. longicornis* females were incubated in triplicate 60 mL borosilicate glass bottles in 0.2 µm FSW containing *R. salina* to determine the respiration rates of feeding copepods (R_f). Triplicate bottles containing *R. salina* in 0.2 µm FSW were incubated in order to determine the respiration rate of the algal food and any bacteria remaining in the 0.2 µm FSW (fed control). Additionally, *T. longicornis* females were incubated in 0.2 µm FSW without *R. salina* to determine the unfed respiration rates (R_u), alongside triplicate bottles of 0.2 µm FSW as a control. All the respiration bottles were incubated in the dark for 18 h and the bottles containing the copepods with the food were placed on a rotating plankton wheel (1 rpm).

Measurements of dissolved oxygen concentration were made with an automated Winkler titration system with a photometric endpoint (Robinson et al., 2002). The sodium thiosulphate titrant was calibrated with 0.1 N potassium iodate standard (replicates average SD ± 0.0001 N) (Carrit and Carpenter, 1966). The salinity of the seawater used in the incubation was measured with a portable salinometer fitted with a salinity/temperature sensor to enable oxygen concentration and saturation to be calculated from the equation of Garcia and Gordon (1992) using the equilibrium constants of Benson and Krause (1984).

The respiration rates for the fed and unfed animals in µL O_2 female^{-1} h^{-1} were calculated as the difference between the means of the triplicate zero time bottles and the triplicate incubated bottles corrected for the controls.

The respiration rates were individually dry weight normalised. The dry weight (DW) was calculated by measuring the prosome length (PL) of 4 % formaldehyde preserved specimens collected at the end of the feeding and respiration incubations. This was then converted to dry weight using Eq. (2) (Castellani and Altunbaş, 2013):

$$\ln \mathrm{DW} = 2.78 \ln \mathrm{PL} - 15.9 \quad (2)$$

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2.4.3 Egg production rates (EPR)

Single adult females were incubated for 24 h in the dark in plexiglass tubes which had a 200 µm nylon screen attached at the lower end. The tubes were inserted in 90 mL amber plastic vessels and filled with 0.2 µm FSW. The nylon screen was small enough to allow the eggs to sink to the bottom of the chamber yet prevent the adults from cannibalising them. At the end of the incubation the number of eggs $female^{-1}$ day^{-1} were counted using a stereoscopic microscope.

Fifteen different single-diet experiments were carried out over a 9 month period from April 2011 to December 2011. In the initial 4 experiments only ingestion, clearance rates and respiration were measured, however in the remaining 11 experiments ingestion, clearance rates, respiration and EPR were measured. During the experiments carried out on 11 and 20 July 2011 a respectively lower and higher volume of *R. salina* was offered to the copepods. Since the study aims to assess the effects of food quality (rather than quantity) on copepod physiology, we did not include these two biased observations in the ingestion and clearance rates dataset. However, since there were no effects of food concentration on respiration and egg production ($r^2 < 0.02$, $P > 0.6$), we included the 11 and 20 July 2011 data in the respiration and EPR datasets.

2.5 Carbon budget

Copepod budgets were calculated in carbon units by converting metabolic rates into carbon equivalents. Since adults do not undergo moulting, egg production was used as an index for growth (G) (Mauchline, 1998). Egg production rate (EPR) was converted to G in carbon units assuming an egg carbon content of 0.14×10^{-6} µg C $µm^{-3}$ (Castellani and Altunbas, 2006) and an average egg diameter of 80 µm (±4 µm SD) (Dam and Lopes, 2003). Ingestion (I), calculated according to Frost (1972), was used as an index of feeding rates. For the experiments on 11 and 20 July, the average ingestion rate from all the experiments was used to calculate assimilation efficiency (AE) and gross

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growth efficiency (GGE). Where applicable the standard deviation was calculated via error propagation using Eq. (1).

Fed (R_f) and unfed (R_u) respiration rates were converted to carbon units using a respiratory quotient (RQ) of 0.97 (Omori and Ikeda, 1992). The adoption of a fixed RQ can result in an uncertainty of about 20 % in the oxygen to carbon dioxide conversion since the quotient varies with the substrate metabolised (Hernandez-Leon and Ikeda, 2005).

Assimilation efficiency ($AE = 100 \cdot (G + R_f)/I$) and gross growth efficiency ($GGE = 100 \cdot G/I$) were determined using µg Carbon (food) µg Carbon (copepod)$^{-1}$. The carbon content of *T. longicornis* was calculated assuming that the carbon content is 40 % of the dry weight (Omori and Ikeda, 1992).

The metabolic increment (MI) of fed copepods was determined as the % increase in carbon consumption between unfed (R_u) and fed (R_f) copepods for each of the *R. salina* N : P ratios ($MI = 100 \cdot (R_f - R_u)/R_u$). In this study MI characterizes the increase in metabolic rate of the organism during feeding, and represents both the additional metabolic cost and the additional energy demand of the copepod's physiology driven by food quality.

The cost of egg production (CoE) was calculated as the % of oxygen needed to produce eggs based on the assumption that the respiration while feeding is a proxy for the oxygen requirement, in excess of basal maintenance, necessary for growth ($CoE = 100 \cdot (R_f - R_u)/G$).

2.6 Time series data

We used time-series data to test whether the ecological implications that are suggested by our laboratory results are consistent with field data and whether other copepod species also respond to a specific seston N : P Threshold Elemental Ratio. While *T. longicornis* is a temperate coastal species, co-located decadal N : P data for both seston and zooplankton biomass were only available from the North Pacific Subtropical Gyre.

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Seston N : P and night-time zooplankton biomass from 1994 to 2010 were obtained from the Hawaii Ocean Time-series Data Organization and Graphical System (HOT-DOGS) website. Zooplankton biomass was separated into 5 size fractions (0.2–0.5, 0.5–1, 1–2, 2–5, > 5 mm). We used a binomial fit for the biomass of each of the 5 zooplankton size classes, as a function of time, to calculate the peak biomass for each of the individual size-fractions. We found that each of the size fractions, except > 5 mm, had significant successive biomass peaks and so we could calculate the year (peak year) in which these different biomass peaks occurred. For each of the peak years we determined the corresponding annual mean seston organic N : P ratio for the upper 150 m of the euphotic zone. This seston, or zooplankton food, N : P ratio (of the peak years of each of the size fractions) was compared with the respective zooplankton N : P, obtained from Hannides et al. (2009).

To investigate this relationship in the habitat of *T. longicornis*, we obtained organic N : P data from the Norwegian Coastal Surveillance Program at Arundel station (58° 23′ N, 8° 48′ E) in the North Sea, between 1991 and 2010. For each of the available years, monthly average PON and POP concentrations ($\mu g\,L^{-1}$) were calculated. Since co-located zooplankton biomass data is not available, we used Continuous Plankton Recorder (CPR) data to calculate *T. longcornis* abundance from 1991 to 2010 in a 2° latitude × 2° longitude area around the Arundel station. Additionally, to investigate spring bloom food quality variation over time, average PON and POP concentrations ($\mu g\,L^{-1}$) selected at chlorophyll *a* values > 2.5 $\mu g\,L^{-1}$ were calculated.

3 Results

3.1 Elemental composition of *R. salina*

The particulate organic matter content (C, N and P) of *R. salina* cells during each of the experiments is presented in Table 1. Although the same species and strain was used throughout, the cell volume measured during the feeding experiments, (averaged

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between the volume at time 0 and after 24 h) differed between experiments (*1-way* ANOVA $F = 36.6$; $p < 0.001$) but was independent of the N : P, C : N or C : P ratio ($r^2 < 0.07$; $p > 0.3$). The N : P ratios of *R. salina* varied from a minimum value of 9.6 : 1 to a maximum value of 22.8 : 1 mol mol^{-1}. The C : N ratios ranged from 5.2 : 1 to 9.9 : 1
5 mol mol^{-1} and the C : P ratios varied from 76.4 : 1 to 166.7 : 1 mol mol^{-1}.

3.2 *T. longicornis* physiological rates

Female size (prosome length; PL) ranged from 6.5 to 9 mm and did not differ significantly between experiments (*1-way* ANOVA $F = 0.89$; $p = 0.57$).

There was no significant difference in algal concentration between experiments (1-
10 way ANOVA $F = 1.27$; $p = 0.34$ with 1 outlier removed). *T. longicornis* clearance rates varied from a minimum of 3.8 mL to a maximum of 6.1 mL filtered female^{-1} day^{-1}. *T. longicornis* ingestion rates varied from 7.5×10^4 to 15.9×10^4 cells female^{-1} d^{-1}. Figures 1a, 2a and 3a show that *R. salina* N : P, C : N and C : P ratios had no significant effect on clearance or ingestion rates ($r^2 < 0.25$; $p > 0.08$). Clearance and ingestion rates were
15 not related to the dry weight normalised respiration of fed and unfed animals or to egg production ($r^2 < 0.04$; $p > 0.5$).

The respiration rates of feeding *T. longicornis* increased exponentially with the N : P ratio of *R. salina* (Fig. 1b). The average respiration rates increase to a maximum of 7 ± 0.4 nL O_2 µg DW^{-1} h^{-1} at 16.5 : 1 N : P (N limited) and exponentially decay as the
20 food N : P increases above 16.5 : 1 (P limited), reaching a minimum respiration rate of 1.6 ± 0.2 nL O_2 µg DW^{-1} h^{-1} at an N : P ratio of 22.8 : 1. Regression equations are given in Table 2.

The respiration of unfed animals ranged from a minimum of 1.6 ± 0.2 nL O_2 µg DW^{-1} h^{-1} to a maximum of 2.5 ± 0.3 nL O_2 µg DW^{-1} h^{-1} (Fig. 1b) The response followed
25 a similar pattern to the respiration of fed animals with an exponential increase at N : P ≤ 16.5 : 1 and an exponential decay at N : P ≥ 16.5 : 1. The maximum fed respiration rate of 7 nL O_2 µg DW^{-1} h^{-1} is 2.9-fold higher than the maximum unfed rate and

3.5-fold higher than the published average basal respiration rate of 2 nL O_2 µg DW^{-1} h^{-1} (Castellani and Altunbaş, 2013). The respiration of fed animals at the minimum and the maximum N : P ratios of 9.6 : 1 and 22.8 : 1 are not statistically different from the unfed values (Student t-test $p = 0.5$; $t = 0.76$ and $p = 0.07$; $t = 2.3$, respectively), suggesting that *T. longicornis* was not feeding on *R. salina* at these ratios.

Egg production varied from a minimum of 0 eggs $female^{-1}$ day^{-1} when fed a nutrient limited diet (9.8 : 1 N : P) to a maximum of 32 eggs $female^{-1}$ day^{-1} when fed a balanced diet (16.5 : 1 N : P). Egg production rates also show a response to *R. salina* N : P ratio (Fig. 1c and Table 2), linearly increasing from a minimum of 2 ± 2.6 eggs $female^{-1}$ day^{-1} at N : P 9.6 : 1 to a maximum of 23.5 ± 7.4 eggs $female^{-1}$ day^{-1} at N : P 16.5 : 1 and then linearly decreasing to a minimum of 10 ± 3.6 eggs $female^{-1}$ day^{-1} at N : P 20.3 : 1.

Figures 2b and 3b show that there was no significant relationship between respiration rates and the C : N (fed $r^2 = 0.2$, $p = 0.1$ a negative trend; unfed $r^2 = 0.001$, $p = 0.9$) or C : P ratios (fed $r^2 = 0.68$, $p = 0.37$; unfed $r^2 = 0.006$, $p = 0.8$) of *R. salina*. Figures 2c and 3c show no relation between EPR and *R. salina* C : P ($r^2 = 0.001$, $p = 0.92$) but a negative correlation with C : N (Table 2) where the minimum of 2 ± 2.6 eggs $female^{-1}$ day^{-1} occurred at a C : N of 10 : 1 and the maximum of 23.5 ± 7.4 eggs $female^{-1}$ day^{-1} occurred at a C : N ratio of 5.2 : 1.

T. longicornis fed and unfed respiration rates were positively correlated with egg production rates (Fed: $r^2 = 0.72$; $p < 0.001$; unfed: $r^2 = 0.50$; $p = 0.02$), consistent with a common physiological response to *R. salina* nutrient ratios.

3.3 *T. longicornis* carbon budget

A carbon budget of *T. longicornis* fed on *R. salina* was constructed from I, G, R_f, AE and GGE (Table 3). Table 4 summarises the significant relationships between the carbon budget of *T. longicornis* and the N : P and C : N ratios of *R. salina*. GGE and AE were highest at a diet of 16.5 : 1 N : P ratio with 21 % and 26 %, respectively (Table 3),

following a similar biphasic pattern to the physiological rates in Fig. 2. AE was lowest at a diet N : P ratio of 22.8 : 1 while GGE was lowest at a diet N : P ratio of 9.6 : 1. In the same manner as EPR, AE and GGE showed a weak significant relationship with diet C : N, linearly decreasing as diet C : N increased (Table 4). AE and GGE showed no significant relationship with C : P ($r^2 < 0.02$; $p > 0.6$).

Respiratory carbon losses and egg production rates peaked at the diet N : P ratio of 16.5 : 1 and decreased with decreasing food quality (Table 3). MI ranged from a minimum of 13 % at an *R. salina* N : P ratio of 22.8 : 1 to a maximum of 189 % at a 16.5 : 1 N : P ratio. MI showed no significant relationship with C : N and C : P ($r^2 < 0.009$; $p > 0.9$).

CoE decreased significantly with increasing N : P ratio ranging from 24.3 % at an *R. salina* N : P ratio of 9.6 : 1 to 4.9 % at 22.8 : 1 N : P (Tables 3 and 4). CoE had no significant relationship with C : N or C : P or the number of eggs produced female^{-1} day^{-1} ($r^2 < 0.09$; $p > 0.3$).

3.4 Time series data

HOT data: regression analyses showed no significant relationship between seston N : P and total zooplankton biomass ($r^2 = 0.04$; $p = 0.45$). However, the binomial relationship between zooplankton biomass and time for the individual size fractions < 5 mm was significant ($r^2 > 0.5$; $p < 0.02$), showing progressively later biomass peaks for smaller size-classes. The smallest size fraction peaked in 2006–2007, while the 2–5 mm biomass peaked in 2001–2002 (data not shown). The relationship between biomass and time for the size fraction > 5 mm was not significant ($r^2 = 0.26$; $p = 0.14$).

Figure 4a shows the highly significant relationship between the N : P ratio of each zooplankton size class and the N : P ratio of the food available in the year of the biomass peak of the respective zooplankton size-class, for the size fractions < 5 mm ($r^2 = 0.96$; $p = 0.02$). The zooplankton > 5 mm did not fit this relationship, which may be because, as indicated above, the peak time of this size class was not well constrained by the available data.

The abundance of *T. longicornis* collected with the Continuous Plankton Recorder in the region of the Arundel time series station is shown in Fig. 4b, alongside monthly averages of particulate organic N : P ratios determined by the Norwegian Coastal Surveillance Program from 1991 to 2010. *T. longicornis* abundance per m^3 reaches a maximum at an N : P ratio of ∼ 16 : 1.

4 Discussion

4.1 Effects of the diet on physiological rates

This study has investigated the effects of a natural range of phytoplankton N : P, C : N and C : P ratios on *T. longicornis* feeding, respiration and egg production rates. *R. salina* organic nutrient stoichiometry varied from 9.6 : 1 to 22.8 : 1 N : P, from 5.2 : 1 to 9.9 : 1 C : N and from 76.4 : 1 to 166.7 : 1 C : P in the experiments. This is within the range (5 : 1 to 38 : 1) of naturally occurring particulate N : P (Geider and LaRoche, 2002; HOT, 2012) and C : N (5 : 1 to 30 : 1) ratios (Tang and Dam, 1999) in the ocean. Phytoplankton N : P, C : N and C : P did not affect *T. longicornis'* ingestion rates, indicating that grazing rates did not increase to compensate for low quality food. Clearance rates were not affected by food N : P, C : N or C : P ratios and remained low and within the values found previously by Dam and Lopes (2003) for *T. longicornis* feeding on a monospecific algal diet.

Previous research shows that phytoplankton concentration has an effect on copepod physiology, with metabolic rates such as respiration and excretion, increasing with increasing ingestion (Kiorboe et al., 1985; Thor, 2002). Therefore, in our study, to avoid the effect of food quantity on metabolism we fed copepods at saturating food conditions. In this way any change in metabolism could be attributed to changes in food quality. In this study, at constant and saturating food abundance, feeding rates did not affect the R_f or EPR of *T. longicornis*.

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Respiration and reproduction were affected by phytoplankton quality, showing similar responses to the same dietary constraints. This is because the growth and reproduction of small neritic copepods is tightly linked to the availability of energy obtained from metabolising organic compounds gained through feeding and produced through respiration and ATP synthesis. Respiration and egg production showed a marked biphasic response to N : P ratio. This highlights the switch from one limiting nutrient (nitrogen) to another (phosphorus), and defines the 16.5 : 1 N : P ratio as the Threshold Elemental Ratio (TER) (Hessen and Anderson, 2008). Although nitrogen is advocated to be the primary limiting nutrient for marine zooplankton (Sterner and Elser, 2002), this study shows that phosphorous also plays an important role and that a balance between nitrogen and phosphorus is needed for optimum metabolism and growth. Importantly phosphorus limitation, by inhibiting RNA synthesis, affects protein biosynthesis (Elser et al., 2003), therefore, together with nitrogen limitation, it can also cause protein limited production (Tang and Dam, 1999; Sterner and Elser, 2002).

Average R_u rates (1.9 nL O_2 DW^{-1} h^{-1}) at 13 °C compared well with published basal metabolic rates of 2 nL O_2 DW^{-1} h^{-1} at 9 °C (Castellani and Altunbaş, 2013). R_f rates reached a maximum, at an *R. salina* N : P ratio of 16.5 : 1 and exponentially decreased, to within the range of unfed values, either side of the peak. The maximum increase (3-fold) in metabolic rate we measured for fed *T. longicornis* compares well with published values of maximum specific dynamic action (SDA) which are typically two to four times higher than unfed rates of oxygen uptake in marine poikilotherms (Whiteley et al., 2001).

Egg production rates reached a maximum of 32 eggs female^{-1} day^{-1}, agreeing with previous laboratory studies where *T. longicornis* fed on monospecific algal diets produced 15–30 eggs female^{-1} day^{-1} (Jonasdottir et al., 2009; Dam and Lopes, 2003). Nevertheless, these laboratory rates are lower than maximum rates of production measured in the field (40–60 eggs female day^{-1}; Castellani and Altunbaş, 2006). Copepods generally produce more eggs on mixed-food diets than on single-food diets (Dam and Lopes, 2003). Egg production rates declined linearly with dietary deficiency. Our results

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agree with previous studies where females fed on a low quality diet produced fewer eggs (e.g. Jonasdottir and Kiorboe, 1996; Kleppel et al., 1998). This follows the concept of optimal nutrition (Hessen and Anderson, 2008) where diet deficiency can cause a shortage in energy supply in the organism. Therefore in this study, the poor nutritional quality of *R. salina* (i.e. lower and higher than 16.5 : 1) did not provide enough energy and specific nutrients essential for egg production, inhibiting growth.

These results show that the variation in metabolic rates is proportional to the quality of food ingested. The scale of these responses is presumably dependent on the availability of the necessary constituents in the diet (e.g. Cowles et al., 1988; Sterner and Schulz, 1998; Mitra and Flynn, 2005); driven by the physiological and structural requirements for biochemical constituents such as protein, carbohydrate and lipid.

Copepod egg production was negatively correlated with the algal C : N ratio. However, C : N ratio alone does not fully characterise the effects of food quality on copepod physiology, as C : N was not significantly related to *T. longicornis* metabolism (although a weak decreasing trend can be seen). Respiration integrates different costs besides that of producing eggs, hence the two patterns may not always coincide (Kiorboe, 2008). Copepod eggs are composed of, on average, 55 % protein derived from the food source (Kiorboe, 2008). Therefore, under food quality constraints, egg production is more limited by nitrogen, (a proxy for cell protein content) compared to other essential nutrients. EPR has been found to be constrained by high C : N ratios and therefore diet induced nitrogen-limitation (e.g. Checkley, 1980; Ambler, 1986; Kiorboe, 1989) suggesting that algal cells with lower C : N ratios represent greater nutritional value to copepods (Ambler, 1986; Tang and Dam, 1999).

Overall food N : P had a greater impact on EPR than food C : N. In the present study however, all the C : N values, except the lowest 5.2 : 1, correspond to either N or P limited values (5.2 : 1 C : N when 16.5 : 1 N : P). We therefore tested the possibility that the relationship between EPR and C : N was an artefact of N : P ratio driven effects. However, even when the peak value of 5.2 : 1 C : N (16.5N : 1P) was excluded from

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the regression, the linear relationship between EPR and C : N was still significant and therefore valid (data not shown).

4.2 Effects of food quality on copepod carbon budgets

Basal metabolism and growth are the major fates of assimilated energy in any organism (Kiorboe et al., 1985; Withers, 1992). Therefore AE is a proxy for the material available for biosynthesis and with increasing AE, more eggs are produced, increasing GGE. Conversely, as AE decreases more material will be lost through excretion and faecal pellets (Ianora and Poulet, 1993). As in previous studies (e.g. Mayzaud et al., 1998), AE increased with increasing food quality. AE values ranged from 2 to 26 %; these values are within the range found by Chervin (1978), but below the value of 51 % reported by Kiorboe et al. (1985) for a monospecific diet. Our results therefore would seem to indicate some dietary deficiency, showing indirectly that poor quality algae are neither properly assimilated nor transformed into production (Ianora and Poulet, 1993) as seen from the corresponding low EPR and GGE (Table 3). The high concentration of food offered to *T. longicornis* perhaps also contributed to this, as AE tends to decrease with increasing phytoplankton concentration (Besiktepe and Dam, 2002).

GGE reached a maximum of 21 % when the food N : P ratio was 16.5 : 1 and C : N was at its lowest of 5.2 : 1. This is in agreement with Straile (1997) who suggested that copepod GGEs typically average 25 %. Both AE and GGE decreased with increasing algal C : N as previously shown by Tang and Dam (1999). Importantly, AE and GGE showed the same biphasic relationship with food N : P ratio as respiration and egg production rates, demonstrating a strong dependence of physiological response on nutrition and therefore N and P limitation. The results of the present study clearly show that copepod metabolism and carbon budgets are influenced by food quality; as respiration decreased, egg production, GGE and AE also decreased. The TER of 16.5 : 1 N : P of the food thus defines the optimal metabolic requirement for *T. longicornis* to maximise reproduction.

MI increased with AE and GGE, showing a linear biphasic response to food N : P. MI is associated with the cost of biosynthesis and reflects the increase of energy requirements and energy flux in the organism, above basal maintenance. Respiration increases to produce additional energy (ATP) to metabolise more protein (Secor, 2008). Therefore, since the cost of protein metabolism is the highest, MI will reflect the cost of protein synthesis for reproduction (Thor, 2000; Kiorboe, 2008). This increased energy intake and increased energy synthesis determines the organism's metabolic rates.

The CoE represents the amount of energy necessary to produce eggs. Interestingly, CoE shows a significant linear decrease with increasing N : P ratio. This indicates that nitrogen plays a major role in the cost of egg production, even above an N : P ratio of 16.5 : 1 where both the numerator ($R_f - R_u$) and the denominator (EPR) indicate P limitation.

4.3 Ecological implications

Ecologically, the physiological responses of *T. longicornis* to food quality can be interpreted as the species' sensitivity to environmental change and determine the adaptive response of the copepod to the habitat it occupies (Kleppel and Burkart, 1995). Assuming that all zooplankton species are just as physiologically sensitive as *T. longicornis* to food N : P, the species GGE will decrease quickly with decreasing food quality and this may eventually affect zooplankton standing stock. A modelling study found a high sensitivity of zooplankton biomass to changes in GGE (Buitenhuis et al., 2006). Thus it is relevant to interrogate available field data to assess any co-variance between zooplankton biomass and particulate organic N : P ratios, to ascertain whether the relationship found in this study (specific TER and sensitivity to dietary N : P) might be applicable to all copepods.

Marine ecosystems and so algal food quality are changing with global warming. Changes in inorganic nutrient concentrations (Falkowski and Davis, 2004; Sarmiento, 2004; Arrigo, 2005) and phytoplankton community structure (Edwards and Richardson, 2004; Arrigo, 2005; Hays et al., 2005) are altering the nutritional value and particulate

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organic nutrient ratios of algae (Geider and LaRoche, 2002; Falkowski and Davis, 2004; Arrigo, 2005). The trend and extent of these stoichiometric variations are dependent on the environmental factors structuring the phytoplankton community. For instance, in the subtropical oligotrophic gyres, characterised by high sea surface temperatures and permanent water column stratification, rising temperatures and increased stratification will reduce the upward nutrient supply (Sarmiento, 2004), promoting an increase in diazotroph abundance and N_2 fixation (Broecker and Henderson, 1998; Bjorkman et al., 2000). This will alter the N : P supply ratio to the euphotic zone, leading to an overall increase in particulate organic N : P ratios (Broecker and Henderson, 1998).

Data from the HOT programme shows that in the oligotrophic North Pacific subtropical gyre (NPSG) the particulate organic N : P in the upper 150 m of the water column has increased from 17 : 1 in 1990 to 38 : 1 in 2010. This was triggered by increased stratification, increasing N_2 fixation and inorganic phosphorus limitation (Karl and Yanagi, 1997). These changes were accompanied by a shift in phytoplankton community structure towards dominance by smaller prokaryotic primary producers (Karl et al., 2001) that have generally higher N : P ratios (Geider and LaRoche, 2002).

It might be expected that the decrease in phytoplankton cell size would lead to an overall decrease in zooplankton size. However, when zooplankton biomass measured at HOT between 1994 and 2010 for each of the size classes (0.2–5 mm) is plotted against time, none of the size classes show a linear decrease in biomass with time. Instead, each size class shows a clear peak in biomass indicating that prey size may not be the only factor affecting the biomass of different zooplankton size classes. These biphasic responses are analogous to the biphasic response seen in *T. longicornis* related to linear changes in dietary N : P. We therefore investigated whether changes in food quality could be contributing to these peaks in size fractionated zooplankton biomass.

The HOT zooplankton biomass data show that the size class which peaks in any particular year decreases with time, so that the biomass of the 2–5 mm size class peaked in 2001–2002 while the biomass of the 0.2–0.5 mm size class peaked in 2006–2007.

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By comparing food quality (N : P) with the N : P ratio of the copepod size fractions (from Hannides et al., 2009) we were able to establish that these temporal changes in biomass peaks were coupled with variations in seston quality (Fig. 4a). Overall the biomass of the smallest size fraction (0.2–0.5 mm) with a higher body N : P ratio (∼ 21.3 : 1 N : P), peaked towards the end of the time series dataset when food with a higher N : P ratio was available (∼ 30.8 : 1 N : P). Interestingly, the peaks in size fractionated biomass coincided with the elemental composition of that particular size fraction to give a constant off-set of ∼ 9.5 N : P between the N : P of the seston and the N : P of the zooplankton size fraction (Fig. 4a). This finding is consistent with our experimental results, showing the high sensitivity of copepod GGE (and therefore biomass) to changes in the N : P ratio of their diet. This also indicates species-specific TERs, i.e. that not all copepods respond to a TER of 16 : 1 N : P as *T. longicornis* did. Therefore different species react differently to ecosystem variability, depending on their specific physiological demands and food quality threshold values shaped by the level of adaptation to the environment occupied.

T. longicornis is a temperate coastal species with a low body N : P ratio ranging from 8 : 1 to 12 : 1 (Pertola et al., 2002), and so we also investigated the available data for long term trends in algal N : P ratios in temperate regions. Data from the Norwegian Coastal Surveillance Program highlights strong interannual variations in N : P ratios in the mesotrophic North Sea (58° 23′ N, 8° 48′ E). However, spring bloom data show an increase in particulate organic N : P ratio from ∼ 10 : 1 in 1991 to ∼ 22 : 1 in 2004 (data not shown). From 2004 to 2010 the particulate organic N : P ratio decreased 49 % (data not shown). These trends are consistent with changes in phytoplankton community composition observed in the nearby waters of the NE Atlantic and northern North Sea. Between 1958 and 2002, phytoplankton community structure shifted from being dominated by diatoms (average group N : P ratio of ∼ 10 : 1 (Quigg et al., 2003) to being dominated by dinoflagellates (average phylum N : P ratio ∼ 30 : 1 and higher C : N ratio, Quigg et al., 2003) (Leterme et al., 2006). From 2006 onwards, there is

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a significant decline in dinoflagellate abundance, resulting in diatom dominance once again (Hinder et al., 2012).

Data from the Continuous Plankton Recorder collected around the Norwegian time-series station, confirm that from 1991 to 2010 the peak abundance for *T. longicornis* coincided with a seston N : P ~ 16 : 1 (Fig. 4b). This validates our laboratory findings and suggests that *T. longicornis* differs from the zooplankton found at HOT in its stoichiometric requirements for homeostatic regulation.

Shifts in the timing of the peak abundance of each phytoplankton species associated with climate warming (Edwards and Richardson, 2004), could also cause changes in the particulate N : P ratio of the phytoplankton present in a particular month each year. The recruitment success of *T. longicornis* and other copepods is highly dependent on synchronization with peak phytoplankton production. This coincides with the phytoplankton N : P increasing from low winter values to ratios closer to Redfield (Menzel and Ryther, 1966; Frigstad et al., 2011). These times of optimal food quality determine the peaks of maximum growth and biomass of the copepods (Pond et al., 1996; Guisande et al., 2000; Vargas et al., 2006; Koski et al., 2010). This study shows that if the N : P ratio of the algal food during the months of maximal *T. longicornis* growth were to increase by 0.5 N : P from the optimal of 16.5 : 1, then the GGE would decrease by ~ 39 % (calculated using data from Table 3 and equations from Table 4).

Therefore changes in food quality may affect the seasonal distribution of copepods, resulting in changes to species-specific peaks of succession and impacting zooplankton biogeography. Changes in the quality and composition of phytoplankton brought about by changes in inorganic nutrient ratios may therefore result in a change in zooplankton community as seen at HOT where, as larger zooplankton biomass decreases, the biomass of smaller species increases. An increase in smaller zooplankton species can lead to a decrease in energy transfer to higher trophic levels, affecting productivity and fisheries stock. For instance, in the North Sea a change in abundance of the larger and more nutritious copepod species, such as *Calanus finmarchicus*, has already resulted in a long-term decline of cod recruitment rates (Beaugrand et al., 2003).

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Variations in zooplankton species composition and size, as seen at HOT, affect nutrient cycles and zooplankton mediated carbon export. Variations in species-specific zooplankton physiological performance will also impact carbon egestion. As the metabolism and AE of larger species decrease, then carbon egestion (through faecal pellet production) will increase, increasing carbon export. However decreased GGE will have a greater impact, decreasing the amount of C available for export (Buitenhuis et al., 2006). At the same time, an increase in smaller species will lead to an increase in the production of smaller, slower sinking faecal pellets that are remineralised faster, so decreasing carbon export below the photic zone (Svensen et al., 2012).

5 Summary and conclusions

This study shows that food quality is an important regulator of copepod physiology and thus potentially of population dynamics.

1. A combination of nutrient ratios provides a more comprehensive description of the effects of nutrient limitation on copepods. Increased consideration of P-limitation is required in future studies.

2. Optimum food quality causes an increased energy demand and metabolic response of the organism. A large decrease in metabolic performance, either side of the Redfield ratio leads to a decrease in copepod GGE affecting the species' biomass.

3. The biphasic response to food N : P of the temperate neritic copepod *T. longicornis* shows that an algal composition of 16.5 : 1 is the optimum diet for the species. However other species have different TERs.

4. A combination of laboratory and time series data can be used to assess the impact of changing food quality on zooplankton biomass and activity. Smaller species appear to be increasing in areas where N : P also increases.

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5. Zooplankton community shifts to smaller species will cause cascade effects on the energy transfer to higher trophic levels, decreasing commercially important fish stocks. However, the scale of the effects on ocean biogeochemical cycles is not yet clear and warrants further study.

6. The lack of time-series data of seston particulate C : N : P stoichiometry limits an investigation of the contribution of food quality, together with temperature and food quantity to zooplankton biomass and community structure. Therefore further study is warranted to assess whether, in combination with temperature, food quality has a significant impact.

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Table 1. Stoichiometric ratios (N : P, C : N and C : P mol mol^{-1}), carbon, nitrogen and phosphorous content (pg cell^{-1}) and cell volume (µm^3) of *R. salina* measured during the 15 experiments. Data are the means of measurements taken at the beginning (T_0) and at the end (T_{24}) of the feeding incubation ± SD. NA = data not available.

Date	N : P	C : N	C : P	C pg cell^{-1}	N pg cell^{-1}	P pg cell^{-1}	Cell µm^3
29 Jun	14.8 : 1 ± 0.7	6.2 : 1 ± 0.2	92.2 : 1 ± 2.9	35.1 ± 0.7	6.5 ± 0.3	1.0 ± 0.06	97.8 ± 2
11 Jul	10.2 : 1 ± 0.7	7.5 : 1 ± 0.3	77.4 : 1 ± 3.9	38.8 ± 1.0	6.0 ± 0.2	1.3 ± 0.12	104.5 ± 3
20 Jul	22.8 : 1 ± 0.5	7.4 : 1 ± 0.2	166.7 : 1 ± 3.4	45.2 ± 0.9	7.1 ± 0.1	0.7 ± 0.01	NA
23 Jul	15.6 : 1 ± 0.8	8.3 : 1 ± 0.2	127.8 : 1 ± 5.6	45.0 ± 0.6	6.3 ± 0.1	0.9 ± 0.05	122.9 ± 2
23 Oct	9.6 : 1 ± 0.6	9.9 : 1 ± 0.7	94.3 : 1 ± 4.1	55.2 ± 2.0	6.5 ± 0.5	1.6 ± 0.05	198.4 ± 6
26 Oct	15.5 : 1 ± 0.4	5.9 : 1 ± 0.2	92 : 1 ± 2.0	34.7 ± 0.6	6.8 ± 0.2	1.0 ± 0.02	172.9 ± 13
31 Oct	14.2 : 1 ± 1.0	6.7 : 1 ± 0.2	90.9 : 1 ± 4.3	33.8 ± 0.4	5.9 ± 0.1	1.0 ± 0.07	180.9 ± 10
5 Nov	12.3 : 1 ± 0.6	7.9 : 1 ± 0.2	94.2 : 1 ± 3.5	50.9 ± 0.8	7.5 ± 0.2	1.4 ± 0.06	204.6 ± 6
11 Nov	13.3 : 1 ± 0.7	5.8 : 1 ± 0.3	76.4 : 1 ± 2.1	33.5 ± 0.6	6.8 ± 0.3	1.1 ± 0.04	170.3 ± 14
14 Nov	17.3 : 1 ± 0.8	8.1 : 1 ± 0.2	137.7 : 1 ± 4.8	46.8 ± 0.6	6.8 ± 0.1	0.9 ± 0.04	201.3 ± 6
17 Nov	20.3 : 1 ± 0.5	5.3 : 1 ± 0.1	107.8 : 1 ± 1.6	38.4 ± 0.5	8.5 ± 0.2	0.9 ± 0.01	177.5 ± 3
26 Nov	16.5 : 1 ± 0.5	5.2 : 1 ± 0.1	86.5 : 1 ± 1.7	33.4 ± 0.4	7.4 ± 0.2	1.0 ± 0.03	173.3 ± 9
29 Nov	17.2 : 1 ± 0.9	6.7 : 1 ± 0.2	114.8 : 1 ± 3.8	39.9 ± 0.5	7.0 ± 0.2	0.9 ± 0.05	176.9 ± 8
13 Dec	19.9 : 1 ± 0.7	6.0 : 1 ± 0.1	122.4 : 1 ± 2.2	37.5 ± 0.2	7.2 ± 0.2	0.8 ± N/A	186.2 ± 6
15 Dec	18.9 : 1 ± 1.2	6.2 : 1 ± 0.1	118.2 : 1 ± 6.7	41.2 ± 0.6	7.7 ± 0.1	0.9 ± 0.06	193.1 ± 12

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Table 2. Significant regression analyses of fed and unfed respiration (R) rates (nL O_2 DW^{-1} h^{-1}) and egg production rates (EPR; eggs $female^{-1}$ day^{-1}) versus food quality. Exponential relationship $y = ae^{bx} + c$ for R and linear relationship $y = ax + c$ for EPR.

Variables	n	Slope (a)	(b)	Intercept (c)	r^2	P
R fed vs. N : P ≤ 16 : 1	7	0.0075	0.39	2.12	0.88	0.005
R fed vs. N : P ≥ 16 : 1	7	22 703.41	−0.52	2.11	0.92	0.006
R unfed vs. N : P ≤ 16 : 1	7	3.59×10^{-7}	0.88	1.74	0.83	0.031
R unfed vs. N : P ≥ 16 : 1	6	856.42	−0.41	1.455	0.86	0.051
EPR vs. N : P ≤ 16 : 1	6	2.661		−24.104	0.86	0.008
EPR vs. N : P ≥ 16 : 1	6	−2.527		60.452	0.75	0.027
EPR vs. C : N	11	−2.380		28.038	0.43	0.029

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Table 3. Average carbon budget of *T. longicornis* fed on *R. salina* at different N : P ratios. G EPR: growth by egg production rates, AE: assimilation efficiency, GGE: gross growth efficiency, R: respiration, MI: metabolic increment, CoE: cost of egg production. The italic numbers represent calculated values using the fitted regression relationships found in Table 2. For the experiments of the 11 July and 20 July the average ingestion rate was used to calculate AE and GGE. Each point represents mean ± SD.

Date	N : P	I Ingestion $\mu g C \mu g C^{-1} d^{-1}$	G EPR $ng C \mu g C^{-1} d^{-1}$	AE % = $G + R_f / I$	GGE % $= G / I$	*R* Fed $ng C \mu g C^{-1} d^{-1}$	*R* Unfed $ng C \mu g C^{-1} d^{-1}$	MI %	CoE %
23 Oct	9.6 : 1	1.28 ± 0.27	13.6 ± 18.1	1.99 ± 1.42	1.06 ± 1.41	11.9 ± 2.1	8.6 ± 1.3	38.24 ± 7.05	24.3 ± 18.3
11 Jul	10.2 : 1	NA	*22.4 ± 5.9*	*4.05 ± NA*	*2.55 ± NA*	13.2 ± 0.8	9.6 ± 2.4	36.95 ± *3.94*	*15.8 ± 11.2*
05 Nov	12.3 : 1	0.88 ± 0.28	49.9 ± 32.0	7.28 ± 3.64	5.68 ± 3.64	14.0 ± 0.0	8.3 ± 1.7	69.96 ± 8.47	11.6 ± 3.3
11 Nov	13.4 : 1	0.82 ± 0.27	79.1 ± 41.8	11.69 ± 5.11	9.66 ± 5.10	16.6 ± 2.2	8.7 ± 1.0	89.84 ± 16.08	9.9 ± 3.1
31 Oct	14.2 : 1	0.42 ± 0.13	61.6 ± 39.4	20.07 ± 9.47	14.74 ± 9.43	22.3 ± 3.4	8.4 ± 2.3	164.30 ± 22.41	22.5 ± 6.6
29 Jun	14.8 : 1	0.76 ± 0.21	*86.2 ± 24.0*	*14.36 ± 3.18*	*11.39 ± 3.18*	*22.5 ± NA*	*9.4 ± NA*	138.21 ± *NA*	*15.1 ± NA*
26 Oct	15.5 : 1	0.85 ± 0.30	94.1 ± 45.7	14.57 ± 5.41	11.04 ± 5.36	30.2 ± 6.4	10.7 ± 1.0	180.92 ± 41.67	20.6 ± 6.9
23 Jul	15.6 : 1	1.27 ± 0.35	*125.5 ± 34.4*	*11.59 ± 2.74*	*9.90 ± 2.72*	21.3 ± 4.6	10.3 ± NA	107.43 ± *NA*	*8.8 ± 3.7*
26 Nov	**16.5 : 1**	**0.74 ± 0.20**	**155.6 ± 65.2**	**25.87 ± 8.86**	**21.14 ± 8.85**	**34.8 ± 2.1**	**12.0 ± 2.5**	**189.18 ± 16.37**	**14.6 ± 2.1**
29 Nov	17.2 : 1	0.80 ± 0.30	86.7 ± 32.6	13.83 ± 4.08	10.84 ± 4.08	23.9 ± 1.6	12.5 ± 1.3	91.87 ± 8.78	13.2 ± 2.4
14 Nov	17.3 : 1	0.78 ± 0.23	88.7 ± 33.9	14.30 ± 4.37	11.40 ± 4.35	22.6 ± 3.6	10.2 ± 2.7	122.42 ± 14.07	14.0 ± 5.0
15 Dec	18.9 : 1	0.88 ± 0.21	59.5 ± 18.4	8.92 ± 2.10	6.77 ± 2.09	19.0 ± 1.6	9.1 ± 2.0	107.93 ± 12.64	16.5 ± 4.3
13 Dec	19.9 : 1	0.82 ± 0.18	64.0 ± 26.7	9.64 ± 3.28	7.79 ± 3.25	15.2 ± 3.8	8.3 ± 0.6	82.76 ± 37.20	10.7 ± 6.0
17 Nov	20.3 : 1	1.14 ± 0.43	70.0 ± 36.2	7.53 ± 3.19	6.15 ± 3.18	15.7 ± 3.5	*8.3 ± NA*	88.37 ± *NA*	*10.5 ± 5.0*
20 Jul	22.8 : 1	NA	*21.0 ± 9.6*	*3.40 ± NA*	*2.39 ± NA*	8.9 ± 1.7	7.8 ± 1.1	13.35 ± *2.84*	*5.0 ± 9.5*

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Table 4. Regression analyses of significant relationships between *T. longicornis* AE (%), GGE (%), MI (%) and CoE (ng C µg C^{-1} d^{-1}) and *R. salina* N : P and C : N ratios. Linear relationship $y = ax + c$. AE: assimilation efficiency, GGE: gross growth efficiency, MI: metabolic increment, CoE: cost of egg production.

Variables	n	Slope (a)	Intercept (c)	r^2	P
AE vs. N : P ≤ 16 : 1	9	0.027	−0.24	0.78	0.002
AE vs. N : P ≥ 16 : 1	7	−0.028	0.65	0.74	0.01
GGE vs. N : P ≤ 16 : 1	9	0.022	−0.21	0.78	0.002
GGE vs. N : P ≥ 16 : 1	7	−0.023	0.53	0.72	0.02
MI vs. N : P ≤ 16 : 1	9	2.50	−22.72	0.79	0.001
MI vs. N : P ≥ 16 : 1	7	−2.97	61.46	0.79	0.007
CoE vs. N : P (all)	15	−0.82	27.27	0.33	0.024
AE vs. C : N	15	−0.03	0.29	0.27	0.049
GGE vs. C : N	15	−0.02	0.23	0.28	0.04

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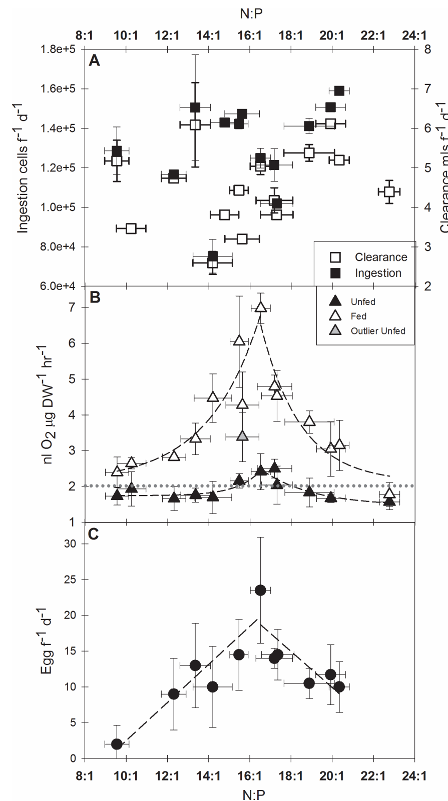

Fig. 1. (A) Relationship between the molar N : P ratio of *R. salina* and clearance and ingestion rates of *T. longicornis*. **(B)** Relationship between the molar N : P ratio of *R. salina* and respiration rate of *T. longicornis* (normalised by dry weight). The dashed lines represent the regression lines. The grey dotted horizontal line represents the average basal respiration rate of *T. longicornis* of 2 nL O_2 DW^{-1} h^{-1} (Castellani and Altunbaş, 2013). **(C)** Relationship between the molar N : P ratio of *R. salina* and EPR of *T. longicornis*. The dashed lines represent the regression lines. All errors are ± SD.

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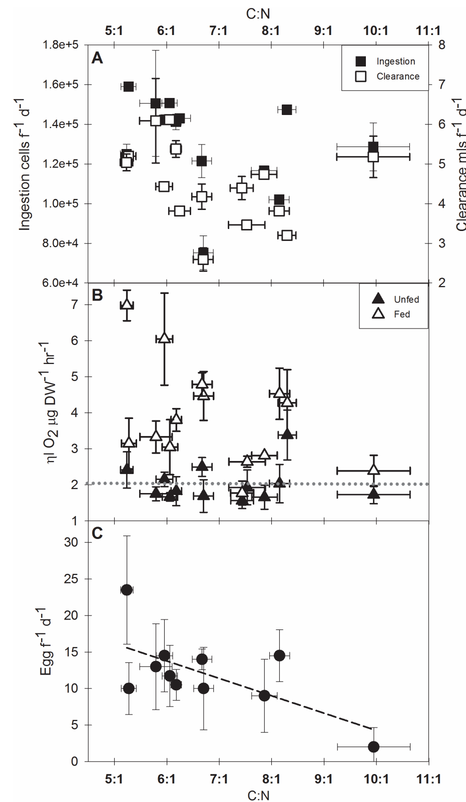

Fig. 2. (A) Relationship between the molar C : N ratio of *R. salina* and clearance and ingestion rates of *T. longicornis*. **(B)** Relationship between the molar C : N ratio of *R. salina* and respiration rate of *T. longicornis* (normalised by dry weight). The grey dotted horizontal line represents the average basal respiration rate of *T. longicornis* of 2 nL O_2 DW^{-1} h^{-1} (Castellani and Altunbaş, 2013). **(C)** Relationship between the molar N : P ratio of *R. salina* and EPR of *T. longicornis*. The dashed line represents the regression line. All errors are ± SD.

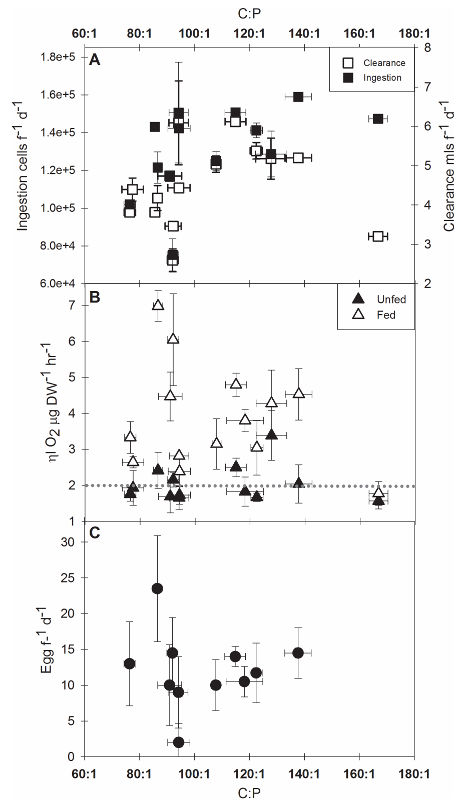

Fig. 3. (A) Relationship between the molar C : P ratio of *R. salina* and clearance and ingestion rates of *T. longicornis*. The dashed line represents the regression line of ingestion rate. **(B)** Relationship between the molar C : P ratio of *R. salina* and respiration rate of *T. longicornis* (normalised by dry weight). The grey dotted horizontal line represents the average basal respiration rate of *T. longicornis* of 2 nL O_2 DW^{-1} h^{-1} (Castellani and Altunbaş, 2013). **(C)** Relationship between the molar N : P ratio of *R. salina* and EPR of *T. longicornis*. All errors are ± SD.

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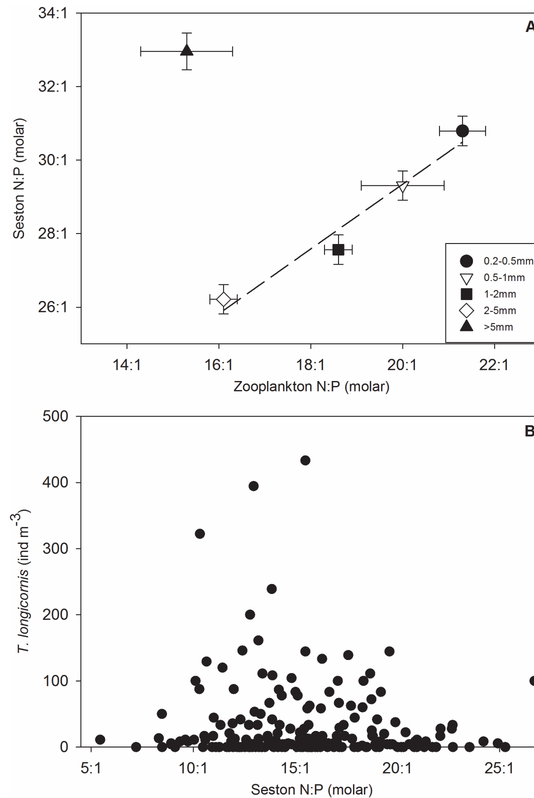

Fig. 4. (A) Relationship between the N : P ratio of size fractionated zooplankton (Hannides et al., 2009) and the corresponding food N : P ratio in the year when the biomass of that zooplankton size-fraction reached a maximum. HOT data from 1994 to 2010. Errors are ± SE. **(B)** Relationship between monthly *T. longicornis* abundance (ind m^{-3}) and monthly average seston N : P ratio (molar) in the North Sea (Arundel station 58° 23′ N, 8° 48′ E) between 1991 and 2010.

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