

Fecundity and development of the benthopelagic copepod *Pseudocyclops umbraticus*: effects of temperature

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ABSTRACT: The shallow-living, benthopelagic copepod species *Pseudocyclops umbraticus* was collected seasonally during nighttime in the brackish waters of Lake Faro (north-eastern Sicily). It showed marked seasonal fluctuations in population abundance, with maximum numbers occurring in summer. In the laboratory, mean daily egg production rates (EPR) of *P. umbraticus* were positively correlated with temperature, with values ranging from (mean \pm SD) 2.3 ± 6.3 eggs female⁻¹ d⁻¹ at 14°C to 6.1 ± 10.2 eggs female⁻¹ d⁻¹ at 24°C. In terms of total egg production over the entire female lifespan, maximum values occurred at 14°C and minimum at 24°C. At 12 and 32°C, females were able to survive, but they stopped egg production. Temperature also dramatically affected female copepod lifespan, which was shorter at higher temperatures. Development time for the eggs decreased with increasing temperature, as did development time from egg to adulthood. Recruitment rate was low at lower temperatures. The distinctive egg-laying behaviour of *P. umbraticus* is described here for the first time. The EPR in *P. umbraticus* is relatively low compared to other pelagic copepods, but within the ranges reported for egg-carrying species. Greater fecundity at higher temperatures compared to other subtemperate species indicates that *P. umbraticus* is well adapted to the higher temperatures of coastal brackish basins, where it contributes to the biodiversity of the plankton community at night as it migrates from the substratum into the water column.

KEY WORDS: Temperature · Egg production rate · Parental care · *Pseudocyclops umbraticus* · Hyperbenthic habitat

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INTRODUCTION

Calanoid copepods are one of the most important constituents of marine pelagic ecosystems, as they are grazers on phytoplankton and prey for animals at higher trophic levels (e.g. fish, Mauchline 1998). The benthopelagic copepods are a rare but interesting component of the calanoids, comprising the most basal species known in this order (Huys & Boxshall 1991). In addition, the benthopelagic calanoids have been referred to in many studies as demersal, benthoplanktonic and hyperbenthic because of their

habitat, which is at and around the sediment–water interface.

Benthopelagic calanoids are only present in the zooplankton community at night (Zagami & Brugnano 2013), which confirms the hypothesis of their circadian vertical migration between the bottom and the water column (Damkaer 1970, Alldredge & King 1985, Ohtsuka 1985, Jacoby & Greenwood 1988, Campolmi et al. 2002). Measurements of the life-history parameters of these copepods, such as development and egg production, will therefore provide useful information about energy transformation in

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pelagic food webs during the night. Benthopelagic coupling of calanoids occurs mostly in autumn, resulting in increased abundance and, in some Mediterranean coastal brackish water environments such as Stagnone of Marsala and Lake Faro (Italy), a marked contribution to functional diversity (Campolmi et al. 2002, Zagami & Brugnano 2013).

The life-history parameters of rare benthopelagic copepods can be difficult to determine from field population data, because of the inadequacy of traditional zooplankton sampling methods in capturing them in their daytime habitat. Therefore, laboratory rearing is often the method used for these analyses. Life-history traits have only been studied in the field for *Pseudocyclops xiphophorus* (Brugnano et al. 2009), which was first recorded in the Mediterranean Sea in Lake Faro (Zagami et al. 2005), as its daytime habitat in the fouling material made it easily available with manual sampling methods.

To date, many experimental studies have shown that water temperature and food conditions are the most important factors affecting the life-history parameters of copepods (see Ban 1994, and references therein). However, egg production and development times can differ between closely related genera, within a genus, and even within the same species (Corkett & McLaren 1978). This is unexpected, because of the general relationship that exists between temperature and metabolic activity.

Pseudocyclops umbraticus is a benthopelagic calanoid (size range 0.6 to 0.7 mm) that lives on the nearshore bottom-water substrate interface of Lake Faro. Its spatial distribution is determined mainly by the substratum rather than by any physical para-

meters (Zagami & Brugnano 2013). Temperature is the only important environmental factor that affects the seasonal abundance pattern of *P. umbraticus*, although potentially in an indirect manner, as higher temperatures induce lower oxygen concentrations at the lake bottom, therefore restricting the available habitat (Zagami & Brugnano 2013).

In the present study, egg production rates (EPR), development and egg-laying behaviours of *P. umbraticus* were studied in the laboratory at different temperatures under food-satiated conditions. The findings were then considered in relation to the natural seasonal fluctuations that occur in the population abundances of *P. umbraticus* in Lake Faro, and also in relation to what is known of the temperature effects and reproductive patterns of the congener species *P. xiphophorus*, which is present in the same environment although it lives on a different substrate (biological material on ropes suspended in the oxygenated layer, and mooring posts all around the lake).

MATERIALS AND METHODS

Study area

Lake Faro is a coastal basin located in the north-eastern tip of Sicily (surface area 263 600 m², max. depth 29 m) (Fig. 1). It has the typical features of a meromictic basin; i.e. an oxic epilimnion (surface to 10–12 m in depth) and an anoxic hypolimnion (15 m to the lake bottom). It is characterised by large fluctuations in its physico-chemical parameters, especially temperature (10 to 28°C), salinity (34 to 37) and dis-

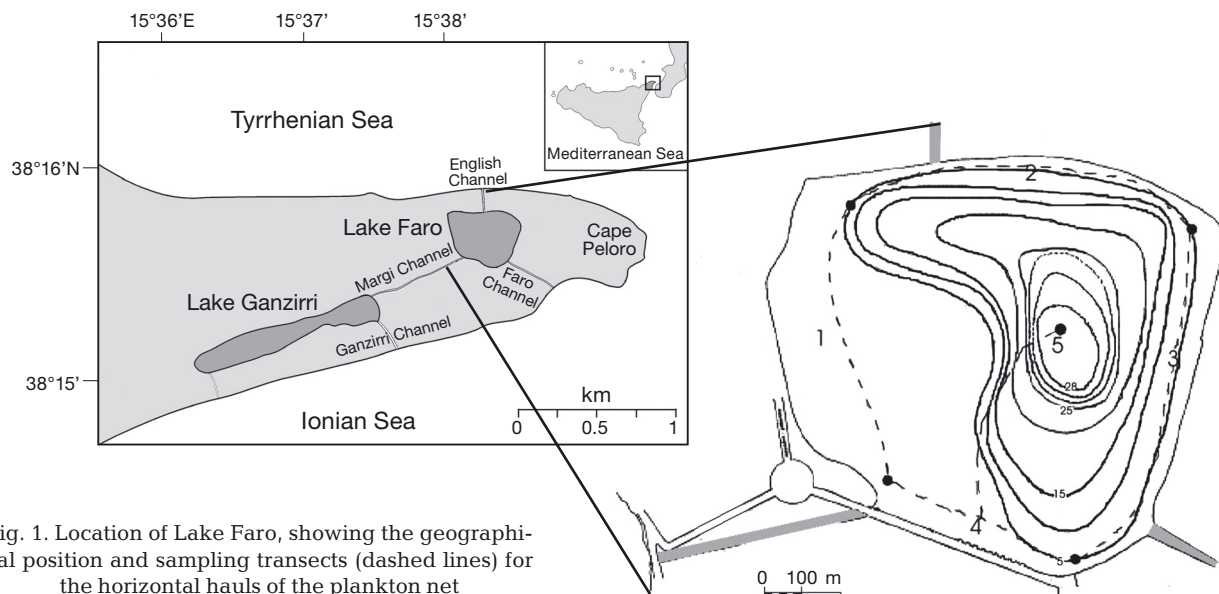


Fig. 1. Location of Lake Faro, showing the geographical position and sampling transects (dashed lines) for the horizontal hauls of the plankton net

solved oxygen (absent near the lake bottom in the central area; 8.3 mg l^{-1} at the surface along the shore). These layers are separated by a metalimnion, within which strong blooms of anoxygenic phototrophic bacteria result in the periodic development of a red water layer (Genovese 1963, Trüpper & Genovese 1968). This physical and chemical stratification is particularly sharp in summer, when the anoxia can typically extend up to the lower mixolimnion, and conspicuous blooms of photosynthetic sulphur bacteria develop. The main component of these blooms has been identified as *Chlorobium phaeovibrioides* (Sorokin & Donato 1975), which is a well-characterised member of the *Chlorobiaceae* that is highly adapted to low light intensities. Lake Faro is connected via a shallow channel to the Straits of Messina, which separate the island of Sicily from the Italian peninsula. Another channel that is silted up for most of the year is artificially opened for a few days during the hottest summer period (June through August), thus establishing communication with the Tyrrhenian Sea to allow better water circulation into the lake.

On the 3 sides of Lake Faro that correspond to Transects 1, 2 and 4 (Fig. 1), there are great masses of the chlorophyte alga *Chaetomorpha linum* (O. F. Müller) Kützinger, and the bottom is muddy and rich in organic detritus. Along Transect 3 of Fig. 1, the lake bottom consists of pebbles and gravel and is covered by *Cymodocea nodosa* (Ucria) Ascherson and *Ulva laetevirens* Areschoug. In the central area of the lake and associated with the anoxic lake-bottom waters, the 'fouling' material is the only substrate type in the oxygenated stratum. This material is comprised of benthic organisms, such as ascidiaceans (*Styela plicata*, *Ciona intestinalis*, and other unidentified species), polychaetes and bryozoans that are attached to submerged ropes, in which a harpacticoid, cyclopoid and hyperbenthic calanoid copepod community lives.

Sampling methods and laboratory experiments

In May, June and November 2004, and in January and April 2005, zooplankton samples were collected from Lake Faro during the day and at night along 5 transects (Fig. 1): 4 along the sides of the lake, and the fifth into the centre of the lake. All of the samples were collected using a rectangular mouth plankton net (mouth area 0.18 m^2 , mesh size $200 \mu\text{m}$) equipped with a flowmeter (Hydrobios). Along the shallow shorelines of the lake (max. depth 1 to 2 m), the net was towed at a few mm from the lake bottom, while in the central area of the lake, it was towed horizon-

tally 2 to 3 m below the water surface. Contemporaneous to each sampling, temperature, salinity and dissolved oxygen were measured using a multiparameter probe (YSI 6920, for temperature, conductivity and dissolved oxygen).

Zooplankton samples were placed into plastic containers and immediately brought to the laboratory where the *P. umbraticus* specimens were sorted out and counted under a stereo microscope and then transferred to a sample flask with 100 ml sterile sea water (salinity = 37). The sample flask was placed in a thermostatic cell at 18°C .

Female-plus-male pairs were taken from the aquarium when they reached the CIV–CV stage and placed as isolated pairs in crystallisation dishes. These dishes were monitored frequently (every ~6 h) for moults to record the initial fertilisation and spawning of the females. Groups of 5 replicate pairs were incubated at each of the following temperatures: 12, 14, 18, 24 and 32°C . Each pair was monitored daily during its entire lifespan, to record EPR in relation to temperature, and to observe egg-laying behaviour. The eggs, nauplii and copepodites from each parental pair were monitored until adulthood. EPRs were calculated as the mean number of eggs produced per female per day, including all of the incubated females. Recruitment rates were calculated by estimating the percentage ratio between the copepods that survived to adulthood and the nauplii that hatched from the eggs.

In the aquarium and in the pair dishes, specimens of *P. umbraticus* were reared on a mixed diet given in excess ($>10^3$) of the incipient limiting concentration for small-size copepods such as *Pseudocyclops* spp. (e.g. Corkett & McLaren 1978, Kiørboe et al. 1985, Runge 1988). The diet consisted of 3 phytoplankton species: *Tetraselmis suecica*, *Pavlova lutheri* and *Isochrysis galbana*. All 3 of these phytoplankton species were grown in Walne medium at 18°C under a 12:12 h light:dark cycle; ~7 d old cultures were used.

Statistical analysis

Kendall's tau correlation analysis was run to evaluate the relationships between the abundance data and the environmental parameters measured in the present study. This analysis is a non-parametric measure of rank association that is based on the number of concordances and discordances in paired observations.

One-way ANOVA was run to test for significant differences in daily egg production across the exper-

imental temperatures; Tukey's pairwise comparisons were used to evaluate the most significant differences in egg production between the temperatures. These tests were performed using the PAST software package (Hammer et al. 2001). To test for differences in the species-specific responses to temperature between *P. umbraticus* and the congeneric species *P. xiphophorus*, linear interpolation was used to calculate the missing values at all corresponding temperatures in both species. Linear regression analysis was used to predict the development time of the eggs, and from eggs to adulthood, at the limiting temperatures of 12 and 32°C. These 2 analyses were performed by computing the relative formulae using an Excel 2010 spreadsheet of the Microsoft Office package. The following formula was used for the linear interpolation, solved for y (temperature):

$$y = y_1 + (x - x_1) \frac{y_2 - y_1}{x_2 - x_1} \quad (1)$$

where x is time, in hours for development time of eggs and in days for development from egg to adulthood.

RESULTS

Seasonal and spatial patterns of abundance and environmental parameters

The temperature of Lake Faro varied between a maximum of 25.6°C in June along Transect 2, to a minimum of 11.8°C in January along Transect 4 (Fig. 2). The salinity reached its peak (36.1) in November along Transect 1, with the lowest salinity (31.5) measured in May along Transect 2 (Fig. 2). Dissolved oxygen concentrations oscillated between 6.4 mg l⁻¹ in May along Transect 1 and 4.2 mg l⁻¹ in November along Transect 2 (Fig. 2).

Pseudocyclops umbraticus occurred in Lake Faro throughout the year, with increasing abundance from May 2004 onwards, at which time the population was almost completely comprised of adults. The population attained a maximum abundance of 2.83 ind. m⁻³ (as adults and copepodites combined) in June 2004 along Transect 2 (Fig. 3). Another peak in abundance of 1.22 ind. m⁻³ (adults plus copepodites) was recorded in November 2004 along Transect 1. Considerable numbers were also recorded in January 2005 along Transect 4, with decreased abundances by April 2005. The seasonal abundance patterns did not show any significant relationships with any environmental parameter with the exception of salinity, which had a significant negative effect on the population abundance (Kendall's tau = -0.33, $p = 0.02$).

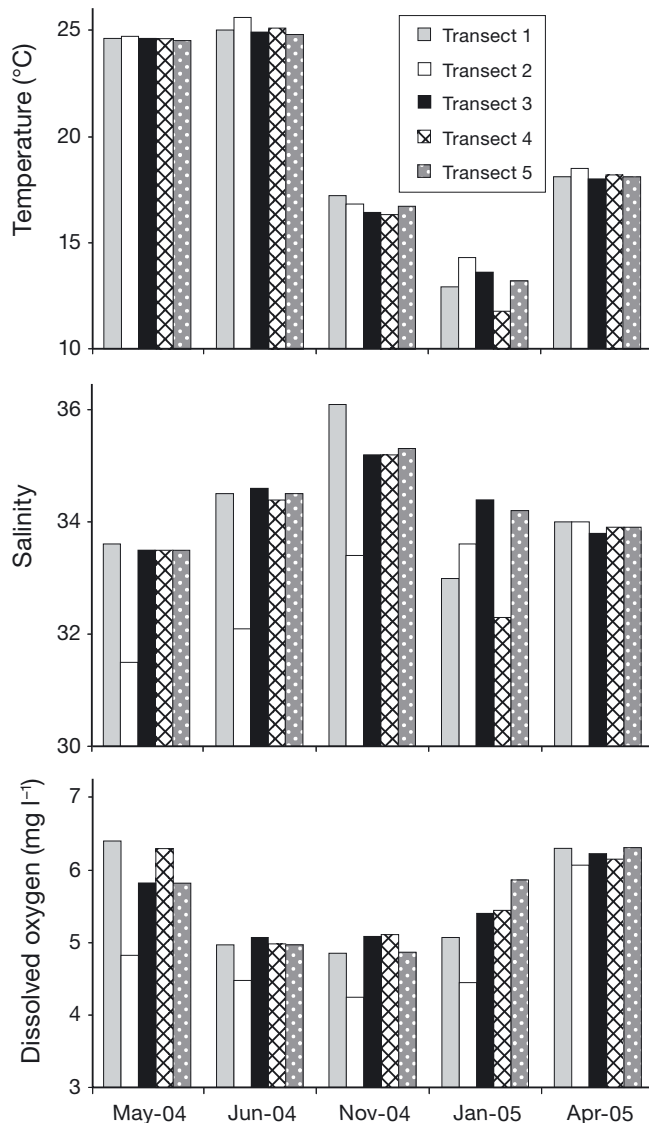


Fig. 2. Environmental parameters of salinity, temperature and dissolved oxygen along the 5 sampling transects during the sampling months

Fecundity experiments in the laboratory

The mean daily EPR throughout the full spawning lifetime of females was (mean ± SD) 2.3 ± 2.9 eggs female⁻¹ d⁻¹ at 14°C, 4.9 ± 5.2 at 18°C, and 6.1 ± 10.2 at 24°C. At 12 and 32°C, *P. umbraticus* females survived but did not reproduce. The mean daily EPR showed marked oscillations throughout the spawning period (Fig. 4). Across the tested temperatures, spawning rates were characterised by 3 phases (although these were less clear at 14°C): a first phase with low initial daily EPR, a second phase in which fecundity reached the maximum levels characterised by each temperature, and a last phase characterised

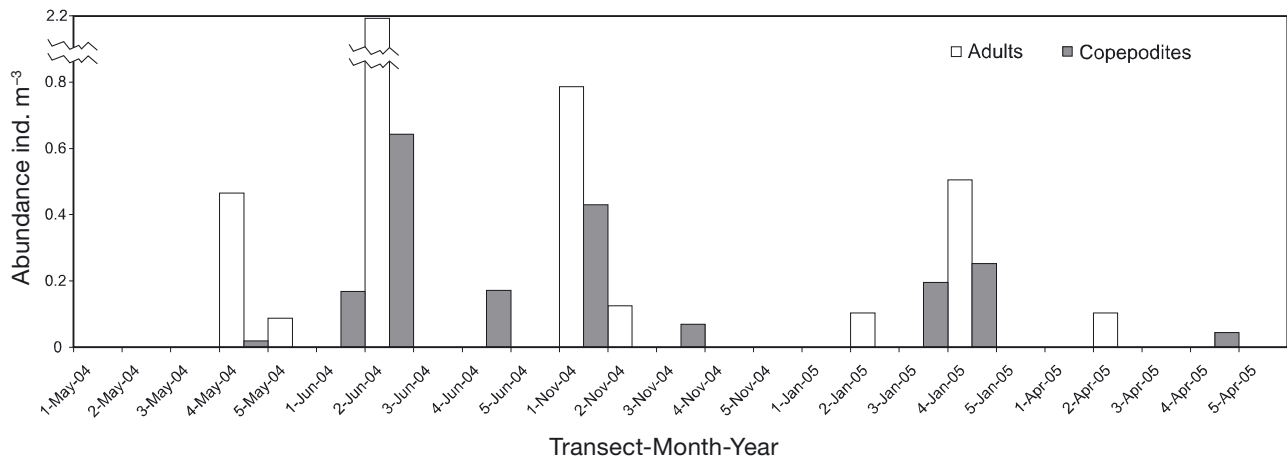


Fig. 3. *Pseudocyclops umbraticus* adult and copepodid abundances (as indicated) along the 5 sampling transects during the sampling months

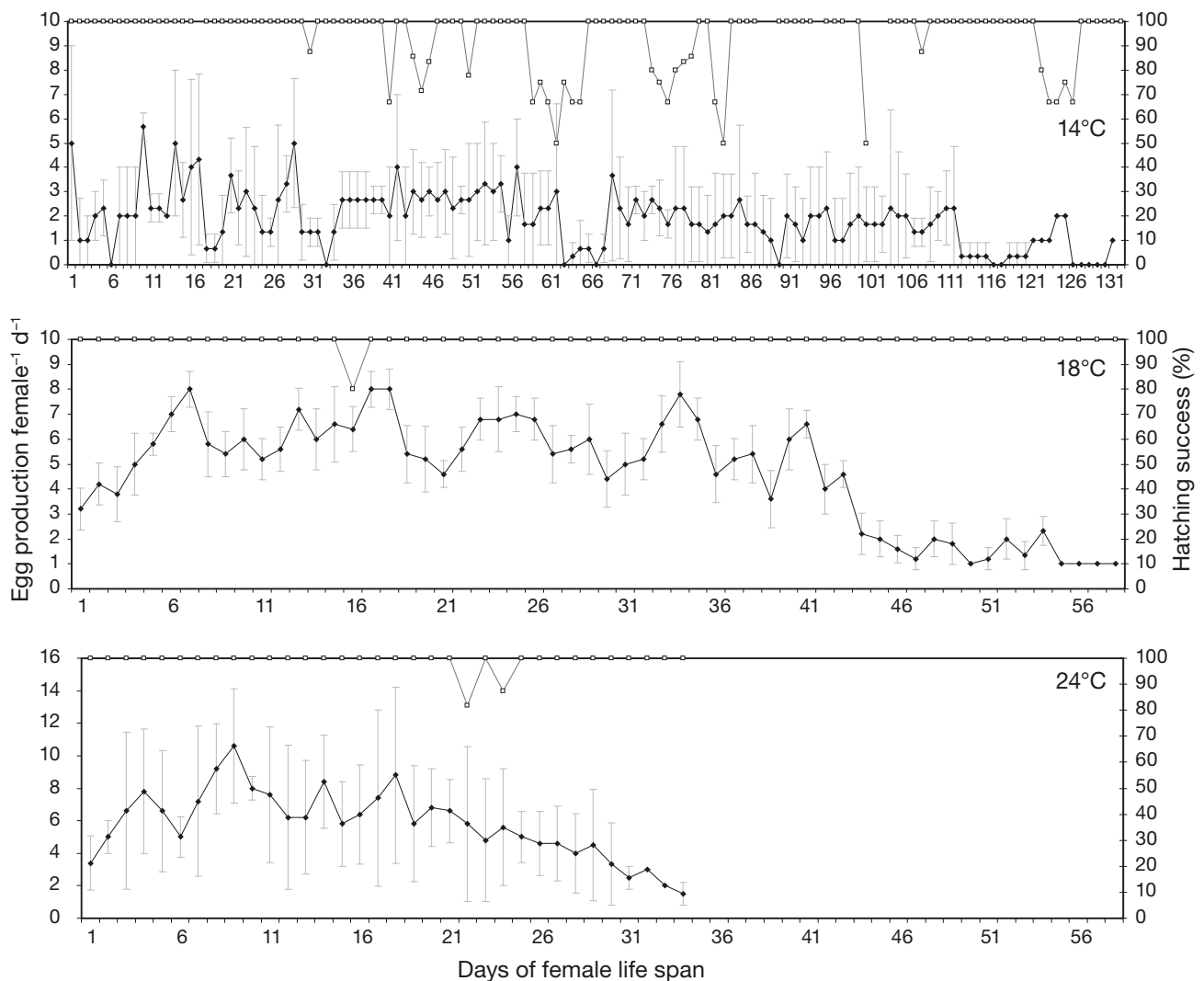


Fig. 4. *Pseudocyclops umbraticus* mean daily egg production rates (●) and hatching success (○) at 14, 18, and 24°C. Note change of y-axis scale for 24°C (due to the higher production), and change of x-axis scale for 14°C (due to the longer spawning period)

Table 1. One-way ANOVA performed on the mean daily egg production of *Pseudocyclops umbraticus* to test for differences in fecundity responses among temperatures. Significant values are highlighted in **bold**. Levene's test for homogeneity of variance based on means: $p(\text{same}) = 0.0138$; based on medians: $p(\text{same}) = 0.01572$. Welch F -test in the case of unequal variances: $F = 67.1$; $df = 63.31$; $p = 2.288 \times 10^{-16}$

	Sum of squares	df	Mean square	F	$p(\text{same})$
Between temperatures	284.337	2	142.168	53.66	1.64×10^{-16}
Within spawning days	262.315	99	2.64965		
Total	546.652	101			

Table 2. Tukey's pairwise comparisons of the differences in *Pseudocyclops umbraticus* mean daily egg production among temperatures. Significant values are highlighted in **bold**

Q \ $p(\text{same})$	14°C	18°C	24°C
14°C		1.00×10^{-4}	1.05×10^{-4}
18°C	12.93		0.933
24°C	12.43	0.5022	

by a slow decline in daily egg production (Fig. 4). One-way ANOVA demonstrated significant differences in mean daily EPR among the temperatures tested (Table 1). Tukey's pairwise comparisons estimated more significant differences in the daily mean EPR between the 14 and 18°C pairs and the 14 and 24°C pairs, than between the 18 and 24°C pairs (Table 2).

Hatching success was very high on average, and ranged from about 100% at 20°C to 94.8% at 14°C. Hatching success was lower at 14°C, although it never dropped below 50% (Fig. 4). The maximum mean

lifetime egg production was seen at 14°C (278.5 ± 30.4 eggs female⁻¹), with the minimum at 24°C (189.0 ± 28.8 eggs female⁻¹). At 18°C, lifetime egg production was 264.8 ± 15.1 eggs female⁻¹.

Increasing temperatures dramatically decreased female lifespan (Fig. 5), which was 121.7 ± 10.5 d at 14°C, 54.4 ± 2.9 d at 18°C, and 31.0 ± 2.8 d at 24°C. Similarly, development times of the eggs decreased with increasing temperatures (Fig. 6a): the eggs hatched after 108 h at 14°C, 84 h at 18°C, and

12 h at 24°C. The development time from egg to adulthood also showed a similar trend, with adulthood attained in 57.3 ± 7.0 d at 14°C, 40.7 ± 6.0 d at 18°C, and 19.3 ± 3.0 d at 28°C (Fig. 6b). In contrast, the recruitment rate was lower at the low temperature ($9.80 \pm 1.44\%$ at 14°C) than the higher temperatures ($17.10 \pm 5.55\%$ at 18°C, $30.25 \pm 2.33\%$ at 28°C), and was significantly related to temperature (Fig. 7).

The EPRs of *P. umbraticus* were significantly higher than those of the congeneric *P. xiphophorus* at all temperatures, as shown by 2-way ANOVA (Table 3), even though the fecundity of both of these species showed the same tolerance limits. For example, the daily mean egg production of *P. umbraticus* at 18°C (4.9 eggs female⁻¹ d⁻¹) was higher than that estimated for *P. xiphophorus* at 20°C. Also, *P. umbraticus* showed a higher correlation coefficient with temperature and a greater slope of the regression line than *P. xiphophorus* (Fig. 8).

The egg-laying behaviour in *P. umbraticus* is similar to that of *P. xiphophorus*, and is very close to being considered parental care. Within 2 to 3 d after achieving maturity, the females release eggs more or less continuously throughout the day, almost until the end of their life. The females do not release these eggs directly into the water; instead, after a pair of eggs is extruded from the paired gonopores, the female swims with the pair of eggs attached on either side of the genital double somite until the eggs are released by a rapid urosome stroke. After releasing the egg pair, the female swims over them with a rotatory motion, apparently secreting a substance that facilitates the adhesion of the eggs to the bottom of the Petri dish or to any hard surface, such as wood fragments, nylon filaments, filamentous algae, or leaves. The female continues to swim over the eggs until they are attached. This adhesive substance is secreted from the female mouthparts, which become so sticky that any encountered fragments can become stuck.

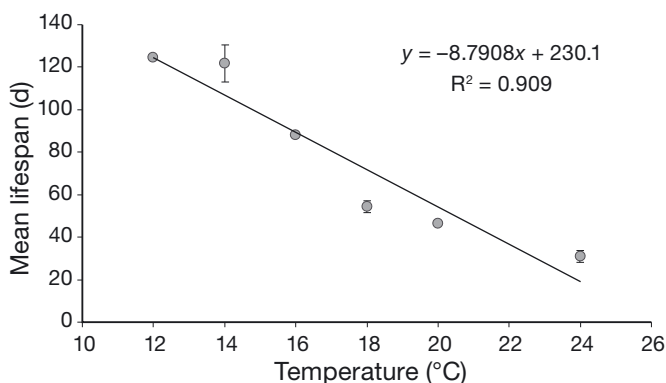


Fig. 5. Relationship between temperature and mean lifespan for female *Pseudocyclops umbraticus*. Circle symbols without error bars represent interpolated values from the regression line

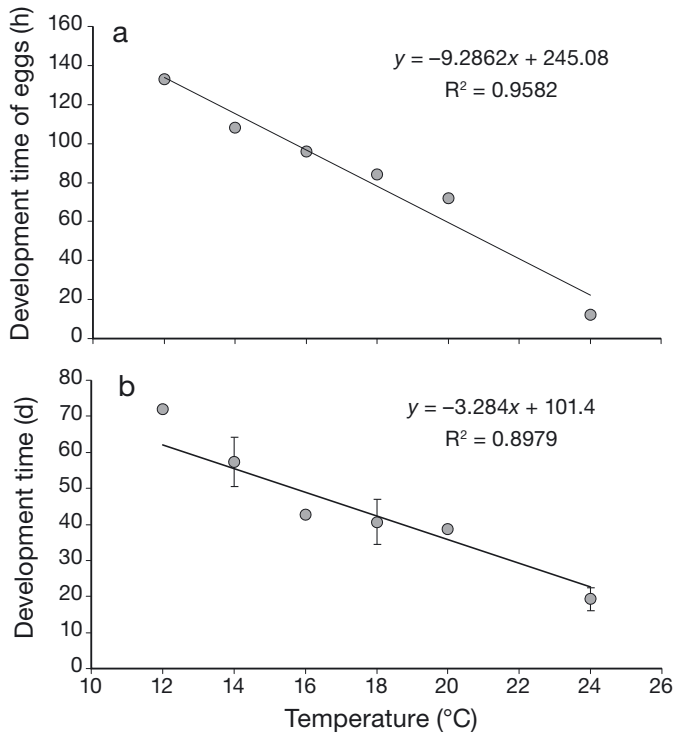


Fig. 6. Relationships between temperature and (a) mean development time of *Pseudocyclops umbraticus* eggs; and (b) development time from naupliar stage to adulthood. Circle symbols without error bars in (b) represent interpolated values from the regression line

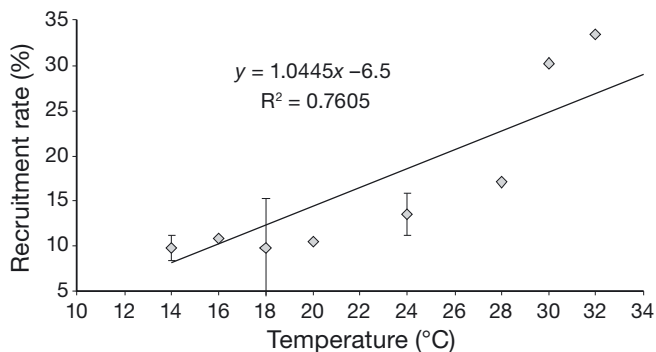


Fig. 7. Relationship between temperature and recruitment rate for female *Pseudocyclops umbraticus*. Symbols without error bars represent interpolated values from the regression line

Table 3. Two-way ANOVA to test the differences in mean daily egg production between *Pseudocyclops umbraticus* and *P. xiphophorus* for each temperature. Significant values are highlighted in **bold**

	Sum of squares	df	Mean square	F	p
<i>P. umbraticus</i> vs. <i>P. xiphophorus</i>	1128.46	2	564.228	105.555	2.0×10^{-7}
Between temp.	295.726	5	59.1452	11.0648	8.0×10^{-4}
Error	53.4537	10	5.34537		
Total	1477.64	17			

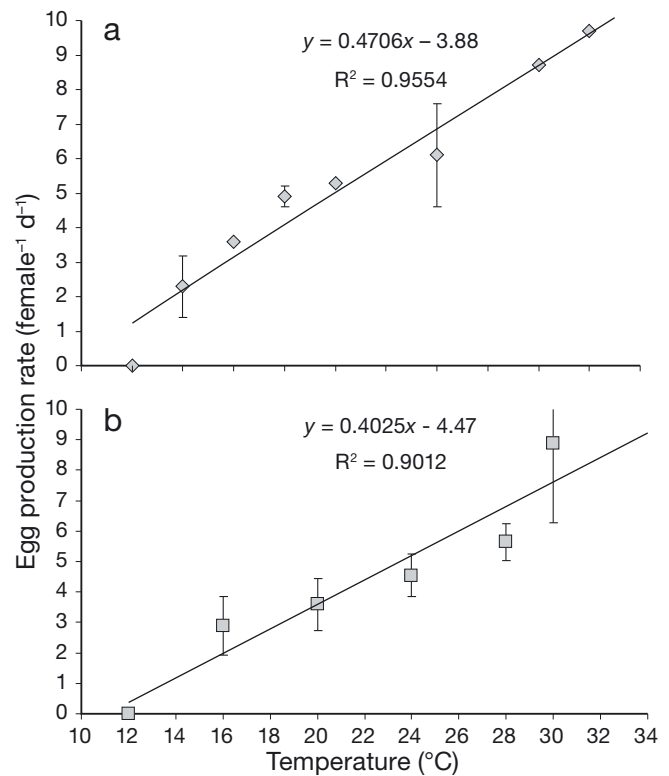


Fig. 8. Relationships between temperature and mean egg production rates in (a) *Pseudocyclops umbraticus* and (b) *P. xiphophorus*. Symbols without error bars represent interpolated values from the regression line

DISCUSSION

Following the work of Brugnano et al. (2006, 2009) who focused on *Pseudocyclops xiphophorus*, the present study represents an additional contribution to our understanding of EPR and life-history parameters of the benthopelagic calanoid *P. umbraticus* reared in the laboratory. We also describe its abundance trends along 5 transects in Lake Faro, where it showed high seasonal variability.

In contrast to the congeneric species *P. xiphophorus*, the maximum abundance of *P. umbraticus* occurred in the summer, although relatively high abundance was also recorded both in autumn and winter, similar to that of *P. xiphophorus*. *P. umbraticus* demonstrated high fecundity during the summer, with a slight decline in winter when the temperatures approached the species' lower tolerance limits. Thus, there is concurrence between adult female abundance and daily EPRs in the summer, similar to Japanese populations of *Acartia clausi* (Uye 1982). This concurrence was only seen for *P. xiphophorus* in November (Brugnano

et al. 2009), when high fecundity coincided with high population density of the females.

Since development time is longer at lower temperatures, *P. umbraticus* appears to overwinter as adults or advanced copepodites, as has also been demonstrated for *P. xiphophorus*. This life strategy would allow both of these populations to survive without any pause in egg production, at least under our different experimental and temperature conditions. Smith & Lane (1985) demonstrated how *Centropages typicus* spends the cooler season as CV, and this temporal pattern is similar to that reported for *Rhincalanus nasutus*, *Pseudocalanus* sp., and *Paracalanus parvus* (Landry 1983). During the winter, when the temperature decreases but remains above 16°C, the abundance of *P. umbraticus* remains relatively high, although EPR declines, and the spawning, laying and development times increase. This mechanism allows the population to sustain a relatively high abundance into January. As temperatures continue to decrease, egg production declines and ultimately stops at 12°C, which results in low abundance of *P. umbraticus* copepodites and adults in the spring. This is corroborated by the low recruitment rates estimated at lower temperatures in our laboratory experiments, which demonstrate that maximum daily EPRs occur when environmental temperatures range from 18 to 24°C. In the spring, as the temperature increases, the *P. umbraticus* population begins to increase in numbers again.

Zagami & Brugnano (2013) reported the total absence of all benthoplanktonic copepod species except *P. xiphophorus* in late summer throughout Lake Faro. They concluded that this absence was caused by benthic anoxia, which was enhanced by the decomposition of benthic macroalgae in the shallow waters along the shores of the lake. In contrast to *P. umbraticus*, *P. xiphophorus* lives in the fouling material associated with ropes and mooring posts in surface water, and is widespread all around Lake Faro in summer (Zagami & Brugnano 2013). In the unfavourable conditions of high temperatures and low oxygen concentrations in the late summer-early autumn, the necessity of resting egg production for *P. umbraticus* could be hypothesized, but this needs further investigation.

The differences in seasonal and spatial abundance patterns between these congeneric species might be due to differences in physiology and/or in predation pressure. The different sensitivity of *P. umbraticus* and *P. xiphophorus* to temperature variation might be a further adaptation to their different substrate types (i.e. the bottom of shallow shorelines, and foul-

ing material suspended in the water column, respectively). Indeed, *P. umbraticus* is more fecund at all temperatures, but less reactive than *P. xiphophorus* to a temperature increment of as little as 6°C (Brugnano et al. 2009). This was demonstrated in this study by the similar (not significantly different) egg production at 18 and 24°C by *P. umbraticus*. It appears well adapted to the high variability within shallow shore waters, which are more likely to undergo rapid changes in environmental parameters, even though it was not possible to demonstrate any significant negative effect of salinity on *P. umbraticus* abundance.

As demonstrated by Uye (1981) for *Acartia omorii*, although the average number of eggs produced by *P. umbraticus* is higher at higher temperatures, the period of egg laying is reduced (as in *P. xiphophorus*; Brugnano et al. 2009), and consequently, lifetime egg production is lower. The average number of eggs produced is higher at higher temperatures because of the acceleration in their metabolic activity and the associated accumulation of material in the developing oocytes, so that egg production is higher and spawning intervals are shortened (Uye 1981). In the laboratory, *P. umbraticus* egg production showed a lower temperature limit below which eggs were not produced (12°C), and an optimal temperature range at which the daily EPR reached its maximum (18 to 28°C). This optimum temperature range appears to be higher than that reported for most common subtemperate copepod species, which ranges from ca. 15°C for *C. typicus*, *Temora stylifera* and *T. longicornis* (Abou Debs & Nival 1983, Van Rijswijk et al. 1989), to 20°C for *Acartia tonsa* and *Sinocalanus tenellus* (Kimoto et al. 1986).

P. umbraticus and *P. xiphophorus* have lower daily EPRs than most planktonic calanoid copepods in subtemperate regions (Ianora 1998). Despite these low EPRs, *P. umbraticus* and *P. xiphophorus* can survive in restricted habitats where interspecific competition, mainly with harpacticoid copepods, is high. The distinctive spawning behaviour whereby females secrete a substance (probably from the mouth apparatus) that aids in the adhesion of their eggs to the substrate appears to be characteristic of these species, and differentiates the genus from the free-spawning and egg-carrying calanoids. This egg-laying behaviour might represent an adaptation to a benthic habitat, to guarantee higher survival rates of the egg stages compared to other planktonic free-spawning and egg-carrying calanoid species (Kjørboe & Sabatini 1994), thereby compensating for its low population abundance and reproduction rates.

By carrying the eggs, the female would be more susceptible to visually hunting predators (Webb & Weaver 1988). What makes the egg-laying behaviour in these species unique is that the female continues to swim over the eggs and contributes to their attachment to the substrate, an action that corresponds to the movement of their mouthparts. To our knowledge, this egg-laying behaviour has never been observed before for copepods.

As the biology and ecology of this particular group of benthic-pelagic calanoid species is poorly known, further studies are currently underway. These include determination of the trophic requirements of *P. umbraticus*, which are of particular interest due to their migratory habits, where *P. umbraticus* move upwards from the daytime bottom-water interface habitat, into the water column at night (Damkaer 1970, Alldredge & King 1985, Ohtsuka 1985, Jacoby & Greenwood 1988, Campolmi et al. 2002), as demonstrated by the diversity increase in the lake during the night (Zagami & Brugnano 2013). Thus, *P. umbraticus* can feed on both planktonic and benthic communities. Brugnano et al. (2009) observed higher *in situ* egg production for *P. xiphophorus* than in laboratory experiments, which they attributed to a deficiency in the diet fed to the copepods in the laboratory.

We were unable to study the *in situ* feeding behaviour of *Pseudocyclops* species because of their rarity, but additional efforts will be made in future studies. However, there is evidence in the literature that mixed diets increase fecundity, and that the combination of a dinoflagellate and ciliate diet contributes more than a monospecific diet. A mixed diet can improve egg production by allowing any lack of nutrients in one food source to be supplemented by another food source (Kleppel et al. 1991). Studies on the effects of different food items on the egg production and hatching success of *P. xiphophorus* have already demonstrated different responses to a phytoplankton diet (Brugnano et al. 2008).

When compared to other more abundant pelagic copepods, *P. umbraticus* is much less fecund, even though the maximum daily EPRs (from 6 to 8 eggs female⁻¹ d⁻¹ at 28 and 30°C) are within the range of values reported for egg-carrying species (Mauchline 1998, Lee et al. 2003). The high hatching success and unique egg-laying behaviour of this benthic-pelagic genus may also contribute to higher survival rates of egg stages compared to free-spawning species (Kjørboe & Sabatini 1994). The greater fecundity at higher temperatures compared to other subtemperate species, which generally show maximum EPRs at 15 to

20°C (Ianora 1998), indicates that *P. umbraticus* is well adapted to the higher temperatures of coastal and brackish water environments, where it contributes to the biofouling community. However, EPRs of *P. umbraticus* are similar to those reported for some key pelagic calanoid species that are typical of these transitional environments, such as *Paracartia latisetosa* (from 0.5 ± 1.1 eggs female⁻¹ d⁻¹ in May, to 6.5 ± 2.1 eggs female⁻¹ d⁻¹ in August; Belmonte & Pati 2007). In any case, they are evenly low when compared to values obtained when rearing the same species on a *Prorocentrum minimum* diet (20.0 ± 12.2 eggs female⁻¹ d⁻¹ at 18°C) under food satiation conditions (C. Brugnano et al. unpubl. data). The reliability of these data is supported by comparing the mean abundances of *P. latisetosa* and *P. umbraticus* in Lake Faro: 195.5 ind. m⁻³, and 0.32 ind. m⁻³, respectively (Zagami & Brugnano 2013).

CONCLUSIONS

We have shown here that, although *Pseudocyclops umbraticus* and *P. xiphophorus* compared are in the same genus, they show significant differences in some important life-history traits, and different physiological adaptations to temperature in relation to substrate type. These benthic-pelagic calanoids can be defined as eurythermic, with a wide range of temperature tolerance, such that they are well adapted to live in the shallow coastal and transitional environments where their low EPRs are balanced by the high naupliar survival rates, which in turn may reflect their unique egg-laying behaviour.

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