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Reproduction and respiration of a climate change indicator species: effect of temperature and variable food in the copepod *Centropages chierchiae*

JOANA CRUZ^{1,2,3*}, SUSANA GARRIDO³, MARTA S. PIMENTEL³, RUI ROSA³, A. MIGUEL P. SANTOS² AND PEDRO RÉ³

¹CENTRO DE CIÊNCIAS DO MAR DO ALGARVE, UNIVERSIDADE DO ALGARVE. CAMPUS DE GAMBELAS, FARO 8005-139, PORTUGAL, ²INSTITUTO PORTUGUÊS DO MAR E DA ATMOSFERA (IPMA), AVENIDA BRÁSILIA S/N, LISBOA 1449-006, PORTUGAL AND ³LABORATÓRIO MARÍTIMO DA GUIA, CENTRO DE OCEANOGRAFIA, FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DE LISBOA, AV NOSSA SENHORA DA GUIA 939, CASCAIS 2750-374, PORTUGAL

*CORRESPONDING AUTHOR: joanacruz23@hotmail.com

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The abundance of the calanoid copepod *Centropages chierchiae* has increased at the northern limits of its distribution in recent decades, mainly due to oceanic climate forcing, suggesting this as a key species in monitoring climate change. Laboratory experiments were conducted to study the combined effect of temperature, food type and concentration on the egg production rate (EPR) and hatching success (HS) of *C. chierchiae*. Females were fed on two monoalgal diets (*Gymnodinium* sp. and *Phaeodactylum tricornutum*) at two food concentrations and at three different temperatures (13, 19, 24°C). Respiration rates of both genders were measured at four different temperatures (8, 13, 19, 24°C). EPR was significantly different between temperatures and food concentrations, the maximum EPR being attained when the copepods were exposed to high food levels and at 19°C. Prey type significantly influenced EPR; feeding on *P. tricornutum* resulted in higher egg production than *Gymnodinium* sp. HS was significantly lower at 13°C than at 19 and 24°C and higher with *Gymnodinium* sp. Respiration rates were sex independent and increased exponentially with temperature. To maintain basal metabolism, the minimum food intake of *P. tricornutum* ranged between 0.4 and 1.8 µg C and for *Gymnodinium* sp. between 0.03 and 0.13 µg C. Food intake was always higher than the metabolic demands, except for the highest temperature tested (24°C). The present results confirm the sensitivity

of *C. chierchiae* to temperature variations and may help in understanding the successful expansion of its distribution towards northern latitudes.

KEYWORDS: *Centropages chierchiae*; reproduction; respiration; temperature; food

INTRODUCTION

Copepods are the dominant component of marine mesozooplankton and the major link between primary production and higher trophic levels (Mauchline, 1998). Furthermore, the fact that they have short life-cycles and are poikilotherms makes them good indicators of climate changes (Hays *et al.*, 2005). Ocean temperature has increased over the past century (Levitus *et al.*, 2000) and is expected to increase 3–5°C over the next century (IPCC, 2007). The responses exhibited by zooplankton to global warming are among the most rapid and greatest of any marine or terrestrial group (Richardson, 2008). Copepods have shown changes in their phenology, geographic distribution, community composition, with likely effects on ecosystem functioning (Edwards and Richardson, 2004; Mackas *et al.*, 2007; Beaugrand *et al.*, 2009). In the eastern North Atlantic Ocean and European shelf seas, calanoid copepods have moved northwards with the increase in water temperature (Lindley and Daykin, 2005).

The calanoid copepod *Centropages chierchiae* Giesbrecht, 1889 occurs in tropical and subtropical waters, restricted to the eastern Atlantic, Mediterranean and western Indian Ocean (Razouls, 1996). Data collected by the Continuous Plankton Recorder (CPR) survey from 1958 to 1999 has shown that this species is distributed in the Northeast Atlantic and western European continental shelf (CPR survey team, 2004). In the early years of the last century, *C. chierchiae* records at the northernmost limit of its distribution, i.e. around 50°N (Lysholm *et al.*, 1945) were very scarce. Conversely, in recent decades its abundance and frequency has increased considerably in the Bay of Biscay, Celtic Sea and English Channel. Lindley and Daykin (Lindley and Daykin, 2005) suggested that *C. chierchiae* has become a resident population in the Celtic sea (latitudes between 48 and 51°N) persisting even during the colder months (6–8°C) (Garcia-Soto and Pingree, 2009). This northward migration has been shown to be related to long-term changes in temperature, its abundance being positively correlated with the strength of the shelf edge current and negatively with the North Atlantic Oscillation. Therefore, this species has been suggested as a key species to better understand the climate changes effects (Lindley and Daykin, 2005).

Centropages chierchiae is reported to occur in higher abundance between 13 and 20°C and to be absent (or present

in low abundance) below 13°C (Bonnet *et al.*, 2007). In northern Portuguese shelf waters, it is one of the most abundant zooplankters in late summer, when upwelling events take place (Fiúza, 1983), while it occurs with lowest abundance in winter (Morgado *et al.*, 2003; Queiroga *et al.*, 2005; Sobrinho-Gonçalves *et al.*, 2013). A recent study has shown that *C. chierchiae* is widespread in the western Iberian shelf and offshore generally in the upper 75 m (Sobrinho-Gonçalves *et al.*, 2013). It is present all year round although its abundance is lowest in winter (mean 20 ind. m⁻³), it increases in spring and summer (mean 73 and 76 ind. m⁻³, respectively), and peaks in late summer (mean 185 individuals m⁻³). Mean temperature during summer months off western Iberia when *C. chierchiae* peaks and upwelling events take place are usually around 19°C; the values increase to 24°C only on the south Portuguese coast, where upwelling is weaker and less frequent. *Centropages chierchiae* is considered an important prey of commercially important small pelagic fish such as *Engraulis encrasicolus* and *Sardina pilchardus* (Plounevez and Champalbert, 1999; Garrido *et al.*, 2008), inhabiting Iberian Atlantic waters. Only a few studies have focused so far on aspects of the biology of *C. chierchiae*, related to feeding selectivity (Vincent and Hartmann, 2001), distribution variability (Lindley and Daykin, 2005) or thermal niche (Bonnet *et al.*, 2007).

The aim of this study is to understand the physiological state of *C. chierchiae* under different environmental conditions and how this can relate to climate change. With this aim, we investigated the effects of temperature, food quality and quantity on egg production and hatching success (HS), as well as the effect of temperature and gender on metabolic rates. Furthermore, metabolic energy demands were estimated at different temperatures in relation to prey concentration and compared with estimates of daily ration. To our knowledge, this is the first attempt to measure respiration rates and reproductive traits of *C. chierchiae*.

METHOD

Collection and organism cultures

Centropages chierchiae adults used in the reproduction experiments were the progeny of copepods collected off

southwestern Portugal in July 2010. Experiments were conducted in October 2010 most likely with individuals from the second and third generations reared in the laboratory starting from the local population. Although it would be preferable to use wild populations, using the second or third generation acclimated to laboratory conditions may not have significantly impacted our results in terms of genetic modification or adaptations to laboratory conditions. The copepods used to measure respiration rates were captured a few days before the beginning of the experiments in the same area in October 2011. At the time of collection, the temperature was 18°C and the salinity was 35. On both sampling dates, two surface plankton tows were performed using a WP-2 net (0.26 m² mouth opening, 200 µm mesh size) with a transparent plastic bag as a codend for collecting copepods with minor damage. Samples were immediately taken to the laboratory where *C. chierchiae* were sorted from the rest of the plankton and placed in a 30 L tank acclimated at 19°C (Salinity of 33, 14L:10D light/dark regime) with gentle aeration. Before the experiments, copepods were fed daily with a mixture of *Rhodomonas baltica*, *Dunaliella* sp., *Phaeodactylum tricorutum* and *Gymnodinium* sp. given *ad libitum*, while during the reproduction experiments only *P. tricorutum* and *Gymnodinium* sp. were used. Before feeding the copepods, about 30% of the water in the tank was siphoned to maintain water quality (by removing organic debris, reducing ammonia and replenish trace elements) through both 45 and 150 µm sieves to prevent the removal of any eggs, nauplii, copepodites or adults. The content of the 150 µm sieve, mostly copepodites and adults, was put back in the tank while the content of 45 µm sieve, mostly containing faecal pellets, was eliminated. At least once per month, the content retained in the 45 µm sieve was observed under a dissecting microscope in order to separate all the eggs and nauplii and begin a new culture.

Algae cultures were kept in 10 L flasks and grown in 0.2 µm filtered and UV sterilized sea water enriched with F/2 medium, under the same illumination regime and temperature as the copepods. The algae were kept in exponential growth phase by frequent dilution of the culture. Concentrations of algal cultures were estimated using a Sedgwick–Rafter counting chamber.

Reproduction experiments: effect of temperature and food

Experiments to quantify the reproductive rate of *C. chierchiae* consisted in estimating daily egg production rate (EPR) and HS of copepods fed with high and low concentrations of two different algae, *Gymnodinium* sp. (dinoflagellate, size ~ 14 µm) and *Phaeodactylum tricorutum*

(diatom, size 17 × 2.3 µm) at three different temperatures (13, 19 and 24°C) over a 5-day period. The two algae used have a similar size and were chosen because previous feeding experiments showed they were an adequate food for the calanoid copepod *C. chierchiae* consumed with high and similar clearance rates (Garrido *et al.*, 2013). Temperature range was chosen according to the *C. chierchiae* thermal niche (Bonnet *et al.*, 2007). In each temperature treatment, the two algae used as food were given separately at two concentrations (60 and 300 µg C L⁻¹). These concentrations were chosen to mimic the natural range of dinoflagellate and diatom densities typically experienced by *C. chierchiae* during summer and autumn in waters off central western Portuguese coast (Silva *et al.*, 2009). Prior to the experiments, the copepods were starved and acclimated during 24 h to the temperature and experimental conditions. Reproduction parameters were measured using triplicates of 600 ml experimental bottles for each temperature × food type × concentration treatment, with four adult copepods (three females and one male) in each bottle. We tried to select ripe females to use in the experiments. The bottles were sealed with no head space and placed on a rotating (1.5 rpm) plankton wheel. On each experimental day, the water in the bottles was sieved with 150 and 45 µm sieves, in order to count eggs and adult copepods, and to estimate EPR and HS. Adult copepods were immediately transferred to the bottles with renewed 0.2 µm filtered and UV sterilized sea water (to reduce the level of contamination) and the corresponding algae species to continue the experiments. Eggs were placed in petri dishes with fresh water at the same treatment temperature. After 48 or 72 h, depending on the temperature (since hatching time is longer at lower temperatures), the contents were fixed with 4% formaldehyde and nauplii were counted using a stereoscope microscope to determine the percentage of hatched eggs.

Respiration experiments: effect of temperature and sex

Respiration experiments consisted of measuring oxygen consumption rates of individual female and male *Centropages chierchiae* at four different temperatures (8, 13, 19 and 24°C). The lower temperature (8°C) was chosen, assumed to be below copepod's optimal thermal tolerance boundaries. Oxygen consumption (µmol O₂ g⁻¹ WW h⁻¹) measurements were performed according to Pimentel *et al.* (Pimentel *et al.*, 2012) and Rosa *et al.* (Rosa *et al.*, 2012), and entailed an endpoint analysis using methods modified from Thuesen and Childress (Thuesen and Childress, 1993) and Marsh and Manahan (Marsh and Manahan, 1999). *Centropages chierchiae* were incubated

in glass gas-tight 10 ml syringes filled with filtered (0.2 μm) and UV sterilized water. In order to compensate for a probable decrease in dissolved oxygen content in the water, the water used in the incubations was aerated prior to the beginning of each experiment, as recommended by Ikeda *et al.* (Ikeda *et al.*, 2000). Control syringes without animals were run simultaneously, to correct for eventual bacterial respiration. Syringes were placed in temperature controlled water baths (Lauda, Lauda-Königshofen, Germany) at the four different experimental temperatures. For each gender, at each experimental temperature, four syringes were used each one with one copepod in a volume of 2 ml. Water samples were taken from each syringe using a Hamilton gas-tight 500-mL syringe and were injected into a micro-respirometry chamber (MC100 Microcell, Strathkelvin). Oxygen concentrations were recorded with Clarke-type O_2 electrode connected to a multi-channel oxygen interface (Strathkelvin, North Lanarkshire, Scotland).

Duration of individual respiratory runs varied from 4 to 19 h. At the end of each experiment, copepods prosome length was measured. Data on dry weight were calculated according to the equation:

$$\log(\text{DW}) = 2.451 \log(\text{PL}) - 6.103$$

where DW is the dry weight and PL the prosome length (Mauchline, 1998).

For each experiment, temperature dependence (Q_{10}) was determined using the standard equation:

$$Q_{10} = [R(T_2)/R(T_1)]^{10/(T_2-T_1)}$$

where $R(T_2)$ and $R(T_1)$ are the oxygen consumption rates at temperatures T_2 and T_1 , respectively. In order to compare results with previous studies units were converted from $\mu\text{mol O}_2 \text{ g}^{-1} \text{ WW h}^{-1}$ to $\mu\text{L O}_2 \text{ mg}^{-1} \text{ DW h}^{-1}$ using the ideal gas law equation.

Energy requirements calculations

The energy required to maintain the routine metabolic rate estimated at the different experimental temperatures was calculated in terms of prey concentrations of *P. tricornutum* and *Gymnodinium* sp. The energetic values (Kcal day^{-1}) were estimated assuming a 4.7 Kcal $\text{L}^{-1} \text{O}_2$ (Childress and Nygaard, 1973). In order to calculate the calories contained in 1 g of wet weight of *P. tricornutum*, the proximate composition (proteins, lipids and carbohydrates) determined by Fábregas *et al.* (Fábregas *et al.*, 1998) was used. The caloric content of *Gymnodinium* sp. used was twice the *P. tricornutum*, assuming that diatoms contain ca. half the calorific value of dinoflagellates of an

equivalent volume (Hitchcock, 1982). The wet weights of a single cell of *P. tricornutum* and *Gymnodinium* sp. used were 2.86×10^{-10} and 1.8×10^{-8} g based on Raymont and Adams (Raymont and Adams, 1958) and Mansour *et al.* (Mansour *et al.*, 2003), respectively. The number of algae necessary per day to sustain the metabolic requirements of the copepods was then calculated assuming an assimilation efficiency for *P. tricornutum* and *Gymnodinium* sp. of 21.5% (Gaudy, 1974) and 34% (Le Ruyet-Person *et al.*, 1975), respectively. To calculate the required food intake ($\mu\text{g C day}^{-1}$) and daily ration (% body carbon), we used a carbon content of 1.5×10^{-5} and 2.2×10^{-4} $\mu\text{g C}$ for *P. tricornutum* and *Gymnodinium* sp., respectively. Carbon content was estimated using equations given in Smayda (Smayda, 1978) for phytoplankton. The carbon content of each copepod was calculated using the formula $\text{CC} = 10^{(2.4492 \log \text{PL} - 6.0984)} \times 0.417$ (Halvorsen *et al.*, 2001) where PL is the prosome length of copepods used in the experiments.

Statistical analysis

A multiway analysis of variance (multiway ANOVA) was used to study the effect of temperature, alga species and alga concentration on the reproduction parameters, EPR and HS. Differences between oxygen consumption for the experiments conducted at the four different temperatures and for both sexes were tested with a two-way ANOVA. When significant differences were found, a Tukey's honestly significant difference *post hoc* test was used for pair-wise comparisons of treatment means. Statistical analyses were carried out using the open source software R 2.14 (R Development Core Team, 2009) and the significance level was set at $\alpha = 0.05$.

RESULTS

Effects of temperature and food on reproduction

The EPR of *Centropages chierchiae* showed significant differences between all the temperatures tested (Table I, Tukey HSD; $P < 0.05$). EPR at 13°C was very low for copepods fed on both phytoplankton species and at both concentrations, decreasing from 4.5 ± 1.8 and 7.6 ± 8.9 to 0 eggs $\text{female}^{-1} \text{day}^{-1}$ on days 3 and 4 with *Gymnodinium* sp. and *P. tricornutum*, respectively. The EPR was higher at the other two temperatures (19 and 24°C) analysed (Fig. 1). Food type and concentration significantly influenced EPR (Table I), with *P. tricornutum* and higher algae concentration inducing a higher production. EPR values reached a maximum of 36 ± 4 and 29 ± 14 eggs

Table I: Results of a three-way ANOVA performed to test for temperature, algae species and concentration effects on the egg production rates and hatching success of Centropages chierchiae

Variable	Factor	df	F	P-value
EPR	Temp	2	205.860	<0.001
	Alga	1	21.460	<0.001
	Conc	1	57.509	<0.001
	Temp:Alga	2	4.050	0.02
	Temp:Conc	2	13.896	<0.001
	Alga:Conc	1	0.710	0.40
	Temp:Alga:Conc	2	0.060	0.94
HS	Temp	2	92.471	<0.001
	Alga	1	15.249	<0.001
	Conc	1	0.090	0.77
	Temp:Alga	2	6.272	0.01
	Temp:Conc	2	1.849	0.18
	Alga:Conc	1	1.430	0.23
	Temp:Alga:Conc	2	0.380	0.54

Significant values are given in bold. EPR, egg production rate (eggs female⁻¹ day⁻¹); HS, hatching success (%); Temp, temperature; Alga, food type; Conc, food concentration.

female⁻¹ day⁻¹ with *P. tricorutum* and *Gymnodinium* sp., respectively, at 19°C and 300 µg C L⁻¹ (Fig. 1). Although EPR varied through the experiments, there was a tendency to be higher at 19°C and high prey concentrations. HS was significantly different between the three different temperatures (Table I), specifically between 13–19°C and 13–24°C (Tukey HSD; *P* < 0.05). In fact, the percentage of nauplii hatched was reduced at lower temperatures (13°C; ranging from 21 to 52%), and it increased significantly at higher temperatures (between 55–95% at 19 and 24°C) (Fig. 2). Food type significantly influenced HS (Table I), with higher values obtained for copepods fed on *Gymnodinium* sp. (Fig. 2). When exposed to *P. tricorutum*, HS showed a decrease throughout the duration of the experiments at 19 and 24°C (Fig. 2).

Effects of temperature and gender on respiration

There was a significant relationship between respiration rates and temperature for both sexes of *C. chierchiae*, which was best fitted with an exponential function (*R*² = 0.83 and *R*² = 0.87 for females and males, respectively) (Fig. 3). Two-way ANOVA analysis showed that temperature significantly influenced the respiration rates while the gender did not (Table II). Oxygen consumption ranged between 2.7 ± 1.0 and 2.8 ± 0.9 µL O₂ mg⁻¹ DW h⁻¹ for females and males, respectively, at 8°C and 13.1 ± 3.5 and 15.3 ± 2.9 µL O₂ mg⁻¹ DW h⁻¹ at 24°C (for an average body dry weights of 32.4 µg for females and 31.1 µg for males; Table III). The Q₁₀

values varied with temperature intervals and genders (females: Q₁₀ [8–13°C] = 2.5; Q₁₀ [13–19°C] = 3.4; Q₁₀ [19–24°C] = 2.2; males: Q₁₀ [8–13°C] = 3.9; Q₁₀ [13–19°C] = 1.8; Q₁₀ [19–24°C] = 3.7); yet when considering the entire range of temperature (8–24°C), the thermal sensitivity values were 2.7 and 2.9 for females and males, respectively.

Energy requirements

The total carbon demands needed to assure growth, molting or reproduction are usually 5–10 times higher than the minimal requirements to maintain the basal metabolic rate (e.g. Perissinotto *et al.*, 1997; Gaudy and Thibault-Botha, 2007). Figure 4 compares the daily ration estimated in complementary experiments (white boxes; Garrido *et al.*, 2013), with the 10-folded daily ration obtained in the present work (grey boxes), showing that average daily ration obtained in the experiments was higher for all temperatures except for 24°C. The energy needed to sustain minimal respiratory metabolic expenditure increased with temperature (Table III). In order to achieve the respiratory requirements of *C. chierchiae*, the minimal food intake on *P. tricorutum* ranged on average from 0.4 to 1.8 µg C and *Gymnodinium* sp. from 0.03 to 0.13 µg C, which were equivalent to a range of 2.78 to 14.72% and 0.21 to 1.09% of the copepods body carbon, respectively (Table III).

DISCUSSION

Effects of temperature on reproduction

Temperature significantly affected the fecundity of *C. chierchiae* females. The egg production was higher at 19°C, intermediate at 24°C and lower at 13°C, in agreement with maximum feeding rates found at 19°C (Garrido *et al.*, 2013). The low productivity observed at 13°C might explain the fact that this temperature determines the lower limit of optimal conditions for the distribution of this species. The higher production rate found at 19°C agrees with the maximum abundance of *C. chierchiae* recorded off western Iberia during warmer months (Sobrinho-Gonçalves *et al.*, 2013) when upwelling events provide rich food environments likely favourable for the reproduction of this species. The fact that *C. chierchiae* was able to exceed its northern limit of distribution in recent years seems to challenge the present results of reproductive performance at lower temperatures (13°C). Lindley and Daykin (Lindley and Daykin, 2005) suggested that by the late 1990's, *C. chierchiae* had developed a resident population in the Celtic Sea and English

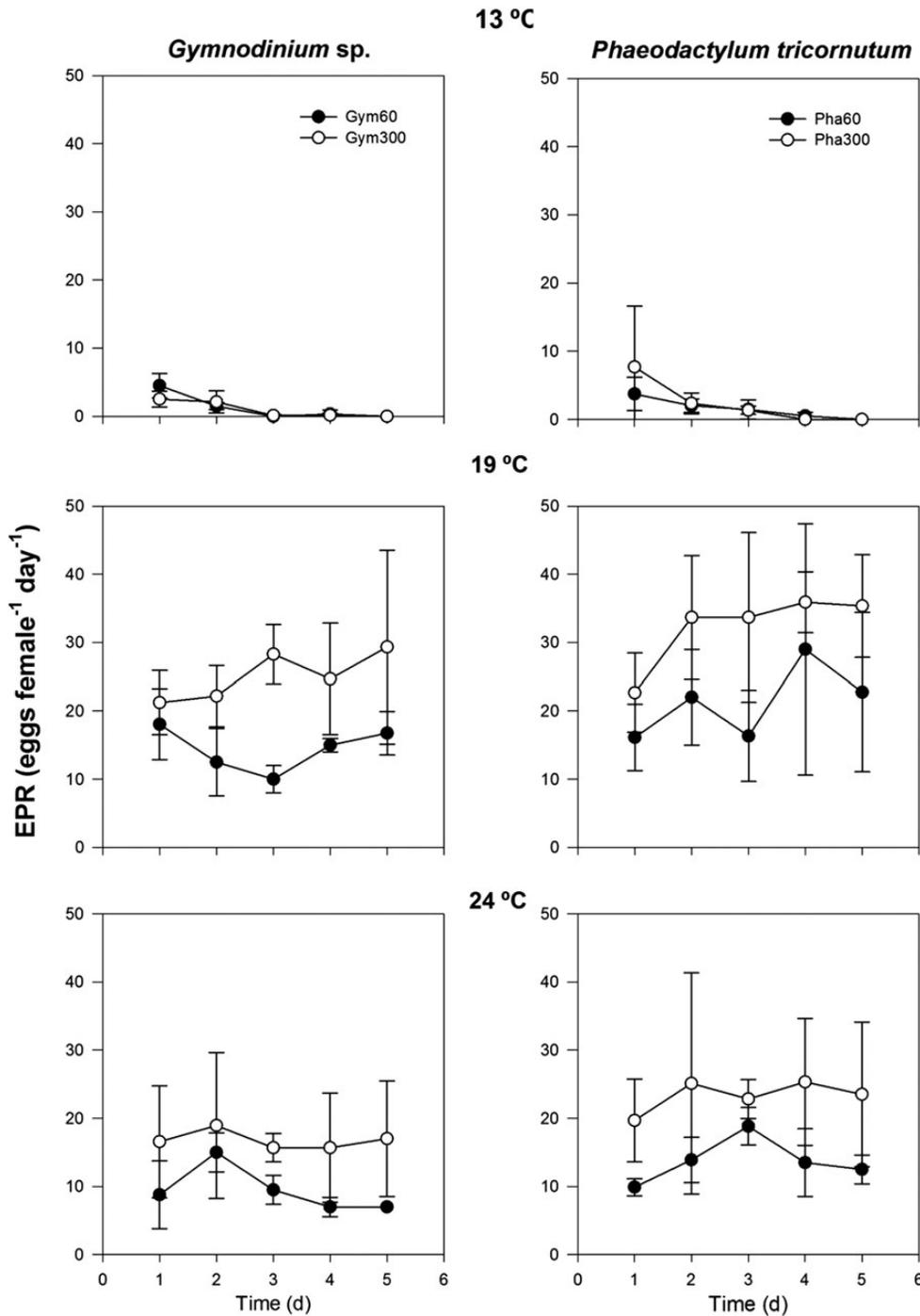


Fig. 1. Egg production rates (EPR, eggs female⁻¹ day⁻¹) of *Centropages chierchiae* at different temperatures fed with two algae, *Gymnodinium sp.* and *Phaeodactylum tricornutum*, during 5 days; black circles are for algae concentration of 60 µg C L⁻¹ and white of 300 µg C L⁻¹. Error bars indicate standard deviation.

Channel and the significant correlations between this species abundance and the minimum temperature also suggest that it persists in the plankton instead of producing over-wintering eggs. However, in this area, higher

abundances of this species are only found in summer when sea water temperature is generally higher than 13°C, reaching values around 16–18°C (García-Soto and Pingree, 2009). Also, the population occurring in

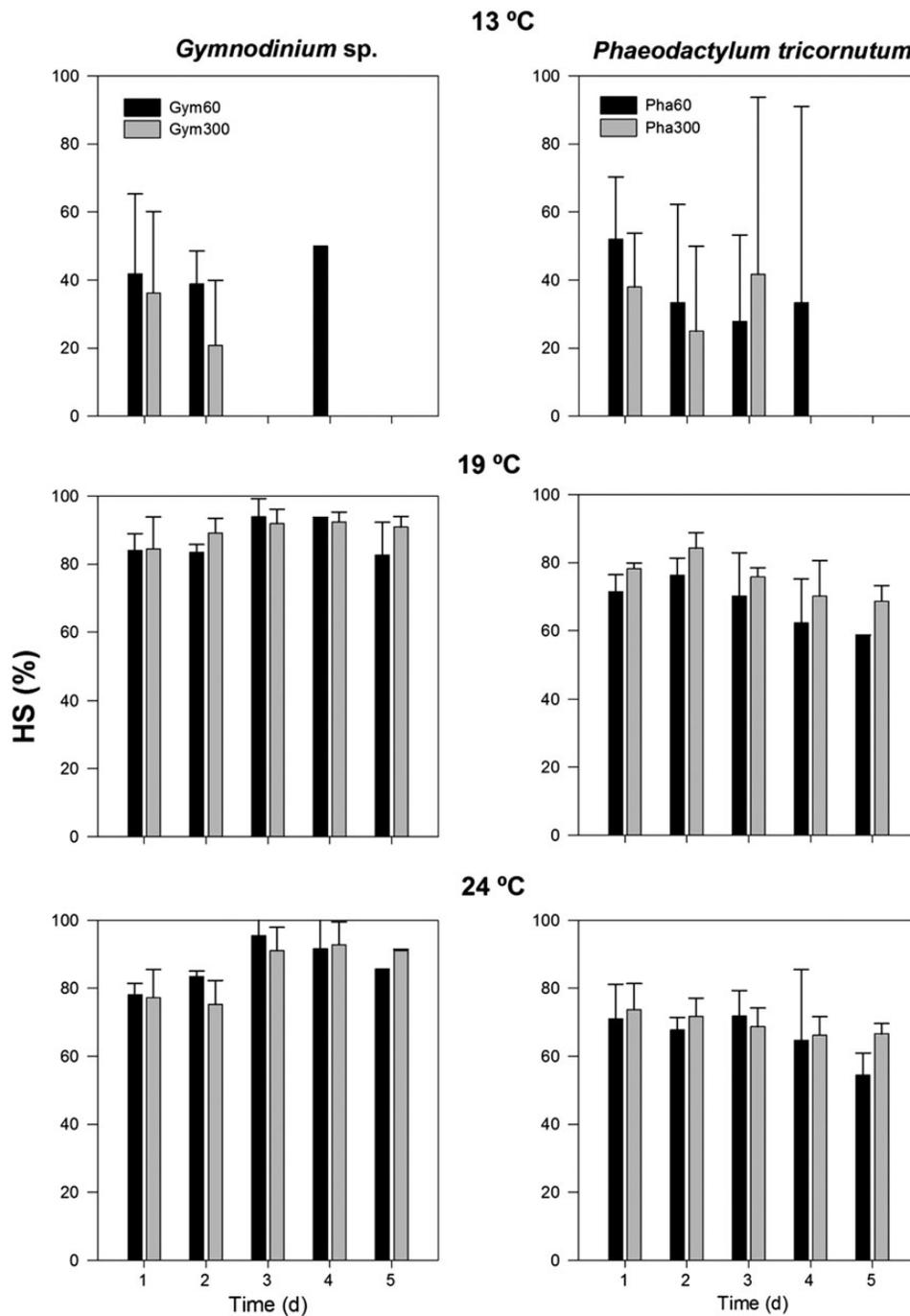


Fig. 2. Hatching success (HS, %) of *Centropages chierchiae* at different temperatures fed with two algae, *Gymnodinium sp.* and *Phaeodactylum tricornutum*, during 5 days; black bars are for algae concentration of 60 µg C L⁻¹ and grey for 300 µg C L⁻¹. Error bars indicate standard deviation.

northern latitudes may have developed adaptation mechanisms to live permanently at lower temperatures that are sub-optimal for southern populations. The congeneric *Centropages typicus* is a very common species that occurs in the Mediterranean Sea and North Atlantic Ocean and is one of the most studied species of calanoid

copepods. The seasonality of the abundance of both species is very similar in the English Channel and Celtic Sea (Lindley and Reid, 2002). Previous studies of *C. typicus* have shown that temperature significantly affects egg production. Smith and Lane (Smith and Lane, 1985) observed that EPR was higher at 15°C than at 10°C,

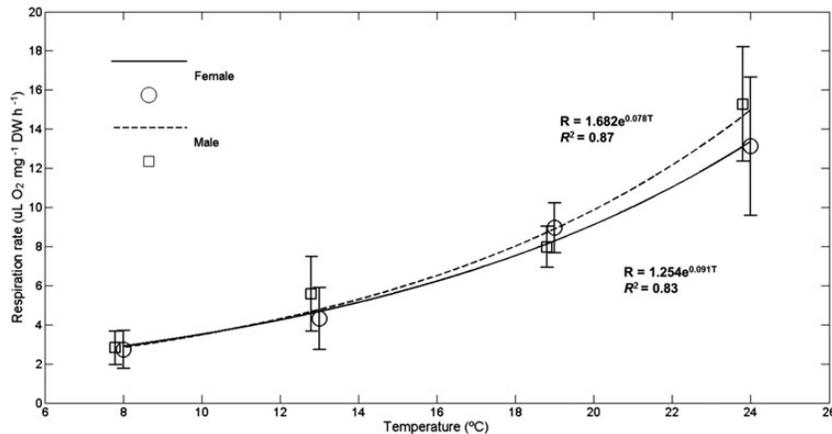


Fig. 3. Variation of mean respiration rates of *Centropages chierchiae* males (squares) and females (circles) for different temperatures. Each point represents the mean value of replicates and error bars indicate standard deviation. The dashed line correspond to the fitted exponential function for males and the continuous for females, in equations T is the temperature (°C) and R is the respiration rate ($\mu\text{L O}_2 \text{ mg}^{-1} \text{ DW h}^{-1}$).

Table II: Results of a two-way ANOVA performed to test for temperature and sex effects on the oxygen rates of Centropages chierchiae

Variable	Factor	df	F	P-value
Oxygen rate	Temp	3	50.363	<0.001
	Sex	1	0.812	0.377
	Temp:Sex	3	0.944	0.435

Significant values are given in bold. Temp, temperature.

which were the only temperatures tested. On the other hand, experiments conducted by Carlotti *et al.* (Carlotti *et al.*, 1997) on the same species revealed that there were no differences of EPR between 15 and 20°C. Halsband-Lenk *et al.* (Halsband-Lenk *et al.*, 2002) compared two distinct populations of *C. typicus* from the North Sea and Mediterranean Sea and found that the optimal temperature for spawning was 20°C, but the lowest temperature at which females were able to produce eggs differed between the two populations, given that *C. typicus* from the North Sea were capable of spawning at 2°C and Mediterranean population only started to produce eggs at 5°C. In order to verify if the same could happen with *C. chierchiae*, comparative studies on the reproductive behavior between populations from northern and southern limits of its distribution are necessary.

Egg HS of copepods is temperature dependent (Holste and Peck, 2006; Hansen *et al.* 2010). Our results showed that HS in *C. chierchiae* was significantly lower at 13°C than at 19 and 24°C. The time used in the 13°C experiment (72 h) until all nauplii were counted following the work of Holste and Peck (Holste and Peck, 2006), which suggests a low probability of copepods requiring more

time to hatch. Smith and Lane (Smith and Lane, 1985) showed that 95% of *C. typicus* eggs hatched within 48 h at 15°C but only 8% at 10°C, with most eggs hatched within 72 h. The lack of remating can also be ruled out since males were always added to the experimental bottles. Another hypothesis would be the production of resting eggs but Marcus (Marcus, 1996) and Lindley and Daykin (Lindley and Daykin, 2005) did not observe any resting egg production in *C. chierchiae*. Ianora *et al.* (Ianora *et al.*, 1992) found no relationship between temperature and HS when studying the copepod *C. typicus* in the field. Regarding other calanoid genera such as the widespread *Acartia*, it has been shown that HS is highly influenced by temperature, and that no eggs are produced at temperatures below 10°C (Castro-Longoria, 2003). Even though the underlying causes of embryonic death, when the development occurs under sub optimal conditions, are not known, decreased membrane permeability, disequilibria of coupled enzyme reactions and limits imposed by kinetics and inactivation of enzyme proteins could be some of the responsible mechanisms (Rosa *et al.*, 2012).

Effects of food on reproduction

Centropages chierchiae had high feeding rates when both *P. tricornutum* and *Gymnodinium* sp. were offered as mono-specific diets in the feeding experiments conducted by Garrido *et al.* (Garrido *et al.*, 2013). Here, a *P. tricornutum* diet induced higher EPR in *C. chierchiae* females than a *Gymnodinium* sp. diet, especially at higher cell concentrations. Similarly, *Centropages typicus* produced significantly more eggs when fed on a high density diet of *P. tricornutum* compared with low concentrations of the same alga (Razouls, 1981). EPR of *C. typicus* increases with food

Table III: Results of respiration rates experiments for the temperatures tested

Sex	Temp (°C)	n	Dry weight (µg)	Oxygen consumption (µlO ₂ mgDW ⁻¹ h ⁻¹)	<i>Phaeodactylum tricornutum</i>		<i>Gymnodinium</i> sp.	
					(µgCday ⁻¹)	% Body Carbon	(µgCday ⁻¹)	% Body Carbon
F	8	5	34.1 ± 3.6	2.73 ± 0.96	0.40 ± 0.10	2.78 ± 0.97	0.033 ± 0.011	0.24 ± 0.09
	13	5	32.8 ± 6.2	4.31 ± 1.59	0.64 ± 0.25	4.31 ± 1.59	0.051 ± 0.017	0.38 ± 0.17
	19	4	30.2 ± 1.8	8.95 ± 1.29	1.13 ± 0.22	8.77 ± 1.26	0.083 ± 0.016	0.65 ± 0.09
	24	5	30.4 ± 3.2	13.13 ± 3.54	1.61 ± 0.53	12.65 ± 3.41	0.133 ± 0.046	1.02 ± 0.29
M	8	4	33.4 ± 1.5	2.81 ± 0.87	0.41 ± 0.13	2.86 ± 0.88	0.030 ± 0.010	0.21 ± 0.07
	13	4	31.7 ± 5.7	5.58 ± 1.90	0.73 ± 0.23	5.58 ± 1.90	0.054 ± 0.017	0.41 ± 0.14
	19	4	31.0 ± 3.5	7.98 ± 1.05	1.02 ± 0.05	7.81 ± 1.03	0.075 ± 0.003	0.58 ± 0.08
	24	4	29.1 ± 3.2	15.23 ± 2.93	1.80 ± 0.32	14.72 ± 2.82	0.133 ± 0.023	1.09 ± 0.21

F, females; M, males; Temp, temperature; n, number of replicates; showing mean values and standard deviation of copepods dry weight (µg), oxygen consumption (µlO₂ mg⁻¹DW h⁻¹) and food intake (µgC day⁻¹) and daily ration (% body carbon) for *Phaeodactylum tricornutum* and *Gymnodinium* sp.

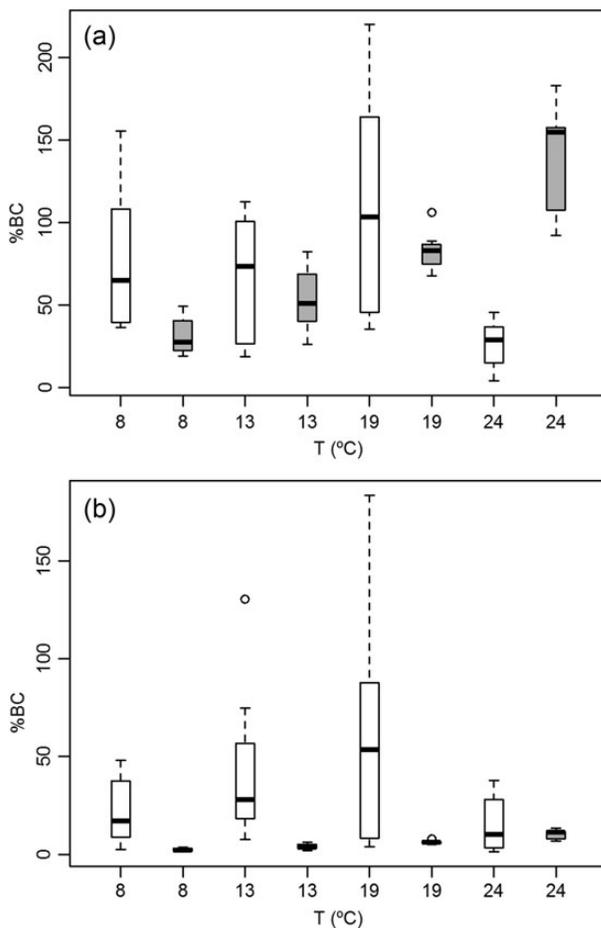


Fig. 4. Box plots of the daily ration of *Centropages chierchiae* (expressed as percentage of body carbon) at the four different temperatures analysed using (a) *Phaeodactylum tricornutum* and (b) *Gymnodinium* sp.; white box plots are for feeding rate experiments results (Garrido *et al.*, 2013) and black for present work.

concentration, although there is a maximum concentration above which egg production remains constant or even decreases (Smith and Lane, 1985; Nival *et al.*, 1990). Moreover, Gaudy (Gaudy, 1971) found that the egg

production of *C. typicus* fed on several diatoms species, including *P. tricornutum* (~80 eggs female⁻¹ day⁻¹), was higher than when fed on a flagellate-based diet. On the other hand, Miralto *et al.* (Miralto *et al.*, 1995) found higher EPRs when *C. typicus* fed on *Thalassiosira rotula* (diatom) and *Gonyaulax polyedra* (dinoflagellate), around 50 eggs female⁻¹ day⁻¹, rather than *P. tricornutum* on which females spawned on average 28 eggs female⁻¹ day⁻¹. Fatty acids as an indicator of nutritional quality of phytoplankton, as well as nutritional components such as vitamins and amino acids, were not analyzed in the present work but may explain the differences on EPR achieved with the two algae.

Although EPR was higher when *C. chierchiae* fed on *P. tricornutum*, HS was found to be lower compared with *Gymnodinium* sp., indicating that these two processes may have different nutritional requirements for their success. Miralto *et al.* (Miralto *et al.*, 1995) found that HS of *C. typicus* was higher using two species of dinoflagellates as prey rather than diatom-based diets including *P. tricornutum*. The authors suggested a blockage of egg development due to the presence of intracellular chemical compounds in diatoms suggesting bottom-up control of recruitment in *C. typicus* populations. Miralto *et al.* (Miralto *et al.*, 1999) reported the presence of three aldehyde compounds in diatoms as the cause of blocked embryonic development in copepods. Nevertheless, a study in which several diatoms were tested including *P. tricornutum* revealed that this alga does not produce these deleterious compounds (Wichard *et al.*, 2005). Egg viability was also lower when a *P. tricornutum* diet was given to several other calanoid species (Lee *et al.*, 1999; Lacoste *et al.*, 2001; Shin *et al.*, 2003). In fact, *P. tricornutum* induced 100% blockage in hatching within 10 days in *C. helgolandicus* (Chaudron *et al.*, 1996; Laabir *et al.*, 1999). In the present work, the 5-day experiments were not designed to detect long-term effects and therefore such low results were not observed. On the other hand, Jónasdóttir and Kiørboe (Jónasdóttir and Kiørboe, 1996)

have suggested that high HS is achieved when eggs receive a favourable balance of required nutrients. They showed particularly low and rapid decline of the viability of *Acartia tonsa* eggs with *P. tricornutum* diets, reporting a positive correlation between hatching and fatty acid composition of prey. Furthermore, Shin *et al.* (Shin *et al.*, 2003) showed that egg viability of *Acartia omorii* declined with a *P. tricornutum* diet, suggesting that changes in egg viability are more closely related to the fatty acid composition of the eggs than to the production of reactive aldehydes blocking the development of copepod embryos proposed by other authors (Miralto *et al.*, 1999).

Nival *et al.* (Nival *et al.*, 1990) proposed that *C. typicus* needs to feed on other particles (e.g. detritus or small metazoa) besides phytoplankton cells to achieve the maximal reproductive potential, because it is not able to attain maximum production at high levels of chlorophyll. In fact, Bonnet and Carlotti (Bonnet and Carlotti, 2001) suggested that omnivory may be the best feeding strategy as mixed diets induced higher egg production. Including a different prey such as ciliates in future experiments would help to investigate a possible enhancing of *C. chierchiae* reproductive potential.

Effects of temperature and gender on respiration rates

Respiration rates of *C. chierchiae* increased with temperature following an exponential function which suggests a low capability to adapt to short-term temperature fluctuations. Most copepods show higher metabolic rates as temperature increases. However, some estuarine species can regulate their physiological activity over a wider temperature range in order to adapt to rapid temperature fluctuations (e.g. Gaudy *et al.*, 2000). Hiromi *et al.* (Hiromi *et al.*, 1988) also found no effect of temperature on respiration of the ubiquitous species *Oithona davisae* over a wide temperature range. Our results agree with results on respiration for *C. typicus* (Table IV). The Q_{10} estimated for

C. chierchiae in this study were highly variable, and for the entire temperature range (8–24°C), 2.7 and 2.9 for females and males, respectively. Similarly, Nival *et al.* (Nival *et al.*, 1974) observed an increase of *C. typicus* respiration off the Moroccan coast in the range from 13 to 23°C with a Q_{10} value of 2.9.

Respiration rates were not influenced by gender in *C. chierchiae*. Previous studies were not conclusive regarding the influence of sex on metabolism in *Centropages* species. Similar to the present study, no differences were found in *C. typicus* between male and female respiration rates (Champalbert and Gaudy, 1972). Yet, Fernandez (Fernandez, 1978) detected small differences with slightly higher values for females (3.095–6.023 $\mu\text{O}_2 \text{ mg}^{-1} \text{ DW h}^{-1}$ for females and 2.874–5.103 $\mu\text{O}_2 \text{ mg}^{-1} \text{ DW h}^{-1}$ for males; T: 10–20°C). Also, Marshall and Orr (Marshall and Orr, 1966) found significantly higher values of female respiration rates of *Centropages hamatus* compared with males, with values two times higher for females than for males. Even though females were larger than males (females: $1545.6 \pm 80.9 \mu\text{m}$, males: $1507.2 \pm 49.7 \mu\text{m}$), the difference in body mass between both genders are probably small enough not to cause differences of individual metabolic rates, which can explain the similar respiration rates found in this work.

Energy requirements

Respiration rates obtained in this work are the minimum carbon requirement of *C. chierchiae* given that copepods were in low activity and with no food during the experiments. Therefore, the food intake and daily ration estimated here are the values necessary to maintain the standard metabolism for the different temperatures tested. The average values of 10-fold minimum daily ration required were lower than the average daily ration estimated from feeding experiments for both algae (Garrido *et al.*, 2013), except at 24°C results, meaning that food intake is usually in excess in terms of the

Table IV: Published data on the respiration rates of *Centropages typicus* including the present results for *Centropages chierchiae*

Temperature (°C)	Sex	Respiration rate ($\mu\text{L O}_2 \text{ mg DW}^{-1} \text{ h}^{-1}$)	Location	References
8, 13, 19, 24	F	2.7, 4.3, 8.9, 13.1	Portuguese West coast	Present work (<i>Centropages chierchiae</i>)
	M	2.8, 5.6, 8.0, 15.2		
15	F	8.34	Woods Hole	Raymont, 1959
7–9	F	3.1	Gulf of Maine	Conover and Corner, 1968
10, 14, 18, 21, 24	F	0.9, 1.2, 1.3, 3.4, 3.3	Moroccan coast	Champalbert and Gaudy, 1972
13, 15, 18, 20, 23	F	7.7, 9.7, 11.7, 13.8, 18.4	Moroccan coast	Nival <i>et al.</i> , 1974
10, 14, 18, 20, 22	F	7.7, 9.7, 11.7, 13.8, 18.4	Marseilles	Gaudy, 1973
10, 15, 20	F	3.1, 5.2, 6.0	Barcelona	Fernandez, 1978
	M	2.9, 4.6, 5.1		

energetic requirements. This means that algae concentrations provided in the feeding experiments are able to fulfill the total energetic requirements of *C. chierchiae* over the entire temperature range except for the highest temperatures. Average values of daily ration obtained at 24°C do not seem to fulfill the energetic requirements. We were also able to compare the present findings with values of phytoplankton densities obtained in nature at a station located in the west Portuguese coast, area where *C. chierchiae* is found in higher abundances (Silva *et al.*, 2009). Dinoflagellates and diatoms concentrations were determined for every season and the genera *Gymnodinium* was considered highly abundant during the summer period, the same as for *C. chierchiae*. On average, dinoflagellate concentration was 28×10^4 cells L⁻¹. Assuming such a concentration of *Gymnodinium* sp., corresponding biomass of this species during this season would be 61 µg C L⁻¹. Making the same assumption, the 10-fold minimum carbon food intake would be 0.8 ± 0.1 µg C day⁻¹ while the average food ingested during the experiments was 12.2 ± 3.3 µg C day⁻¹ (Garrido *et al.*, 2013), when *Gymnodinium* sp. is in concentrations similar to the one found by Silva *et al.* (Silva *et al.*, 2009), and for 19°C (temperature more often registered during this season and area). This means that the ingested food, when *C. chierchiae* is exposed to a concentration of *Gymnodinium* sp. around 61 µg C L⁻¹, is higher than the food intake needed. Probably during the periods when this temperature occurs, dinoflagellates such as *Gymnodinium* sp. are sufficient to maintain *C. chierchiae* metabolism. Nonetheless, it seems that this dinoflagellate species is not the most efficient to guarantee a good production of *C. chierchiae*, as it was shown in the reproduction experiments.

Centropages chierchiae as a climate change indicator

The temperature range (8–24°C) used in this study had a significant effect on the reproduction and respiration rates of *C. chierchiae*. The fact that the reproductive response was highly temperature dependent may be indicative that the poleward movement of this copepod species is linked to climate change. More specifically, we suggest that such extended distribution is related to the ocean warming that has been occurring, in the northern European seas, for the past decades (MacKenzie and Schiedek, 2007). Moreover, in a future warming scenario, *C. chierchiae* will probably become more abundant in northern latitudes, while in the Iberian Peninsula coast will suffer a shift in its production time. It is worth noting that this warming effect may correspond to maximum

temperature tested in this study (24°C). As a consequence of the potential temporal population displacement, there may be a mismatch between *C. chierchiae* and their predators or prey. Although the thermal sensitivity data (Q_{10}) are within the normal temperature effects on metabolism, the projected near future warming (24°C) will require *C. chierchiae* to consume more food per unit body size and feeding may be critical since this genus shows high metabolic rates and low levels of metabolic reserves (Dagg, 1977). At low temperatures, food intake always exceeded the metabolic requirements for this species, which probably explains the northern shift of this species, even though EPRs were reduced when compared with higher temperatures. Based on the extremely low rates found at 13°C, one may argue that the northern populations have probably adapted to colder temperatures, involving different life history traits and thermal stress tolerance. It would be of great interest to compare both *C. chierchiae* populations from the Iberian Peninsula and northern areas, in terms of their respiration physiology, reproductive biology and feeding ecology, in order to understand the phenotypic acclimation processes of this copepod species.

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