Journal of
Plankton Research

plankt.oxfordjournals.org

Effects of temperature, food type and food concentration on the grazing of the calanoid copepod *Centropages chierchiae*

SUSANA GARRIDO[1,2]*, JOANA CRUZ[1,3,4], A. MIGUEL P. SANTOS[3], PEDRO RÉ[1] AND ENRIC SAIZ[2]

[1]CENTRO DE OCEANOGRAFIA, LABORATÓRIO MARÍTIMO DA GUIA, FACULDADE DE CIÊNCIAS, UNIVERSIDADE DE LISBOA, AVENIDA NOSSA SENHORA DO CABO, NO 939, 2750-374 CASCAIS, PORTUGAL, [2]INSTITUT DE CIÈNCIES DEL MAR - CSIC, PS. MARÍTIM DE LA BARCELONETA 37-49, E-08003 BARCELONA, SPAIN, [3]INSTITUTO PORTUGUÊS DO MAR E DA ATMOSFERA IPMA, AV. BRASILIA S/N, 1449-006 LISBON, PORTUGAL AND [4]CCMAR, UNIVERSITY OF ALGARVE, CAMPUS DAS GAMBELAS, 8005-139 FARO, PORTUGAL

*CORRESPONDING AUTHOR: garridosus@gmail.com

Laboratory experiments were conducted to study the combined effect of temperature (8, 13, 19 and 24°C), food type and food concentration on the grazing rates of the adult stages of the calanoid copepod *Centropages chierchiae*. As prey, the diatom *Phaeodactylum tricornutum* and the dinoflagellate *Gymnodinium* sp. (both ca. 15 μm cell diameter) were used at a range of carbon concentrations similar to the ones experienced in nature (6.4 to 393.8 $\mu C\,L^{-1}$). Ingestion rates increased linearly with food concentration and did not differ between prey types. When comparing the effect of temperature, highest clearance and ingestion rates were obtained at 19°C, whereas no difference was observed among the other temperatures. Daily rations varied between 1.2 and 183.5% body carbon day^{-1}. Additional experiments were conducted to study the selective feeding behaviour of *C. chierchiae* when offered a mixture of different prey types. Selective feeding was dependent on food concentration; at low food levels, large cells were selected (*Ditylum brightwellii*), whereas at medium and high food concentrations no clear selection patterns were observed. In contrast to other studies, no positive selection of dinoflagellates over other algal food was found.

INTRODUCTION

The calanoid copepod *Centropages chierchiae* typically inhabits tropical and subtropical waters of the eastern Atlantic Ocean, the Mediterranean Sea and the western Indian Ocean (Razouls, 1996). From the northern limit of its distribution south to the Bay of Biscay, *C. chierchiae* occurs preferentially during the warm summer months (Lindley and Daykin, 2005), whereas during the rest of the year it can also be found in high concentrations off western Iberia (Sobrinho-Gonçalves *et al.*, 2013). This copepod species constitutes one of the most important prey items in the diet of small pelagic fish in the productive, upwelling waters off the Iberian Peninsula, such as adult sardines (Garrido *et al.*, 2008) and anchovies (Plouvenez and Champalbert, 1999). A better understanding of the dynamics of the coastal food webs that sustain small pelagic fish stocks involves not only knowing the kinds and amounts of food required for fish larval survival, but also aspects of the biology and ecology of the key organisms upon which fish feed (Turner, 1984). Additionally, the implications that fish pressure on certain key copepod species may have shaping the trophic web structure are relevant. Despite its importance, however, information on the biology and ecology of this copepod species is lacking, in particular when compared with the better studied congeneric species *Centropages hamatus* and *Centropages typicus* (e.g. Kiørboe *et al.*, 1982; Carlotti *et al.*, 1997; Calbet *et al.*, 2007).

In addition to the importance of *Centropages chierchiae* in southern Atlanto-European waters, this species has expanded its distribution northward during the last decade. Before 1988, *C. chierchiae* occurred rarely in the Bay of Biscay, Celtic Sea and English Channel, whereas since 2000 it is frequently found and at greater abundance in these areas (Lindley and Daykin, 2005). This northward shift in the eastern North Atlantic Ocean and European shelf seas has been associated with an increase in water temperature in these areas (Lindley and Daykin, 2005). Range shifts exhibited by zooplankton in response to global warming are reported as among the fastest and largest of any marine or terrestrial group (Richardson, 2008). Such ecosystem modifications arising from changes in the distribution of species at the base of the marine food web may have dramatic impacts for exploited resources, as indicated for cod in the North Sea (Beaugrand *et al.*, 2003).

In this study, we aim to provide basic ecophysiological knowledge on the feeding rates and behaviour of *Centropages chierchiae*, with the goal of improving our understanding of its capability to cope with environmental variability. Understanding the physiological limits and plasticity of an organism are essential traits required in order to forecast its direct impact on the trophic food webs of newly colonized ecosystems. We will focus on two of the major factors driving copepod feeding in the oceans, temperature and food type and availability (Saiz and Calbet, 2011). We also studied the selective feeding behaviour in the presence of different prey types provided at different concentrations. The rate of food intake of one prey may be affected by the presence of alternative prey choices. Because of time restrictions due to handling of alternative prey, selectivity patterns and/or behavioural switches may occur associated with changes in the relative prey abundances (Kiørboe *et al.*, 1996; Gentleman *et al.*, 2003). Copepods can discriminate between different types of food and select preferred food items. Usually, larger particles are positively selected over small cells (e.g. Frost, 1972; Meyer *et al.*, 2002), although discrimination between similar-sized items has also been reported (e.g. Fernández, 1979; Henriksen *et al.*, 2007). Other, more complex feeding behaviours have been described for copepods, where selection depends on the interplay between cell size and abundance (e.g. Wilson, 1973; Kiørboe *et al.*, 1996).

To our knowledge, this is the first time that the functional response of *Centropages chierchiae* has been described in relation to temperature, and one of the few works studying such relationships for this genus (Calbet *et al.*, 2007).

METHOD

Copepod and microalgae cultures

Zooplankton samples were collected in coastal waters near Lisbon (western Iberian Peninsula) during November 2009 and July 2010, with a 200-μm mesh size WP2 net with a plastic bag as cod end to avoid copepod damage. The samples were immediately transported to the laboratory, and *Centropages chierchiae* individuals were sorted under the stereomicroscope. The sorted copepods were kept in culture in 30 L tanks with filtered, autoclaved seawater (35 salinity) at 20°C, and fed a mixture of different microalgae at saturated conditions (matching those used in the feeding experiments, described below).

Two sets of experiments were conducted on adult *Centropages chierchiae*: (i) unialgal experiments to study the effect of temperature on the feeding rates, using the diatom *Phaeodactylum tricornutum* ($\sim 17.0 \times 2.3$ μm) and the dinoflagellate *Gymnodinium* sp. (~ 14 μm) as prey, and (ii) plurialgal experiments to study the feeding selectivity behaviour. The size of the prey used in the selective-feeding experiments ranged between 8 and 50 μm, and

Table I: Size and estimated carbon content of Centropages chierchiae *and of the prey species used*

Species	Size (μm)	Carbon content (μg)
Centropages chierchiae adult female	1545.6 ± 80.89SE[a]	17.3
Centropages chierchiae adult male	1507.2 ± 49.72SE[a]	16.1
Ditylum brightwellii	50[b]	5.1×10^{-3}
Rhodomonas baltica	8[b]	7.8×10^{-5}
Gymnodinium sp.	14[b]	2.2×10^{-4}
Phaeodactylum tricornutum	17×2.3[c]	1.5×10^{-5}

Carbon content was estimated from the equations provided by van der Lingen (van der Lingen, 2002) and Smayda (Smayda, 1965) for *C. chierchiae* and microalgae, respectively.
[a]Prosome length.
[b]Mean diameter.
[c]Minor and major axes.

comprised the cryptophyte *Rhodomonas baltica*, the dinoflagellate *Gymnodinium* sp. and the diatoms *P. tricornutum* and *Ditylum brightwellii*.

All microalgae used as prey were obtained from cultures growing exponentially at 19°C at a 12:12 h light/dark regime in the f/2 medium. Phytoplankton cell size ($n = 30$) and copepod prosome length ($n = 80$) were measured from digital pictures using the software Visilog Expert 6.300 (Table I). The carbon content of microalgae and copepods were estimated using the equations of, respectively, Smayda (Smayda, 1978) and van der Lingen (van der Lingen, 2002) (Table I).

Unialgal feeding experiments and temperature effects

Unialgal experiments were conducted at four different temperatures (8, 13, 19 and 24°C), using as prey *Gymnodinium* sp. and *Phaeodactylum tricornutum* at concentrations between 29.0 and 1790.0 cells mL^{-1}, equivalent to 6.4 to 393.8 μgC L^{-1}. These food concentrations were chosen with the aim of mimicking the natural range of dinoflagellate and diatom densities typically experienced by *Centropages chierchiae* during summer and autumn in the waters off the central western Portuguese coast, as reported by Silva *et al.* (Silva *et al.*, 2009). In the case of the experiments conducted at 19°C, the upper bound of the range was further expanded to food concentrations (6.4 and 457.5 μgC L^{-1}) higher than those found in the wild, to be able to describe the functional response of this species at the temperature when its abundance peaks in Iberian waters.

The copepods and microalgae were acclimated to the experimental temperature and light conditions for at least 24 h prior to the beginning of the feeding experiments.

Experiments were conducted in 600-mL Pyrex screw-cap bottles filled with filtered seawater enriched with the f/2 medium, which were amended with aliquots of the prey stock cultures in order to achieve the desired experimental concentrations. Five bottles were set for each prey concentration, two acting as control bottles (no copepods added) and three acting as experimental ones (in which three adult females and two adult males were added); the sex ratio used in the incubations is similar to the average sex ratio of adult copepods in the sea, as reported by Hirst and Kiørboe (Hirst and Kiørboe, 2002). The bottles were rotated at 1 rpm on a plankton wheel, and incubation ended after 24 h. Phytoplankton samples were collected at the start and end of the experiments, preserved with 2% Lugol's solution and counted by triplicate using a Sedgewick-Rafter counting chamber under an inverted microscope. The number of dead and live copepods at the end of the incubation was also recorded.

Plurialgal feeding experiments and selective behaviour

The plurialgal feeding experiments were conducted by offering the copepods simultaneously four prey species (*Gymnodinium* sp., *Phaeodactylum tricornutum*, *Rhodomonas baltica* and *Ditylum brightwellii*), at three prey mixture concentrations (respectively 56.1, 454.7 and 1107.9 μg C L^{-1}). These experiments were conducted only at 19°C, and the remaining procedures were similar to the ones followed for the unialgal experiments, using two control and three experimental (three females and two males added) bottles for each mixture concentration tested.

Data analysis

Calculations of average food concentration, clearance and ingestion rates followed the equations of Frost (Frost, 1972). Weight-specific ingestion rates were estimated as daily ration (i.e. percentage of body mass ingested daily), taking into account the carbon content of the microalgae and the copepod. Prior to calculations, we compared the prey growth rates between experimental (prey with added copepods) and control (prey without copepods) bottles using *t*-tests. Only when (apparent) growth rates were significantly lower in the experimental bottles were calculations done (i.e. feeding took place).

An exponential decay equation was fitted to the functional response of clearance rates

$$F = F_{max} \times e^{-bC}$$

where F_{max} is the maximum clearance rate, b a prey-specific constant and C the food concentration (μg C L^{-1}).

In the case of ingestion rates, the functional response was fitted to the Ivlev equation (Ivlev, 1961)

$$I = I_{\max} \times (1 - e^{-aC})$$

where $I_{\max}$ is the maximum ingestion rate, a is a constant describing the rate at which ingestion I approaches the maximum, and C is the food concentration (μg C L^{-1}). In both cases, fitting was done on non-transformed data using an iterative non-linear regression routine. Additionally, to compare the ability of *Centropages chierchiae* to approach saturation under the different diets, we estimated the half-saturation rate concentration (Isari *et al.*, 2011), which is the concentration at which ingestion equals half of the ingestion maximum rate:

$$\frac{CI_{\max}}{2} = \frac{\ln(0.5)}{-\alpha}$$

In the unialgal experiments, differences between the feeding rates obtained with the two algae and the four temperatures tested were determined using the analysis of covariance (ANCOVA). The ANCOVA was constructed with the initial prey concentration, prey type (*Gymnodinium* or *Phaeodactylum*) and temperature (8, 13, 19, 24°C) as predictor variables, and ingestion rate as dependent variable (using both per individual and carbon-specific rates). The comparison of feeding rates under different temperature regimes was done using data only from the common range of initial prey concentrations (i.e. it did not included the extended range of food concentrations used in the experiments conducted at 19°C). Multiple comparisons of means between pairs of categories were tested *a posteriori* using the Tukey test for unequal sample sizes at a confidence level of $P < 0.05$.

To evaluate if there was prey selection in the plurialgal experiments, selectivity was determined using the Vanderploeg and Scavia relativized electivity index (E') (Vanderploeg and Scavia, 1979), calculated as

$$Ei = \frac{Wi - 1/n}{Wi + 1/n}$$

where n is the number of prey types, i is prey type i, and Wi is the selectivity coefficient for prey type i calculated as:

$$Wi = \frac{Ri/Pi}{\sum_{i=1}^{n} (Ri/Pi)}$$

where Ri is the proportion of prey type i ingested, and Pi is proportion of prey type i in the water. Ei values range between $+1$ and -1; values close to 1 indicate positive selection, close to 0 represent 'random' selection and negative values close to -1 indicate avoidance.

All graphics and statistical analyses were performed using the open source software R version 2.9.2 (R Development Core Team; www.r-project.org).

RESULTS

Unialgal feeding experiments and temperature effects

The clearance rates of *Centropages chierchiae* in the unialgal experiments, fed either *Gymnodinium* sp. or *Phaeodactylum tricornutum*, were very variable and did not show a clear relationship with food availability, ranging from 6.3 to 150.0 mL cop^{-1} day^{-1}. Maximum clearance rates increased from 8 to 19°C (69.3 $\pm$ 11.82, 93.9 $\pm$ 11.71 and 116.1 $\pm$ 10.47 SE mL cop^{-1} day^{-1} at, respectively, 8, 13 and 19°C) and decreased at 24°C (62.1 $\pm$ 6.14 mL cop^{-1} day^{-1}) (Fig. 1). Maximum clearance rates were all significantly different (*t*-tests using the mean and SE obtained from the iterative non-linear regressions at $P < 0.005$). Ingestion rates of *C. chierchiae* in the unialgal experiments ranged between 0.2 and 28.9 μg C cop^{-1} day^{-1} (Fig. 2), and increased linearly with food concentration at all temperatures tested. Maximum individual ingestion rates were 24.4 $\pm$ 2.59, 20.9 $\pm$ 3.05, 32.1 $\pm$ 1.83 and 7.5 $\pm$ 0.73 SE μg C day^{-1} at, respectively, 8, 13,19 and 24°C (Fig. 2). Maximum ingestion rates were all significantly different (*t*-tests using the mean and SE obtained from the iterative non-linear regression at $P < 0.005$) except those obtained at 8 and 13°C.

The ingestion rates of *Centropages chierchiae* on *Gymnodinium* sp. and *Phaeodactylum tricornutum* in the unialgal experiments conducted at four temperatures showed no significant effect of prey type, whereas there were significant differences for temperature and prey concentration (Table II). There was also no significant interaction between temperature and food concentration (ANCOVA test, F = 1.239, P = 0.301), indicating that the slopes of the linear relationship between food concentration and ingestion were similar across temperatures. Ingestion rates obtained at 19°C were significantly higher than those achieved at the other temperatures, which did not show significant differences among them (*a posteriori* Tukey test, Fig. 3). Similarly to ingestion rates, clearance rates of *C. chierchiae* showed no significant differences between prey types and significant effects of temperature, with higher clearance rates at 19°C when compared with the other temperatures; in this case, however, no significant relationship with food concentration was found (Table II, Fig. 3).
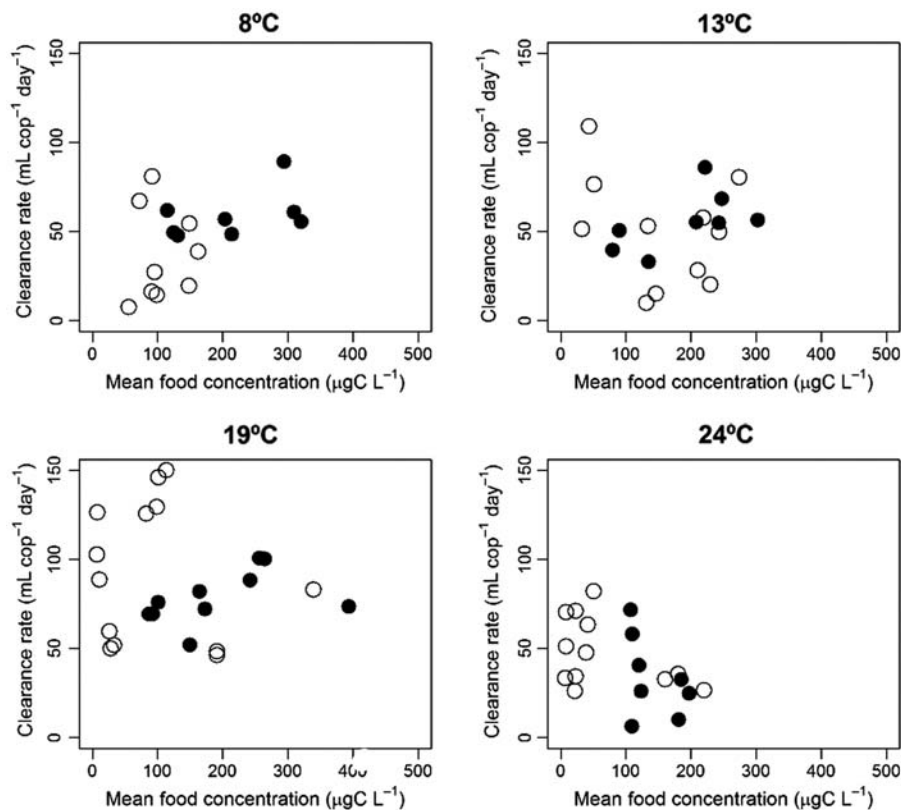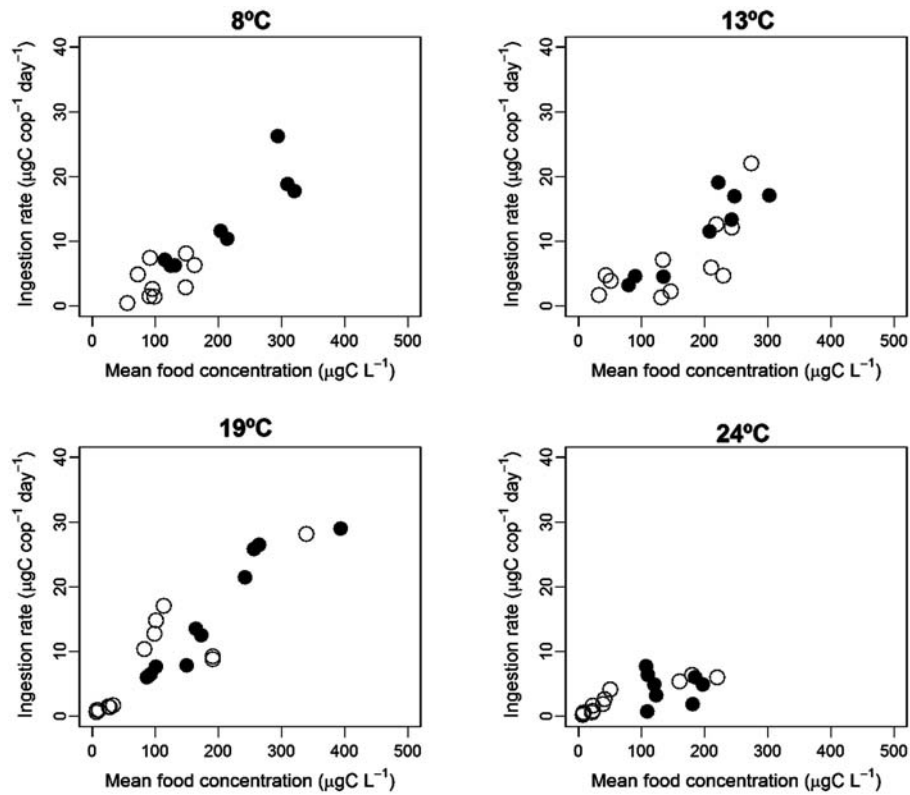
Fig. 1. Clearance rates (mL cop^{-1} day^{-1}) of *Centropages chierchiae* feeding on *Phaeodactylum tricornutum* (filled circles) and *Gymnodinium* sp. (open circles) in the experiments conducted at 8, 13, 19 and 24°C.

As no differences were found regarding prey type (*Gymnodinium* vs. *Phaeodactylum tricornutum*), the broader-range data set for feeding rates conducted at 19°C was analysed by pooling data for both prey types (Fig. 4). The maximum clearance rate was 150 mL^{-1} cop^{-1} day^{-1}, whereas at prey concentrations >400 μg C L^{-1} ingestion rates reached a plateau (I_{max}: 37.9 ± 3.58 SE μg C cop^{-1} day^{-1}). The half-saturation concentration was 206 μg C L^{-1}.

In terms of daily rations, feeding rates varied between 1.2 and 183.5% body carbon day^{-1} (Fig. 5). Similarly to the per-individual feeding rates, daily rations showed no significant effect of prey type (ANCOVA test, $P = 0.63$), whereas significant effects of prey concentration ($P < 0.0001$) and temperature ($P < 0.0001$) were found. Daily ration was significantly higher at 19°C and similar among the other temperatures tested (Fig. 5; Tukey test, data not shown). Mean daily rations ($\pm$ SE) estimated within the range of prey concentrations common to all temperatures (<410 μgC L^{-1}) were 48.7 ± 41.38, 52.4 ± 38.33, 73.2 ± 48.83 and 19.5 ± 19.56% body carbon ingested day^{-1} at, respectively, 8, 13, 19 and 24°C.

Plurialgal feeding experiments and selective behaviour

When offered a mixture of different prey types, the total carbon ingestion rates of *Centropages chierchiae* adults varied from 4.7 ± 1.56 μg C cop^{-1} day^{-1} at the lowest concentration used (56.1 μg C L^{-1}) to 18.2 ± 3.03 μg C cop^{-1} day^{-1} at the intermediate concentration (454.7 μg C L^{-1}) and up to 85.6 ± 15.31 μg C cop^{-1} day^{-1} when at the highest prey concentration (1107.9 μg C L^{-1}) (Table III). Maximum clearance rates (159 ± 87.20 mL cop^{-1} day^{-1}) were obtained at the low concentration treatment when fed the diatom *Ditylum brightwellii*. Regarding the electivity index, there was a shift in selectivity patterns as food concentration increased (Fig. 6). In the experiments performed at the lowest food concentration, the large diatom *Dytilum brightwellii* was selected over the other smaller algae; however, at the intermediate and high food concentrations, the electivity index for *D. brightwellii* indicated no selection or weak avoidance (below threshold line; Fig. 6). The other prey types were not consistently selected nor avoided at any of the prey concentrations used (Fig. 6).

Fig. 2. Ingestion rates (μg C cop^{-1} day^{-1}) of *Centropages chierchiae* feeding on *Phaeodactylum tricornutum* (filled circles) and *Gymnodinium* sp. (open circles) in the experiments conducted at 8, 13, 19 and 24°C.

*Table II: ANCOVA analysis of the effect of prey concentration (μgC L^{-1}), temperature and prey type (*Gymnodinium* sp. and* Phaeodactylum tricornutum*) on the ingestion and clearance rates of* Centropages chierchiae

| Response variable	Independent variables	DF	F-value	P-value
Ingestion rate	Initial concentration (μgC L^{-1})	1	338.1	<0.0001
	Temperature	3	11.2	<0.0001
	Prey type	1	0.3	0.6164
Clearance rate	Initial concentration (μgC L^{-1})	1	338.1	0.9860
	Temperature	3	11.2	<0.0001
	Prey type	1	0.3	0.9674

DISCUSSION

Feeding rates

The feeding rates of *Centropages chierchiae*, in terms of carbon ingested per individual, obtained in this work ranged from 0.2 to 37.9 μg C ind^{-1} day^{-1}. The mean ingestion rate across temperatures was 3.0 μg C ind^{-1} day^{-1} at low food concentrations (<100 μg C L^{-1}) and 12.4 μg C ind^{-1} day^{-1} at high food concentrations (100–500 μg C L^{-1}). Our estimated ingestion rates are consistent with *in situ* determinations of *C. chierchiae* feeding rates at 11°C in the Bay of Biscay at (natural) low prey concentrations (Vincent and Hartmann, 2001), and slightly lower than those previously reported in the laboratory in experiments conducted at 15°C (Schnack, 1983). The ingestion rates obtained here for *C. chierchiae* also fall within the range of values typically reported for similarly sized copepods (a copepod with body mass of 16 μg C, like *C. chierchiae*, is expected to eat 10–50 μg C ind^{-1} day^{-1}; Saiz and Calbet, 2011). In spite of the low food concentrations tested in our experiments (6.4 μg C L^{-1}), no zero feeding was observed, in agreement with the observations of Schnack (Schnack, 1983) who reported feeding by *C. chierchiae* at concentrations as low as 0.9 μg C L^{-1}.

The clearance rates of *Centropages chierchiae* obtained in the unialgal experiments were quite variable, and ranged between 6.3 and 150.0 mL^{-1} cop^{-1} day^{-1}. The expected decline in clearance rate with increasing food availability was only observed for the 19°C data set. At the other temperatures, the narrow range of (low) food concentrations used very likely precluded the identification of clearer declining trends. Overall, the clearance
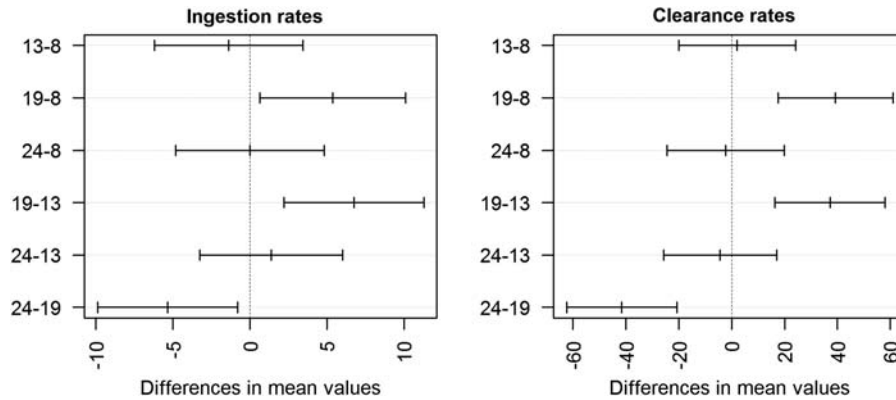
Fig. 3. Pairwise comparison of ingestion (left panel) and clearance rates (right panel) between each level of the factor temperature (8, 13, 19, 24°C). Differences in mean values and 95% confidence intervals of the Tukey test for unequal sample size are shown. A given pairwise comparison of means is significant if the confidence intervals do not overlap the 0 difference. Only data from experiments conducted at food concentrations $<410.0\ \mu g\ C\ L^{-1}$ are used.
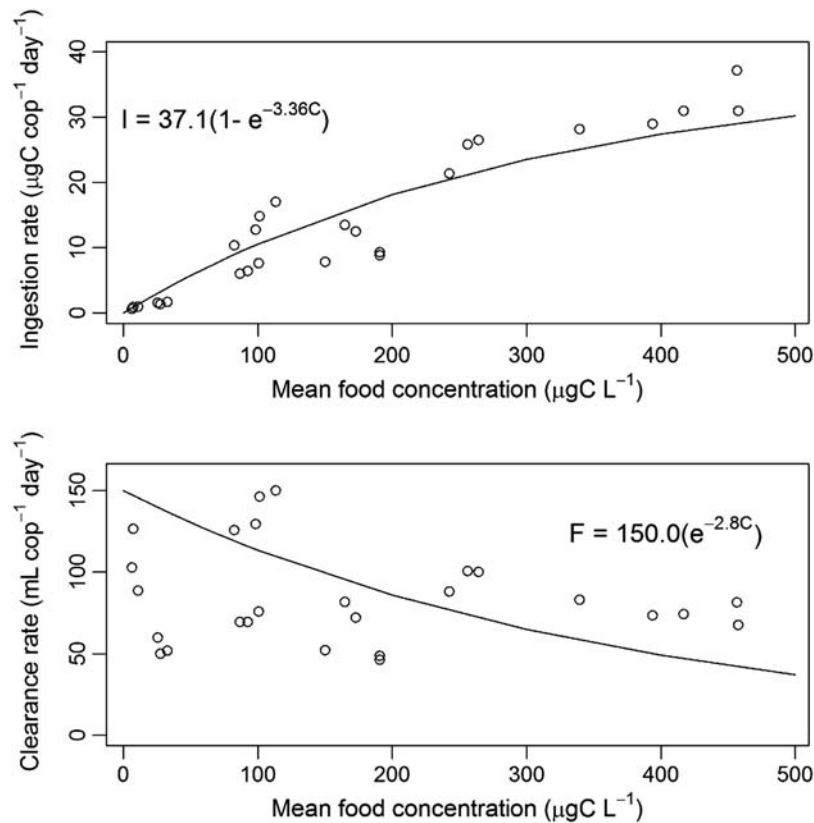
Fig. 4. Functional feeding response of *Centropages chierchiae* at 19°C using *Gymnodinium* and *Phaeodactylum tricornutum* as prey. Data of both algae are pooled since no significant differences were found of the feeding rates for both algae (Table II). Ingestion (I; $\mu g\ C\ cop^{-1}\ day^{-1}$) in the upper panel, and clearance (F; $mL\ cop^{-1}\ day^{-1}$) in the lower panel.

rates obtained in this study were lower than those reported by Schnack (Schnack, 1983) for *C. chierchiae* fed the diatom *Thalassiosira partheneia* (offered as single cell, $24-340\ mL\ cop^{-1}\ day^{-1}$) and the dinoflagellate *Scrippsiella trochoidea* ($192-570\ mL\ cop^{-1}\ day^{-1}$) at low food concentrations ($<100\ \mu g\ C\ L^{-1}$). This was quite unexpected because *T. partheneia* given as single cells is much smaller (9 μm diameter) and *S. trochoidea* is similarly sized (20 μm diameter) to the prey we have used to describe the functional response of *C. chierchiae*. Moreover,

7
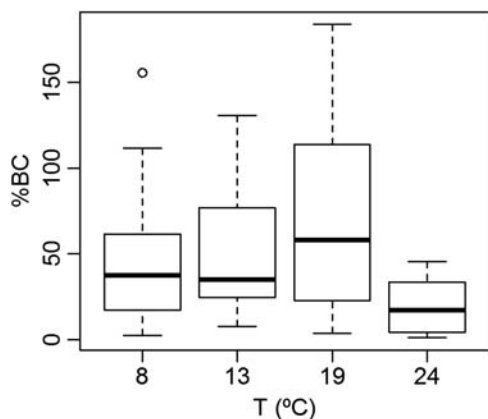
Fig. 5. Box-plots of *Centropages chierchiae* weight-specific ingestion rates expressed as daily ration (%BC, % of body carbon ingested day^{-1}) at the four different temperatures studied (only data from experiments conducted at food concentrations $<410.0\,\mu g\,C\,L^{-1}$ are shown). Median values are indicated by the horizontal line in the boxes.

Table III: Ingestion (I; $\mu gC\,cop^{-1}\,day^{-1}$) and clearance rates (F; $mL\,cop^{-1}\,day^{-1}$) of Centropages chierchiae *in the plurialgal experiment*

Total prey concentration	Prey	Species-specific prey concentration	I	F
56.1 ± 3.39	dyt	13.6 ± 6.25	2.4 ± 1.61	159 ± 87.20
	gym	3.3 ± 0.50	0.2 ± 0.12	56 ± 30.79
	pha	37.8 ± 3.43	2.1 ± 0.25	54 ± 1.89
	rho	1.43. ± 0.14	0.1 ± 0.09	58 ± 56.37
454.7 ± 12.99	dyt	128.6. ± 6.25	2.5 ± 0.23	19 ± 3.00
	gym	41.1 ± 0.50	1.4 ± 0.67	33 ± 13.66
	pha	268.0 ± 5.81	13.9 ± 3.34	51 ± 13.40
	rho	16.99 ± 0.14	0.4 ± 0.33	24 ± 19.89
1107.9 ± 237.08	dyt	297.8. ± 46.31	18.8 ± 5.60	63 ± 14.86
	gym	229.7 ± 123.02	17.6 ± 8.25	88 ± 59.02
	pha	380.1 ± 22.62	29.0 ± 8.81	75 ± 18.21
	rho	200.2 ± 160.2	20.2 ± 11.66	121 ± 87.37

Total and prey-specific food concentrations ($\mu gC\,L^{-1}$) are shown. rho: *Rhodomonas baltica;* gym: *Gymnodinium* sp.; pha: *Phaeodactylum tricornutum*; dyt: *Ditylum brightwellii*.

our experiments were conducted at higher temperatures (19 and 24°C) than the experiments described in Schnack (15°C ; Schnack, 1983), and therefore one may expect higher rates in our study. The experiments described by Schnack (Schnack, 1983) only lasted 8–10 h during daylight, so daily rations might be underestimated, assuming that copepods might show a diel rhythm and eat more during the night (Saiz *et al.*, 1992; Calbet *et al.*, 1999). Differences in feeding rates between experiments can be explained by several factors such as the physiological condition and previous feeding history of the copepods, as well as the composition of the prey

(Huntley, 1988), which are difficult to repeat between experiments. In the review by Calbet *et al.* (Calbet *et al.*, 2007) on *Centropages* feeding, clearance rates obtained for congeneric species in different studies also showed highly variable results, even if restricted to the same species. For example, clearance rates of *Centropages typicus* reported in the literature can vary from a few mL up to 1221 mL cop^{-1} day^{-1} (Calbet *et al.*, 2007). Using *Phaeodactylum tricornutum* as prey, Gaudy (Gaudy, 1974) reported clearance rates in the range 0.5–2.5 mL cop^{-1} day^{-1} for *C. typicus* at 18°C, which is much lower than our values; conversely, our clearance rates are similar to those found for *C. typicus* feeding on the same algae in a different study (3.7–21 mL cop^{-1} day^{-1}; Tomasini and Mazza, 1978).

The range of daily ration values found in our study (1.2–183.5% body carbon ingested day^{-1}) reaches the upper bound of feeding rates reported for copepods feeding at intermediate and high concentrations of food (Saiz and Calbet, 2011). In the only previous feeding experiment conducted in the laboratory with *Centropages chierchiae*, Schnack (Schnack, 1983) determined maximum daily food intakes in the range of 29 to 56% body carbon. However, these results are difficult to compare with ours, as one would expect much lower rates since these experiments were conducted at very low food concentration (ca. 55 μg C L^{-1}). Also, the *C. chierchiae* used differed in size, having larger body mass (28 μg cop^{-1}) than the ones in our study (16.9 μg cop^{-1}). Regarding other congeneric species, our daily rations were slightly higher than those reported for *Centropages hamatus* (e.g. 85% body carbon ingested day^{-1} at 15°C; Kiørboe *et al.*, 1982), and similar to the ones reported for *Centropages typicus* [from ca. 50% body carbon ingested day^{-1} at low food concentrations to values up to 380% body carbon ingested day^{-1} at high food concentrations (Gaudy, 1974)]. In general, the feeding rates of *Centropages chierchiae* determined in this study are in the upper range of those previously determined for congeners and other copepod species (Calbet *et al.* 2007, Saiz and Calbet, 2011), which might represent an important advantage for *C. chierchiae* when competing for food with co-habiting copepod species.

Temperature effects on feeding rates

In our study, feeding rates increased significantly with temperature up to 19°C and then decreased at 24°C, following a dome-shaped pattern (Fig. 5). Such patterns are the result of optimal temperature windows driven by species-specific physiological tolerance to temperature, and have been observed in several copepod species, both in the upper bound (too high temperature, e.g. *Calanus*
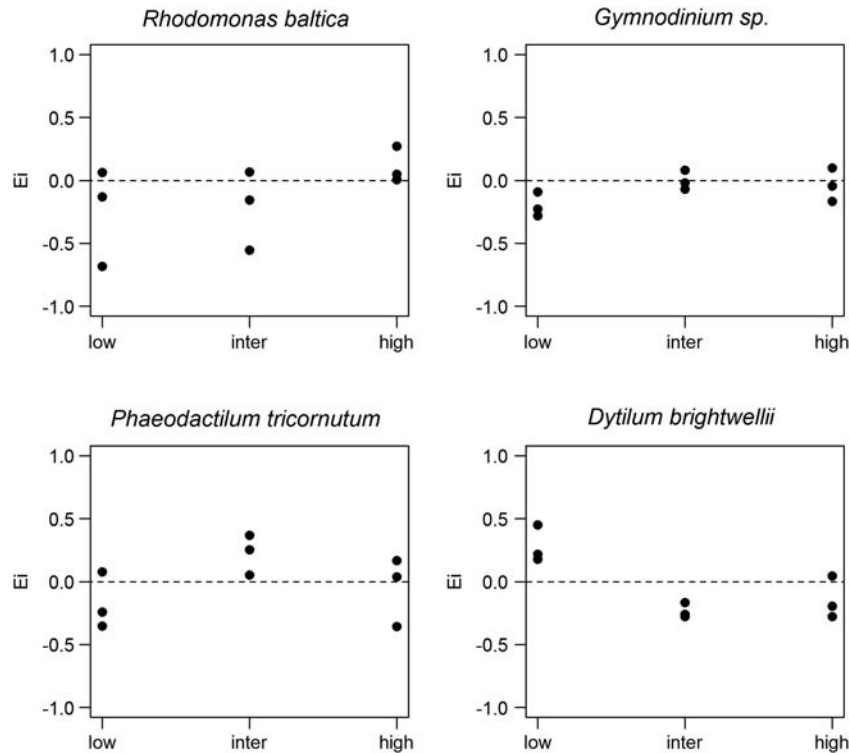
Fig. 6. Electivity index (Ei) for the (plurialgal) selective feeding experiment, using a mixed assemblage of four prey (*Ditylum brightwellii*, *Gymnodinium* sp., *Phaeodactylum tricornutum* and *Rhodomonas baltica*) at three total prey concentrations (low: 56.1 ± 3.39, inter: 454.7 ± 12.99 and high: 1107.9 ± 237.08 μg C L^{-1}). Each prey level has three replicates. The dotted line represents the threshold level (i.e. *Ei* = 0) at which a given prey is neither selected nor avoided.

helgolandicus and *Calanus finmarchicus*, Møller *et al.*, 2012; *Temora stylifera*, Thébault, 1985) or the lower bound (*Oithona davisae*, Almeda *et al.*, 2010b). Our results suggest that 19°C falls in the range of optimal temperatures for *Centropages chierchiae*, likely as the result of an adaptation of the species to the prevailing habitat conditions. This hypothesis is indeed confirmed by the fact that this temperature corresponds to the mean temperature in surface waters off western Iberia during the warm months when *Centropages chierchiae* peaks its abundance (Sobrinho-Gonçalves *et al.*, 2013). In terms of temperature dependence, the Q_{10} values for maximum clearance rates obtained here for *Centropages chierchiae* ($Q_{10} = 1.6$) were similar to those described for *Acartia hudsonica* ($Q_{10} = 1.8$; Durbin and Durbin, 1992), lower than the $Q_{10} = 3.9$ and $Q_{10} = 2.45$ obtained for the ingestion rates of, respectively, *Centropages hamatus* (Kiørboe *et al.*, 1982) and *Oithona davisae* (Almeda *et al.*, 2010a), and within the range of variation of the temperature dependence of ingestion rates for *Temora stylifera* (Q_{10}:1.2−4.4), *Calanus helgolandicus* (Q_{10}:0.78−4.3) and *Clausocalanus arcuicornis* (Q_{10}:1.28−2.56) (Fernández, 1978). In this regard, some of the very high Q_{10} values found in laboratory experiments reported in the literature might be a consequence

of a response to rapid changes in temperature without enough time for conditioning (thermal stress). Empirical analyses of copepod feeding field data (Peters and Downing, 1984; Saiz and Calbet, 2011) have shown that, compared with other variables such as food concentration, temperature had a weaker control over copepod feeding rates, likely as a consequence of physiological adaptation to habitat condition. This fact may suggest that the populations invading the North Atlantic in recent years might be better adapted to colder waters than the population we worked with, caught off western Iberia. Examples of physiological adaptation in copepods have been well studied in the calanoid *Eurytemora affinis* (e.g. Ketzner and Bradley, 1982; Petersen and Lee, 2003). There is also evidence that co-existing copepod species, as demonstrated for some *Temora* and *Centropages* species, may have more similarities in their abundance, body size and reproduction cycles than congeners living in different habitats (Halsband-Lenk *et al.*, 2004).

Plurialgal experiments and selective feeding

Feeding rates of copepods depend on a variety of factors such as body mass, temperature, food type, quality and

concentration, and also previous feeding history (Mullin, 1963; Price and Paffenhöfer, 1985; Saiz and Calbet, 2011). Although copepod feeding has been a key subject in zooplankton research for decades, one of the aspects that is less studied is the mechanisms involved in prey selection (e.g. Cowles *et al.*, 1988; DeMott, 1988), particularly when copepods are offered a variety of prey as occurs in nature (e.g. Vanderploeg *et al.*, 1984). Field studies of copepod feeding, in which they are exposed to a range of natural prey, are relatively scarce in the literature (Saiz and Calbet, 2007) and have the drawback that complex interactions between various factors such as prey concentration, size, motility and composition (Kleppel, 1993) are difficult to discern within a natural assemblage. On the other hand, laboratory experiments of copepod feeding are typically conducted with single prey suspensions, and very few studies have used multiple prey approaches (e.g. Donaghay and Small, 1979; Kiørboe *et al.*, 1996; Meyer *et al.*, 2002). Generally, copepods are described as being able to selectively ingest large cells over small cells (e.g. Frost, 1972). In this regard, Vincent and Hartmann (Vincent and Hartmann, 2001) reported that in feeding incubation experiments with natural prey assemblages, *Centropages chierchiae* cleared large ciliates (>40 μm) at higher rates than small ciliates. In our study, however, we have found that across a range of prey sizes between 5 and 50 μm, the pattern of prey-size selectivity for this copepod species depended on prey abundance. For instance, when food availability was low *C. chierchiae* cleared the largest prey (*Ditylum brightwellii*) at higher rates than the smaller cells, in agreement with the observations by Vincent and Hartmann (Vincent and Hartmann, 2001). However, at intermediate and high prey concentrations, there was no clear positive selectivity for any of the prey. Food-concentration-dependent selectivity patterns have also been described for *Centropages brachiatus* (Cowles, 1979) and the group *Para-Pseudocalanus* (Fileman *et al.*, 2007). The fact that in our experiments *Rhodomonas baltica* was, in most cases, not positively selected, is probably a consequence of its small size, likely below optimum, in agreement with a general preference for >10 μm prey reported for the congeneric *Centropages typicus* (Tomasini and Mazza, 1978).

General patterns of copepod feeding show that ciliates are strongly selected prey, followed by dinoflagellates, while selective feeding patterns on diatoms may range from negative to positive selection (Saiz and Calbet, 2011). Vincent and Hartmann (Vincent and Hartmann, 2001) also observed that that *Centropages chierchiae* cleared dinoflagellates (4.9 mL cop^{-1} day^{-1}) and ciliates (4.3 mL cop^{-1} day^{-1}) at higher rates than phytoplankton cells (0.7 mL cop^{-1} day^{-1}); these authors also highlighted the preference for dinoflagellates exhibited by *C. chierchiae* when compared with parallel incubations conducted with *Calanus helgolandicus* and *Temora longicornis*. In this regard, Schnack (Schnack, 1983) also found that the clearance rates of *C. chierchiae* were higher for the dinoflagellate *Scrippsiella trochoidea* than for the diatom *Thalassiosira partheneia*; we think that this latter result, however, might be biased by the larger size of the dinoflagellate in that study. Our results did not support any preference for dinoflagellates over other chlorophyll-pigmented cells by *C. chierchiae*, and actually showed that similarly sized diatoms and dinoflagellates were cleared at similar rates. From an ecological point of view, diatoms represent the major component of the phytoplankton biomass available from the summer months to early autumn in the productive upwelling region off the western Iberian Peninsula (Silva *et al.*, 2009), when *C. chierchiae* abundance peaks (Sobrinho-Gonçalves *et al.* 2013). Therefore, it would make sense that this copepod species might benefit from the high abundance of diatoms in that period of the year. In fact, in productive ecosystems, the contribution of diatoms to the diet of copepods is higher than in oligotrophic environments, where it is considered to be very low (Saiz and Calbet, 2011).

Conclusions

This study has improved our understanding of the functional response and feeding behaviour of the calanoid copepod *Centropages chierchiae*, particularly under food concentrations similar to the ones experienced *in situ*. Our results show that this copepod species has high feeding rates for both diatoms and dinoflagellates, and that its selective-feeding behaviour is dependent on food concentration, larger prey being selected only at low food concentrations. Feeding rates were temperature dependent, showing a dome-shaped response with optimal temperature values at 19°C, similar to the mean temperature in surface waters off western Iberia during the warm months when *C. chierchiae* peaks in abundance.

FUNDING

REFERENCES

Almeda, R., Augustin, C., Alcaraz, M. *et al.* (2010a) Feeding rates and gross growth efficiencies of larval developmental stages of *Oithona davisae* (Copepoda, Cyclopoida). *J. Exp. Mar. Biol. Ecol.*, **387**, 24–35.

Almeda, R., Calbet, A., Alcaraz, M. *et al.* (2010b) Effects of temperature and food concentration on the survival, development and growth rates of naupliar stages of *Oithona davisae* (Copepoda, Cyclopoida). *Mar. Ecol. Prog. Ser.*, **410**, 97–109.

Beaugrand, G., Brander, K. M., Lindley, J. A. *et al.* (2003) Plankton effect on cod recruitment in the North Sea. *Nature*, **426**, 661–664.

Calbet, A., Carlotti, F. and Gaudy, R. (2007) The feeding ecology of the copepod *Centropages typicus* (Kroyer). *Prog. Oceanogr.*, **72**, 137–150.

Calbet, A., Saiz, E., Irigoien, X. *et al.* (1999) Food availability and diel feeding rhythms in the marine copepods *Acartia grani* and *Centropages typicus*. *J. Plankton Res.*, **21**, 1009–1015.

Carlotti, F., Rey, C., Javanshir, A. *et al.* (1997) Laboratory studies on egg and faecal pellet production of *Centropages typicus*: Effect of age, effect of temperature, individual variability. *J. Plankton Res.*, **19**, 1143–1165.

Cowles, T. J. (1979) The feeding response of copepods from the Peru upwelling system: food size selection. *J. Mar. Res.*, **37**, 601–622.

Cowles, T. J., Olson, R. J. and Chisholm, S. W. (1988) Food selection by copepods: discrimination on the basis of food quality. *Mar. Biol.*, **100**, 41–49.

DeMott, W. R. (1988) Discrimination between algae and artificial particles by freshwater and marine copepods. *Limnol. Oceanogr.*, **33**, 397–408.

Donaghay, P. L. and Small, L. F. (1979) Food selection capabilities of the estuarine copepod *Acartia clausi*. *Mar. Biol.*, **52**, 137–146.

Durbin, E. G. and Durbin, A. G. (1992) Effects of temperature and food abundance on grazing and short-term weight change in the marine copepod *Acartia hudsonica*. *Limnol. Oceanogr.*, **37**, 361–378.

Fernández, F. (1978) Metabolismo y alimentación en copépodos planctónicos del Mediterráneo: Respuesta a la temperatura. *Invest. Pesq.*, **42**, 97–139.

Fernández, F. (1979) Particle selection in the nauplius of *Calanus pacificus*. *J. Plankton Res.*, **1**, 313–328.

Fileman, E., Smith, T. and Harris, R. (2007) Grazing by *Calanus helgolandicus* and *Para-Pseudocalanus* spp. on phytoplankton and protozooplankton during the spring bloom in the Celtic Sea. *J. Exp. Mar. Biol. Ecol.*, **348**, 70–84.

Frost, B. W. (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.*, **17**, 805–815.

Garrido, S., Ben-Hamadou, R., Oliveira, P. B. *et al.* (2008) Diet and feeding intensity of sardine *Sardina pilchardus*: correlation with satellite-derived chlorophyll data. *Mar. Ecol. Prog. Ser.*, **354**, 245–256.

Gaudy, R. (1974) Feeding four species of pelagic copepods under experimental conditions. *Mar. Biol.*, **25**, 125–141.

Gentleman, W., Leising, A., Frost, B. *et al.* (2003) Functional responses for zooplankton feeding on multiple resources: a review of assumptions and biological dynamics. *Deep-Sea Res. II*, **50**, 2847–2875.

Halsband-Lenk, C., Carlotti, F. and Greve, W. (2004) Life-history strategies of calanoid congeners under two different climate regimes: a comparison. *ICES J. Mar. Sci.*, **61**, 709–720.

Henriksen, C. I., Saiz, E., Calbet, A. *et al.* (2007) Feeding activity and swimming patterns of *Acartia grani* and *Oithona davisae* nauplii in the presence of motile and non-motile prey. *Mar. Ecol. Prog. Ser.*, **331**, 119–129.

Hirst, A. G. and Kiørboe, T. (2002) Mortality of marine planktonic copepods: global rates and patterns. *Mar. Ecol. Prog. Ser.*, **230**, 195–209.

Huntley, M. (1988) Feeding biology of *Calanus*: a new perspective. *Hydrobiologia*, **167/168**, 83–99.

Ivlev, V. S. (1961) *Experimental Ecology of Feeding Fishes*. Yale University Press, New Haven.

Ketzner, P. A. and Bradley, B. P. (1982) Rate of environmental change and adaptation in the copepod *Eurytemora affinis*. *Evolution*, **36**, 298–306.

Kiørboe, T., Møhlenberg, F. and Nicolajsen, H. (1982) Ingestion rate and gut clearance in the planktonic copepod *Centropages hamatus* (Liljeborg) in relation to food concentration and temperature. *Ophelia*, **21**, 181–194.

Kiørboe, T., Saiz, E. and Viitasalo, M. (1996) Prey switching behaviour in the planktonic copepod *Acartia tonsa*. *Mar. Ecol. Prog. Ser.*, **146**, 65–75.

Kleppel, G. S. (1993). On the diets of calanoid copepods. *Mar. Ecol. Prog. Ser.*, **99**, 183–195.

Lindley, J. A. and Daykin, S. (2005) Variations in the distributions of *Centropages chierchiae* and *Temora stylifera* (Copepoda : Calanoida) in the north-eastern Atlantic Ocean and western European shelf waters. *ICES J. Mar. Sci.*, **62**, 869–877.

Meyer, B., Irigoien, X., Graeve, M. *et al.* (2002) Feeding rates and selectivity among nauplii, copepodites and adult females of *Calanus finmarchicus* and *Calanus helgolandicus*. *Helgoland Mar. Res.*, **56**, 169–176.

Møller, E. F., Maar, M., Jónasdóttir, S. H. *et al.* (2012) The effect of changes in temperature and food on the development of *Calanus finmarchicus* and *Calanus helgolandicus* populations. *Limnol. Oceanogr.*, **57**, 211–220.

Mullin, M. (1963) Some factors affecting the feeding of marine copepods of the genus *Calanus*. *Limnol. Oceanogr.*, **8**, 239–250.

Peters, R. H. and Downing, J. A. (1984) Empirical analysis of zooplankton filtering and feeding rates. *Limnol. Oceanogr.*, **29**, 763–784.

Petersen, C. H. and Lee, C. E. (2003) Effects of developmental acclimation on adult salinity tolerance in the freshwater-invading copepod *Eurytemora affinis*. *Physiol. Biochem. Zool.*, **76**, 296–301.

Plouvenez, S. and Champalbert, G. (1999) Feeding behaviour and trophic environment of *Engraulis encrasicolus* (L.) in the Bay of Biscay. *Estuarine Coastal Shelf Sci.*, **433**, 177–191.

Price, H. J. and Paffenhöfer, G.-A. (1985) Perception of food availability by calanoid copepods. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, **21**, 115–124.

Razouls, C. (1996) Diversité et répartition geographique chez les copépodes pelagiques. 1. Calanoida. *Ann. Inst. Oceanogr. (Paris)*, **71**, 81–404.

R Development Core Team. (2011) *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Richardson, A. J. (2008) In hot water: zooplankton and climate change. *ICES J. Mar. Sci.*, **65**, 279–295.

Saiz, E. and Calbet, A. (2007) Scaling of feeding in marine calanoid copepods. *Limnol. Oceanogr.*, **52**, 668–675.

Saiz, E. and Calbet, A. (2011) Copepod feeding in the ocean: scaling patterns, composition of their diet and the bias of estimates due to microzooplankton grazing during incubations. *Hydrobiologia*, **666**, 181–196.

Saiz, E., Rodríguez, V. and Alcaraz, M. (1992) Spatial distribution and feeding rates of *Centropages typicus* in relation to frontal hydrographic structures in the Catalan Sea (Western Mediterranean). *Mar. Biol.*, **112**, 49–56.

Schnack, S. B. (1983) On the feeding of copepods on *Thalassiosira partheneia* from the Northwest African upwelling area. *Mar. Ecol. Progr. Ser.*, **11**, 49–53.

Silva, A., Palma, S., Oliveira, P. B. *et al.* (2009) Composition and inter-annual variability of phytoplankton in a coastal upwelling region (Lisbon Bay, Portugal). *J. Sea Res.*, **62**, 238–249.

Smayda, T. J. (1978) From phytoplankters to biomass. In Sournia, A. (ed.), *Phytoplankton Manual. UNESCO Monographs on Oceanographic Methodology.* Vol. 6. UNESCO, Paris, pp. 273–279.

Sobrinho-Gonçalves, L., Moita, M. T., Garrido, S. *et al.* (2013) Environmental forcing on the interactions of plankton communities across a continental shelf in the Eastern Atlantic upwelling system. *Hydrobiologia*, doi: 10.1007/s10750-013-1500-2.

Thébault, J. M. (1985) Etude expérimentale de la nutrition d'un copépode commun (*Temora stylifera* Dana). Effets de la température et de la concentration de nourriture. *J. Exp. Mar. Biol. Ecol.*, **93**, 223–234.

Tomasini, J. A. and Mazza, J. (1978) Feeding habits of 2 calanoid copepods (*Centropages typicus* and *Acartia clausi*) in nutritive medium with single alga. *J. Cons. Int. Explor. Mer.*, **38**, 154–179.

Turner, J. (1984) The feeding ecology of some zooplankters that are important prey items of larval fish. *NOAA Tech. Rep. NMFS*, **7**, 1–28.

van der Lingen, C. D. (2002) Diet of sardine *Sardinops sagax* in the Southern Benguela upwelling ecosystem. *S. Afr. J. Mar. Sci.*, **24**, 301–316.

Vanderploeg, H. A. and Scavia, D. (1979) Two electivity indices for feeding with special reference to zooplankton grazing. *J. Fish. Res. Board Can.*, **36**, 362–365.

Vanderploeg, H. A., Scavia, D. and Liebig, J. R. (1984) Feeding rate of *Diaptomus sicilis* and its relation to selectivity and effective food concentration in algal mixtures and in Lake Michigan. *J. Plankton Res.*, **6**, 919–941.

Vincent, D. and Hartmann, H. J. (2001) Contribution of ciliated microprotozoans and dinoflagellates to the diet of three copepod species in the Bay of Biscay. *Hydrobiologia*, **443**, 193–204.

Wilson, D. S. (1973) Food size selection among copepods. *Ecology*, **54**, 909–914.