INTRODUCTION

Respiration is a fundamental property of organisms and ultimately represents the main energy loss pathway within ecological systems. Thus, as the most abundant and diverse aquatic metazoan, marine copepods play an important role, through their respiration, in shaping the structure and dynamics of food-webs and the flow of carbon in the ocean. Therefore, knowledge of how copepod respiration changes with in situ conditions is central to understanding the mechanisms determining community diversity, secondary production and biogeochemical cycles in the ocean.

In temperate latitudes, copepods experience considerable fluctuation in ambient conditions over the seasonal cycle, which exerts a profound effect on their metabolism (e.g. Mauchline 1998). In copepods, as in poikilotherms in general, respiration rate is considered to vary mainly as a function of body mass and ambient temperature (Peters 1986, Ikeda et al. 2001, Brown et al. 2004). Published data, however, suggest that other factors, such as food quantity, food quality and temperature acclimatisation, are also important (Conover 1959, Conover & Corner 1968, Marshall & Orr 1958, 1966, Butler et al. 1970, Gaudy 1973). Respiration is also closely coupled with growth and reproduction (Mc Neill & Lawton 1970), both of which are influenced by in situ conditions.
which can represent a substantial energetic cost to an organism (Parry 1983, Kjærboe et al. 1985, Clarke 1993). Hence, several authors have argued that seasonal changes in the respiration rate of poikilotherms could be mainly a reflection of the effects of temperature on growth and reproduction rather than a direct effect of temperature on respiration (Parry 1983, Clarke 1993). Poikilotherms may also display species-specific physiological plasticity and genetic adaptation which enable them to cope with seasonal and latitudinal changes in temperature (Pretch 1958, Somero 2012, Dam 2013).

Despite previous studies, the relative importance of different environmental factors on copepod respiration remains unclear. Understanding how copepod metabolism varies with ambient conditions is also important for the correct parameterization of ecosystem models (Stock & Dunne 2010). Some models have proposed simple equations to describe the metabolic rate of all organisms from ‘first principles’, using a combination of body mass scaling and thermodynamic laws (Gillooly et al. 2001, Enquist et al. 2003, Brown et al. 2004). These predictive models generally use fixed body mass scaling, such as $b = 0.75$, and metabolic thermal coefficient values, such as the activation energy ($E_a$) = 0.63 eV derived from the Arrhenius formulation. Similarly, other modelling studies commonly use empirically derived values of the thermal coefficient $Q_{10}$ between 2 and 4 (e.g. Olonscheck et al. 2013). However, these $E_a$ and $Q_{10}$ values are typically derived from acutely measured metabolism-temperature rates (i.e. M-T curves) of animals maintained under optimal feeding conditions (Peters 1986). In nature, however, copepods can often be food limited (Hirst & Bunker 2003), and under such circumstances, the respiration rate is reduced and does not respond to temperature changes in the same way as that of well-fed organisms (Mayzaud 1976, Thor 2003). Hence, Cossins & Bowler (1987) have argued that eco-physiological studies should measure acclimatised metabolic rates as these are ecologically meaningful. Acclimatised rates refer to the physiological rates of poikilotherms measured at in situ conditions. In contrast, acclimated rates refer to the rates of poikilotherms maintained in the laboratory at a given temperature, whereas ‘acute’ rates refer to measurements made on poikilotherms maintained in the laboratory and exposed to a sudden change in ambient conditions, generally temperature (Cossins & Bowler 1987).

Overall, published data on respiration rates of copepods acclimatised to in situ conditions are very scarce. One of the reasons for such scarcity is that the methods adopted by previous investigators to measure seasonal changes in respiration rate have been often inadequate; for instance, most studies have disregarded the effect of the nutritional condition and acute temperature exposure on metabolism by measuring copepods maintained under different feeding conditions at fixed arbitrary temperatures (Berner 1962, Conover 1962, Marshall & Orr 1966, Gaudy 1973, Gaudy & Thibault-Botha 2007). Thus, differences in methodological approaches used in the literature to measure copepod respiration rates make comparisons and synthesis of published data difficult and their use in predictive ecological models unreliable.

The small copepod Temora longicornis often dominates the spring and early summer zooplankton communities of coastal temperate waters of the North Atlantic (Peterson 1985, Fransz et al. 1991, Castellani & Lucas 2003). In its environment, this species experiences a wide fluctuation in temperature and feeding conditions over the seasonal cycle (Castellani & Altunbaş 2006). Because of its reduced ability to store body lipids, T. longicornis closely depends on its food supply to meet basal energy requirements, let alone to grow and reproduce (Clarke & Walsh 1993, Kreibich et al. 2008). Such physiological trait makes this species an ideal model organism to study how environmental change affects the respiration rate of small neritic copepods. Hence, the aim of the present study was to investigate how the acclimatised respiration rate of adult copepod $T. longicornis$ varies in relation to seasonal changes in body mass, temperature, salinity, prey availability and reproduction. In addition, we explore whether temperature-acclimatised respiration rates differ from acclimated and acutely measured rates of copepods maintained in the laboratory under high food concentrations.

**MATERIALS AND METHODS**

**Sampling**

Zooplankton were collected weekly, between April 1996 and April 1997, with a 200 μm mesh WP-2 plankton net fitted with a non-filtering cod-end from the St. George Pier, Menai Strait (53° 13’ N, 4° 09’ W), eastern Irish Sea. After collection, the plankton was immediately re-suspended into an opalescent, polypropylene aspirator containing 20 l of natural seawater pre-screened through a 200 μm mesh filter to exclude predators. Salinity (S, ppt) and temperature ($T, °C$) were measured with a CTD (Braystoke, Series...
600) at 2 m intervals from the surface to the seabed (i.e. maximum of 20 m water depth) during each sampling. Water samples for chlorophyll a concentration (Chl, μg l−1) and microplankton community characterisation were collected with a 2 l Niskin bottle at ~2 m depth from the surface. Chl was determined from water samples of 100 to 750 ml, filtered onto GF/F filters, extracted in neutralised 90% acetone solution for 24 h at 4°C in the dark and measured using a Turner 10 fluorometer (Tett 1987). Microplankton samples were immediately fixed with Lugol’s iodine to 2% final concentration (Kiorboe & Nielsen 1994), stored in 100 ml dark glass bottles in the dark at 4°C and analysed by the Utermöhl (1958) technique within 1 mo.

Copepod maintenance prior to respiration rate experiments

Adults Temora longicornis were sorted from the catch within 1 h of collection in a walk-in cold room set at in situ temperatures between 5 and 17.5°C. The copepods were used for 1 of the 3 following experiments: to investigate (1) seasonal changes in acclimatised respiration rate, (2) the respiration rate of copepods acclimated to temperature in the laboratory and (3) the respiration rate of copepods exposed to a sudden (i.e. acute) temperature change. Here, we refer to ‘acclimatised’ rates for the respiration rates of copepods measured at in situ conditions, to ‘acclimated’ rates for measurements on copepods maintained in the laboratory at a temperature different from in situ and to ‘acute’ rates for measurements made on copepods maintained in the laboratory and exposed to a sudden temperature change (Cossins & Bowler 1987). Thus, copepods were maintained under different conditions according to the type of experiment performed. The T. longicornis measured for (1) the seasonal respiration rate experiment were kept in batches of up to 10 ind. l−1 in 5 l glass jars filled with natural seawater pre-screened through a 250 μm mesh sieve and in temperature-controlled water baths for a maximum of 1 d at their original in situ temperature. The respiration rates of copepods that were (2) acclimated and (3) acutely exposed were measured over the temperature range of 4 to 20°C, and these copepods were maintained in the laboratory on an ad libitum diet of the cultured flagellate alga Rhinomonas reticulata (Novarino 1991) for 1 wk before measurements. The water in the jars was changed every other day by sieving the copepods in a submerged 200 μm sieve to avoid damage to the animals and gently re-suspended with some remaining water in a cleaned jar containing UV-treated filtered (0.2 μm) seawater (UFSW) and fresh cultured micro-algae. Jars were cleaned overnight with a 1% solution of sodium hypochlorite and thoroughly rinsed with hot tap water to remove chemical residues.

We also carried out a preliminary experiment to assess the time required by freshly caught copepods to attain a stable routine respiration rate once deprived of food to avoid the effect of the increase in oxygen consumption resulting from ‘stress’ after capture (Marshall & Orr 1966) and from feeding metabolism (viz. specific dynamic action [SDA]; Kiorboe et al. 1985, Secor 2009). Freshly caught copepods were placed in filtered seawater and their respiration rate measured continuously until the respiration rate stabilised. An inverse function fitted to the data set obtained from this time course experiment indicated that copepod respiration rate reached a stable routine rate ~10 h after capture and that such respiration rate remained stable at ~1.7 nl μg−1 dry weight (DW) h−1 over at least the following 24 h (Fig. 1). Hence, a minimum time of 10 h since the start of fasting was allowed to elapse prior to all respiration rate measurements carried out in the 3 experiments outlined above.

Respiration rate measurements

Seasonal variation in copepod respiration rate in the field

We measure the seasonal variation in the respiration rate of adult Temora longicornis between April 1996 and April 1997. Here, respiration rate corresponds to routine rate, that is, the oxygen consumed...
over time by fasting copepods at minimum uncontrolled motor activity (Ikeda et al. 2001). The respiration rate of fasting copepods was measured individually using a polarographic oxygen electrode (pO₂-electrode, Strathkelvin model 1302) sensitive to changes in oxygen tension in a fluid media (Kanowski 1959). The pO₂-electrode was fitted to the base of a closed transparent glass micro-respirometer chamber of 100 to 150 μl in volume to allow regular monitoring of animal activity and behaviour. The temperature inside the respirometer was kept constant within ± 0.2°C by a thermostat through a recirculating water bath system allowing a continuous water flow in the chamber water jacket. The pO₂-electrode was connected to an O₂ meter (Strathkelvin inst. model 781) to display the change in oxygen tension inside the respirometer and through this to a chart recorder to obtain a plot of the respiratory activity of the copepods over the time of observation. The pO₂-electrode was regularly calibrated with distilled water containing sodium dithionite to set the zero point on the O₂-meter, and then with air-saturated UFSW to set the 100% air saturation level. The electrode response was linear over the range 0 to 100% air saturation; hence, the amount of oxygen present in the chamber was calculated with the algorithm of Green & Carrit (1967). The 1302 microcathode oxygen electrode is a high precision electrode with a very small oxygen consumption rate (i.e. $0.5 \times 10^{-10}$ to $3 \times 10^{-10}$ mg O₂ min⁻¹). We used a low permeability polypropylene membrane so most of the resulting oxygen gradient is confined to the distance between the outside of the membrane and the cathode surface. Consequently, there is no need for vigorous physical movement of the solution to replenish the oxygen at the outer surface of the membrane. However, correction for electrode oxygen consumption was performed at regular intervals by running an oxygen respiration measurement without the animal (i.e. the blank). The blank was then subtracted from the oxygen rate measurement of the copepod. Copepods were measured under dim light conditions since bright light has been shown to increase the respiration rate in some species (Marshall et al. 1935). Respiration measurements were run for 1 h maximum.

Copepod DW vs. length relationship

At the end of each experiment, the prosome length (PL) of copepod was measured with an eye-piece graticule under a dissecting microscope (Wild M5). Lengths were converted to body mass from the regression $\ln \text{DW} = 2.79 \ln \text{PL} - 15.9$ ($r = 0.92$, $p < 0.001$, df = 29), constructed using copepods from the present study and published by Castellani & Altunbaş (2006), where PL is prosome length in μm, and DW is the dry weight in μg. The relationship was obtained by measuring 30 groups of adult copepods consisting of between 20 and 30 individuals of similar prosome length (±20 μm) over the full size range. The copepods were dried in pre-weighed aluminium cups in an oven at 50°C until the weight stabilised, and they were weighed to the nearest 1 μg with a microbalance (Cahan). Copepod DW was converted to carbon assuming a specific-C content of 40% (Omori & Ikeda 1984).

Copepod egg production rate

Measurements of egg production rates (EPR) of Temora longicornis were carried out at in situ conditions concomitantly to respiration rate measurements as described by Castellani & Altunbaş (2006). Briefly, between 25 and 30 intact active female T. longicornis randomly selected from the catch were incubated individually in 250 crystallising dishes filled with natural seawater pre-screened through a 53 μm mesh. The dishes were kept for 24 h in a temperature-controlled water bath at the ambient surface temperature ± 0.2°C (i.e. 1 m depth), under artificial lighting, with an ambient light/dark regime. After incubation, the females were gently removed from the crystallising dishes with a pipette, the content of each dish was filtered through a 53 μm sieve, and the eggs and nauplii were retained, stained with Lugol’s iodine and counted in a Bogorov’s tray under a dissecting microscope (Wild M5). The egg counts of copepods found dead or moribund at the end of the incubation time were discarded. EPR was calculated as the total number of eggs produced per female over the 24 h incubation period.

Calculation of the thermal coefficients $Q_{10}$ and $E_a$

The relationship between the seasonal changes in the respiration rate of Temora longicornis with temperature was investigated determining the thermal coefficient $Q_{10}$, which is commonly used to describe the increase in physiological rates over a 10°C change (Schmidt-Nielsen 1990) (Eq. 1):

$$Q_{10} = \left( \frac{R_T}{R_2} \right)^{10/(T_2 - T_1)} \quad \text{(Eq. 1)}$$
where $R_1$ and $R_2$ are the respiration rates at the temperatures $T_1$ and $T_2$ respectively.

We also investigated the dependence of respiration on temperature using the Arrhenius equation (Eq. 2) by plotting the reciprocal of the absolute temperature, $\theta$, expressed in kelvin (K) against the respiration rates:

$$ R = A e^{-E_a/R_0} \quad \text{(Eq. 2)} $$

where $R$ is the rate constant, $A$ is the frequency factor constant, $e$ is the base of the natural logarithm (i.e. $2.718$), $\theta$ is the absolute temperature in K, $R$ - is the gas constant (i.e. $8.31 \text{ J mole}^{-1} \text{ K}^{-1}$), and $E_a$ (i.e. in J mol$^{-1}$) is the activation energy. The change in rate constant with temperature can be predicted from the proportionality constant $E_a$ of an integrated form of the Arrhenius equation, that is:

$$ \ln \left( \frac{R_2}{R_1} \right) = \left( \frac{1}{\theta_1} - \frac{1}{\theta_2} \right) \cdot \frac{E_a}{R} \quad \text{(Eq. 3)} $$

where $R_1$ and $R_2$ are the rate constants at temperatures $\theta_1$ and $\theta_2$ in K. The Arrhenius plot of $\ln R$ against the reciprocal of the absolute temperature gives a straight line with a slope equal to $E_a/R$ from which $E_a$ can be calculated, as shown in Eq. 4:

$$ E_a = \text{slope} \times 8.31 \text{ (J mol}^{-1}) \quad \text{(Eq. 4)} $$

### RESULTS

#### Environmental conditions and microplankton composition

The sampling area is characterised by shallow water depth between 1 and 20 m, strong tidal currents and high tidal ranges between 3.4 m and 6.6 m, resulting in a well-mixed water column throughout the year. Table 1 and Fig. 2 summarise the seasonal changes in ambient variables at the sampling site already reported by Castellani & Altunbaš (2006). Briefly, in situ temperature ($T$, °C) recorded during the present study varied from a minimum of 5°C in February to a maximum of 17.5°C in August (Fig. 2a). Salinity changed little, reaching maximum values of 34.1 ppt in summer and a minima of 31.7 ppt in autumn (Table 1), during the highest local annual rainfall (i.e. 110 to 126 mm mo$^{-1}$; Eden 1997).

The early spring increase of Chl from low winter concentrations of 0.5–0.9 to ~3–8 μg l$^{-1}$ (Fig. 2b) was almost exclusively diatomaceous and included species like *Ditylum brightwelli*, *Skeletonema costatum*, *Chaetoceros sp.*, *Asterionella sp.* and *Thalassiosira sp.* Between April and June, the microplankton community was replaced by the mixed diatom-flagellates bloom of *Phaeocystis sp.* and *Rhizosolenia delicatula* during which Chl increased to ~16 to 26 μg l$^{-1}$. The spring bloom was followed by euglenoids and cryptomonad-like flagellates. A series of monospecific diatom blooms of *Leptocylindrus danicus*, *Rhizosolenia styliformis* and *Guinardia flaccida* developed in summer and autumn, with Chl ranging between ~3 and 8 μg l$^{-1}$. Ciliate biomass, mostly belonging to the genus *Strombidium*, also peaked between spring and early summer and was correlated with Chl ($r = 0.67$, $p < 0.05$, df = 33). Dinoflagellates, on the other hand, peaked in summer after the Chl maxima (Castellani & Altunbaš 2006).

### Seasonal variation of copepod respiration with biotic and abiotic variables

*Temora longicornis* respiration rates began to increase between January and April, reached a maximum in August ($R$, mean ± SE, 83.2 ± 7.6 nl O$_2$ ind.$^{-1}$ h$^{-1}$) and a lower secondary peak in October–November before declining to the annual minimum.

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**Table 1.** Sampling dates for *Temora longicornis* used for the respiration rate experiments and corresponding environmental variables. Dates given as dd/mm/yyyy

| Sampling date | $T$ (°C) | Salinity | Chl a (μg l$^{-1}$) |
|---------------|----------|----------|---------------------|
| 17/05/1996    | 10.0     | 34       | 12.80               |
| 27/05/1996    | 10.0     | 34       | 25.92               |
| 02/06/1996    | 12.0     | 34       | 15.55               |
| 10/06/1996    | 13.0     | 34       | 8.99                |
| 12/06/1996    | 13.0     | 34       | 8.99                |
| 08/08/1996    | 17.5     | 33.8     | 4.84                |
| 11/08/1996    | 17.5     | 33.8     | 4.84                |
| 14/08/1996    | 17.5     | 33.8     | 2.76                |
| 14/09/1996    | 16.0     | 34       | 3.28                |
| 17/09/1996    | 16.0     | 34       | 3.28                |
| 06/10/1996    | 15.0     | 33.7     | 1.05                |
| 10/10/1996    | 15.0     | 33.7     | 1.52                |
| 30/10/1996    | 15.0     | 33.7     | 1.35                |
| 17/11/1996    | 10.1     | 33.6     | 0.48                |
| 28/11/1996    | 7.0      | 31.76    | 1.05                |
| 01/12/1996    | 7.0      | 33.1     | 0.62                |
| 12/12/1996    | 7.0      | 33.6     | 0.76                |
| 01/02/1997    | 5.0      | 33.04    | 0.33                |
| 05/02/1997    | 5.0      | 33.04    | 0.95                |
| 05/04/1997    | 8.0      | 33.8     | 2.11                |
| 12/04/1997    | 8.0      | 33.7     | 8.42                |
| 16/04/1997    | 9.0      | 33.7     | 8.42                |
in December ($R$, mean ± SE, 40.4 ± 5.7 nl O$_2$ ind.$^{-1}$ h.$^{-1}$) (Fig. 2c). In contrast, copepod DW (mean ± SE) ranged between 19.6 ± 1.1 μg in September and 42.9 ± 3.7 μg in February (Fig. 2c,d).

The seasonal change in EPR at the study site has already been reported in detail by Castellani & Altunbaş (2006). Briefly, *Temora longicornis* produced eggs all year round. A mean (± SE) EPR maximum of 48 ± 7 eggs female$^{-1}$ d$^{-1}$ occurred in spring–early summer during the phytoplankton bloom, whereas a minimum of 1.3 ± 0.5 eggs female$^{-1}$ d$^{-1}$ was recorded in autumn–winter (Fig. 2e).

*In situ* copepod respiration rate followed the variation in DW, chl a, EPR and $T$ (Fig. 2). Logarithmically transformed respiration rate (ln $R$) was significantly correlated with ln DW ($r = 0.44$; df = 297; $p < 0.001$), ln Chl ($r = 0.33$; df = 297; $p < 0.001$), ln EPR ($r = 0.33$; df = 186; $p < 0.001$), $T$ ($r = 0.23$; df = 297; $p < 0.001$) and salinity ($r = 0.19$; df = 297; $p < 0.001$). Table 2 shows the results of the multiple regression analysis between ln $R$ and ln DW, $T$, ln Chl, and ln EPR for the temperature intervals 5 to 17.5 and 8 to 13°C (i.e. the temperature range within which respiration rate increased linearly with temperature, see Fig. 4). The normalised β-coefficient from the multiple regression analysis, over both temperature intervals, indicated that ln DW ($β = 0.6$ to 0.5) explained the largest proportion of variability in copepod $R$ followed by $T$ ($β = 0.4$ to 0.5), ln Chl ($β = 0.2$) and ln EPR ($β = 0.1$), whereas salinity was not significant. Table 2 also shows that the goodness of fit of the regression is higher for the 8 to 13°C temperature interval where respiration rate increases linearly with temperature.
Table 2. Multiple regression analysis between ln-transformed *Temora longicornis* respiration rate (ln* R*, nl O₂ ind.⁻¹ h⁻¹) with copepod body dry weight (ln* DW*) in situ temperature (*T*), chlorophyll *a* (ln* Chl*) and egg-production rate (ln (EPR + 1), eggs female⁻¹ d⁻¹). The thermal coefficient *Q*₁₀ and the percentage of body carbon requirement calculated from the equation models are also shown. θ: absolute temperature (in kelvin, K); *E*ₐ: activation energy. Note that the *F*-test for all regression models is statistically significant at the 1% level. *p < 0.10, **p < 0.05, ***p < 0.01

| Regression model | ln DW (µg) | *T* (°C) | ln Chl (µg l⁻¹) | ln (EPR+1) | Intercept | n      | r²    | *F*-value | *Q*₁₀ | 5°C  | 10°C  | 17.5°C |
|------------------|-----------|---------|----------------|------------|-----------|--------|-------|-----------|-------|------|-------|--------|
| **Temperature range: 5 to 17.5°C** |           |         |                |            |           |        |       |           |       |      |       |        |
| 1) ln *R* = 1.00 ln DW + 0.06 *T* + 0.03 | 1.00***   | 0.06*** | 0.03           | 299        | 47.6      | 180.94 | 1.89  | 4.46      | 6.02  | 9.16 |
|                  | (0.056)   | (0.005) |               | (0.218)    |           |        |       |           |       |      |       |        |
| 2) ln *R* = 0.96 ln DW + 0.06 *T* + 0.05 ln Chl + 0.21 | 0.96***   | 0.06*** | 0.05***        | 299        | 49.6      | 124.40 | 1.75  | 4.51      | 7.25  | 10.25|
|                  | (0.058)   | (0.005) | (0.014)        | (0.225)    |           |        |       |           |       |      |       |        |
| 3) ln *R* = 0.87 ln DW + 0.05 *T* + 0.05 ln Chl + 0.04 ln (EPR + 1) + 0.56 | 0.87***   | 0.05*** | 0.05**         | 188        | 43.9      | 36.79  | 1.57  | 4.75      | 7.62  | 9.90 |
|                  | (0.099)   | (0.007) | (0.017)        | (0.377)    |           |        |       |           |       |      |       |        |
| **Temperature range: 8 to 13°C** |           |         |                |            |           |        |       |           |       |      |       |        |
| 4) ln *R* = 1.04 ln DW + 0.11 *T* − 0.5 | 1.04***   | 0.11*** | −0.50*         | 189        | 53.2      | 146.81 | 2.88  | 18.2      |       |      |       |        |
|                  | (0.067)   | (0.010) |               | (0.272)    |           |        |       |           |       |      |       |        |
| 5) ln *R* = 1.00 ln DW + 0.09 *T* + 0.05 ln Chl − 0.25 | 1.00***   | 0.09*** | 0.05***        | 189        | 54.7      | 99.33  | 2.39  | 7.07      |       |      |       |        |
|                  | (0.071)   | (0.011) | (0.017)        | (0.290)    |           |        |       |           |       |      |       |        |
| 6) ln *R* = 0.83 ln DW + 0.07 *T* + 0.06 ln Chl + 0.03 ln (EPR + 1) + 0.46 | 0.83***   | 0.07*** | 0.06**         | 121        | 52.5      | 32.35  | 2.01  | 6.83      |       |      |       |        |
|                  | (0.119)   | (0.014) | (0.025)        | (0.474)    |           |        |       |           |       |      |       |        |
| **Temperature range: 5 to 17.5°C** |           |         |                |            |           |        |       |           |       |      |       |        |
| 7) ln *R* = 0.99 ln DW − (5163/K*T*) + 18.9 | 0.99***   | −5163*** |              | 299        | 47.8      | 180.16 | 42.93 | 0.45      |       |      |       |        |
|                  | (0.055)   | (385.64)|               |           |           |        |       |           |       |      |       |        |
| 8) ln *R* = 0.95 ln DW − (4585/K*T*) + 0.05 ln Chl + 16.9 | 0.95***   | −4585*** | 0.045***       | 299        | 49.5      | 122.25 | 38.12 | 0.39      |       |      |       |        |
|                  | 0.058     | 395.83  | 0.014          | 1.31       |           |        |       |           |       |      |       |        |
| 9) ln *R* = 0.85 ln DW − (3670/K*T*) + 0.05 ln Chl + 0.03 ln (EPR + 1) + 14.01 | 0.85***   | −3670*** | 0.046**        | 14.01      | 35.8      | 43.7   | 30.51 | 0.31      |       |      |       |        |
|                  | 0.099     | 570     | 0.0198         | 1.83       |           |        |       |           |       |      |       |        |
| **Temperature range: 8 to 13°C** |           |         |                |            |           |        |       |           |       |      |       |        |
| 10) ln *R* = 1.01 ln DW − (8627/K*T*) + 31.1 | 1.01***   | −8627*** |              | 189        | 53.6      | 144.02 | 71.72 | 0.74      |       |      |       |        |
|                  | 0.067     | 812     | 2.83           |           |           |        |       |           |       |      |       |        |
| 11) ln *R* = 0.99 ln DW − (7259/K*T*) + 0.04 0.989* −7259*** + 0.041** ln Chl + 26.3 | 0.071     | 912   | 0.0177         | 3.15       |           |        |       |           |       |      |       |        |
|                  | 0.071     | 912     | 0.0177         | 1.35       |           |        |       |           |       |      |       |        |
| 12) ln *R* = 0.81 ln DW − (5740/K*T*) + 0.05 0.81* −5740*** + 0.054** ln Chl + 0.03 ln (EPR + 1) + 21.48 | 0.11   | 1182 | 0.025          | 4.02       |           |        |       |           |       |      |       |        |

*Note that the *F*-test for all regression models is statistically significant at the 1% level. *p < 0.10, **p < 0.05, ***p < 0.01*
Relationship between respiration and body DW

Copepod respiration rate increased with DW with the largest copepods showing the widest range in rates (Fig. 3). Table 3 summarises the relationship between respiration rate and body DW of Temora longicornis for different months and in situ temperatures. Following logarithmic transformation, the slopes (i.e. b) of the equations ranged from 0.83 to 1.35, and t-test analysis indicated that they were not significantly different from unity. Comparison of the slopes using analysis of covariance (ANCOVA) (generalized linear model [GLM], df = 9, F = 0.44, p = 0.915) also showed that they were not significantly different from each other. Overall, the total yearly variation in copepod respiration rates with DW was larger than the variation for each individual month, and a positive trend in respiration rate with temperature was evident (Fig. 3). Statistical analysis using ANCOVA (df = 9, F = 25, p < 0.0001) confirmed that the intercepts of the regression equations in Table 3 were significantly different from each other.

Relationship between respiration and in situ temperature

The variation in mean monthly weight-specific respiration rate ($R_{sp}$, nl O$_2$ g$^{-1}$ DW h$^{-1}$) with temperature was characterised by a sigmoid pattern (Fig. 4); $R_{sp}$ remained at its lowest between 5 and 7°C from December to April, it increased between 8 and 13°C from April to May and reached a maximum plateau between 15 and 17.5°C from June to August. The pattern of $R$ with temperature did not change for different DW, suggesting that the sigmoid trend was not caused by seasonal variation in copepod body mass. A logistic function model was fitted to the data set as such model fit statistically ($r^2 = 93\%$, see Fig. 4) better than the linear regression model ($r^2 = 86\%$). The multiple regression model shown in Table 2 predicts that the highest rate of increase in the acclimatised respiration of Temora longicornis occurs between 8 and 13°C (i.e. $Q_{10} = 2.01$ to 2.88). In contrast, above and below this temperature range, $R_{sp}$ remains virtually constant (i.e. $Q_{10} = 1$). Overall, the change in $R_{sp}$ over the whole in situ annual temperature range was characterised by $Q_{10}$ varying from 1.57 to 1.89 depending on the variables included in the regression model (Table 2a).

Table 2b shows the $E_a$ values calculated from the Arrhenius equation (i.e. Eq. 4). $E_a$ varied from 30.5 to 42.9 KJ mol$^{-1}$ (or 0.31 to 0.45 eV) over the whole in situ temperature range reaching the highest values...
between 47.7 and 71.7 KJ mol⁻¹ (or 0.49 to 0.74 eV) when calculated over the 8 to 13°C temperature range.

**Relationship between respiration with chl a and EPR**

Figs. 5 & 6 show the pattern of increase in $R$ with Chl and EPR. Both relationships were characterised by an asymptotic trend with $R$ rates reaching a maximum around 5 μg Chl l⁻¹ and 15 egg female⁻¹ d⁻¹. These results suggest that $R$ reaches its maximum values during the phytoplankton bloom, i.e. when feeding conditions for copepods are most favourable (Fig. 2b). Moreover, the relatively constant $R$ (i.e. energy consumption) for EPR increasing from ~15 to 50 eggs female⁻¹ d⁻¹ suggests higher reproduction efficiency possibly linked to higher food quality, such as the increase in nitrogen-rich ciliate diet during the spring bloom.

**Changes in respiration rate with gender**

Adult males represented ~8% of the total copepods in the population sampled during the present study, and they were smaller than females. As a result, the mean $R_p$ of *Temora longicornis* males (1.67 ± 0.12 nl O₂ μg⁻¹ DW h⁻¹) was on average lower than that of females (2.27 ± 0.048 nl O₂ μg⁻¹ DW h⁻¹). However, comparison of copepods of similar DW measured at the *in situ* temperature of 6.5°C showed that the respiration rate of the males did not differ significantly from that of the females (Table 4).

Table 3. Summary of regression analysis between *Temora longicornis* ln-transformed respiration rate (ln $R$, nl O₂ ind⁻¹ h⁻¹) and body dry weight (ln DW, μg) (see Fig. 3). The temperature ($T$, °C) at which respiration rate was measured, the number of measurements ($n$), the mean (SD) copepod prosome length (PL, μm), DW and $R$ are also shown. *p < 0.10, **p < 0.05, ***p < 0.01; coeff.: coefficient

| Date   | $T$ | n  | PL Mean (SD) | DW Mean (SD) | $R$ Mean (SD) | Intercept Coeff. (SE) | Slope Coeff. (SE) | $r^2$ (%) | $F$-value |
|--------|-----|----|--------------|--------------|---------------|-----------------------|-------------------|----------|-----------|
| Feb 1997 | 5   | 24 | 1081.7 (126.1) | 37.5 (12.3) | 60.6 (23.8) | 1.07* (0.581) | 0.83*** (0.164) | 46.86 | 25.46 |
| Dec 1996 | 7   | 62 | 1043.6 (97.6) | 33.5 (8.4)  | 50.5 (22.0) | 0.02 (0.481) | 1.09*** (0.139) | 38.96 | 61.54 |
| Apr 1997 | 8   | 25 | 1111.2 (82.9) | 39.7 (8.5)  | 68.9 (19.2) | 0.78 (0.750) | 0.93*** (0.203) | 47.21 | 21.29 |
| May 1996 | 10  | 35 | 1016.0 (110.7)| 31.4 (9.5)  | 73.2 (28.7) | 0.75 (0.474) | 1.02*** (0.137) | 53.05 | 55.08 |
| Nov 1996 | 10.1| 20 | 966.0 (41.1)  | 26.6 (3.2)  | 52.6 (11.9) | 0.96 (1.077) | 0.91*** (0.333) | 26.39 | 7.49  |
| Jun 1996 | 12  | 17 | 1007.1 (80.0) | 30.2 (7.3)  | 69.3 (21.9) | 1.00* (0.537) | 0.94*** (0.151) | 39.67 | 39.32 |
| Jun 1996 | 13  | 30 | 986.0 (82.9)  | 28.5 (6.5)  | 78.4 (23.3) | 1.24** (0.570) | 0.93*** (0.170) | 49.42 | 29.72 |
| Oct 1996 | 15  | 29 | 910.3 (56.0)  | 22.6 (3.9)  | 63.5 (14.3) | 0.92* (0.539) | 1.03*** (0.170) | 60.11 | 36.81 |
| Sep 1996 | 16  | 29 | 872.4 (65.6)  | 20.2 (4.0)  | 60.2 (25.0) | −0.01 (0.652) | 1.35*** (0.213) | 47.6  | 40.19 |
| Aug 1996 | 17.5| 28 | 960.0 (65.3)  | 26.3 (5.1)  | 73.2 (18.7) | 1.40*** (0.463) | 0.88*** (0.143) | 46.16 | 38.21 |
DISCUSSION

Seasonal variation of copepod respiration rate

We investigated the seasonal variation in the respiration rate of *Temora longicornis* acclimatised to field conditions, during a 1yr study, by measuring individually 299 adults over the full range of copepod DW, EPR, *T*, salinity and chl a level as a proxy for prey availability. In our study, *T. longicornis* respiration increased from a minimum in December to a maximum during May and August (Fig. 2). Multiple regression analysis showed that seasonal changes in ln *R* were significantly related to ln DW, ln *T*, ln Chl and ln EPR (Table 1a). A spring increase in the respiration rate of *T. longicornis* has been reported before by Marshall & Orr (1966) in the Clyde Sea and by Conover (1959) in Southampton waters; whereas Marshall & Orr (1966) attributed the rise in the respiration rate of *T. longicornis* mainly to an increase in copepod body size, Conover (1959) argued that copepod metabolic rates may be ‘geared to the phytoplankton bloom’. However, neither of these authors analysed their field data statistically or tested experimentally the factors that might have affected copepod respiration, rendering conclusions from their study speculative. Critically, all previous field investigations disregarded the effect of acute temperature exposure on copepod respiration rate by measuring *T. longicornis* at a fixed arbitrary temperature (Conover 1959, Raymont 1959, Berner 1962, Marshall & Orr 1966; see Table 5). In addition, these studies were mainly limited to between March and July. Thus, limited data sets and differences in methodology make the interpretation of published data on copepod respiration difficult and their use in predictive models questionable. In this respect, our investigation represents one of the largest and most comprehensive studies ever conducted on the seasonal changes in the respiration rate of a single copepod species acclimatised to *in situ* conditions.

Dependence of respiration on body size

In our study, copepod DW explained the largest proportion of variability in copepod respiration rate. This finding is not surprising since body mass is an important determinant of the metabolic rate of an organism (Peters 1986, Schmidt-Nielsen 1991). On the other hand, the weight exponent *b* of the power function (i.e. the slope of the ln function) between respiration rate and body weight has been reported to vary widely across taxa (Glazier 2006). In our study, *b* varied from 0.83 to 1.34 (Tables 2 & 3, Fig. 3), and it was not significantly different from unity, indicating direct proportionality (i.e. isometric scaling) between respiration rate and body mass. Conover (1959) reported a weight exponent of 0.76 for *Temora longicornis* from Southampton waters. Using the data published by Marshall & Orr (1966), we calculated a weight exponent (±1 SE) of 1.22 ± 0.23 (df = 6; *F* = 27.9; *r*² = 81.8%; *p* < 0.003) for *T. longicornis* from the Clyde Sea. Thus, the weight exponent we estimated for *T. longicornis* in our study is similar to values reported by previous authors for this species and to the range of 0.5 and 1 generally reported for copepods (Mauchline 1998, Ikeda et al. 2000).

Although it is generally assumed that metabolic rate increases as the body mass increases to the power of $\frac{2}{3}$ (i.e. 0.66, proportional to body surface) or $\frac{3}{4}$

Table 4. *Temora longicornis*. Result of the 2-sample *t*-test statistic comparing the mean (±1 SE) respiration rate (*R*, nl O₂ ind.−¹ h⁻¹) and weight-specific respiration rate (*R*_sp, nl O₂ μg⁻¹ DW h⁻¹) of individual (n) female and male *T. longicornis* of similar prosome length (PL, μm) and dry weight (DW, μg) at 6.5°C

| Gender | n  | PL (±10.4) | DW (±0.93) | *R* (±4.4) | *R*_sp (±0.11) |
|--------|----|------------|------------|------------|---------------|
| Female | 13 | 1060       | 34.4       | 65.9       | 1.91          |
| Male   | 15 | 1048 (± 9.11) | 33.32 (± 0.81) | 56 (± 5.1) | 1.68 (± 0.15) |
| *t*-value | 1.48 | 1.23 | | |
| *p*-value | 0.15 | 0.23 | | |
Castellani & Altunbaş: Acclimatised respiration rate of *Temora longicornis*

(i.e. 0.75, intermediate proportionality between body weight and surface), there is still no broad consensus regarding the value of this coefficient (Kleiber 1961, Brown et al. 2004, Downs et al. 2008, Glazier 2010, Kolokotrones et al. 2010, Agutter & Tuszynski 2011). West et al. (1997) have argued that the ¾ metabolic scaling is the result of the fractal geometry of the internal network present in organisms (fractal network theory [FNT]), including the circulatory and respiratory systems. However, recent theoretical and empirical research has questioned the ¾-power law and the FNT model proposed to explain it by showing that such model is based on questionable or unsubstantiated assumptions (Glazier 2009, 2010, Kolokotrones et al. 2010, Agutter & Tuszynski 2011). West et al. (1997) have argued that the ¼ metabolic scaling is the result of the fractal geometry of the internal network present in organisms (fractal network theory [FNT]), including the circulatory and respiratory systems. However, recent theoretical and empirical research has questioned the ¾-power law and the FNT model proposed to explain it by showing that such model is based on questionable or unsubstantiated assumptions (Glazier 2009, 2010, Kolokotrones et al. 2010, Agutter & Tuszynski 2011). West et al. (1997) have argued that the ¼ metabolic scaling is the result of the fractal geometry of the internal network present in organisms (fractal network theory [FNT]), including the circulatory and respiratory systems. However, recent theoretical and empirical research has questioned the ¾-power law and the FNT model proposed to explain it by showing that such model is based on questionable or unsubstantiated assumptions (Glazier 2009, 2010, Kolokotrones et al. 2010, Agutter & Tuszynski 2011).

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Kolokotrones et al. (2010) have shown that the relationship between metabolic rate and body mass has a convex curvature on a logarithmic scale, suggesting that the metabolic coefficient is highly sensitive to the body mass range used. Moreover, Agutter & Tuszynski (2011) advocated that the quantum metabolism (QM) theory, which adopts a molecular-cellular perspective, can be used to predict the large variations in body scaling exponents and to predict the temperature dependence of the proportionality constant, issues that have eluded models such as the FNT. Interestingly, isometric scaling of metabolic rate appears to be common in planktonic animals, and Glazier (2006, 2009) argued that it probably represents an adaptation to the high-energy cost of continual swimming to stay afloat, rapid growth rates and high reproductive rates in response to high levels of mortality in open water.

### Relationship between respiration rate and temperature

The respiration rate of *Temora longicornis* increased with temperature (Fig. 4), similar to the pattern generally reported for other poikilotherms (Schmidt-Nielsen 1991, Mauchline 1998, Castellani et al. 2005). Interestingly, respiration rates from our study overlap with rates reported by Berner (1962) and Marshall & Orr (1966) at 10°C and Le Ruyet-Person et al. (1975) at 16 to 17°C, but they are lower than rates reported by Raymont (1959) at 15°C and by Conover (1959) at 20°C for *T. longicornis* of comparable DW (Fig. 7) measured over a similar temperature range (Fig. 8). One of the reasons for such discrepancy may be attributed to methodological differences between studies; whereas we measured individually the respiration rate of fasting copepods acclimatised to environmental conditions, previous authors measured groups of copepods exposed to variable feeding conditions at a fixed arbitrary temperature (see Table 5). Berner (1962) and Marshall & Orr (1966) measured the respiration rate of fasting

| PL (μm) | DW (μg) | FT | RV (ml) | Cop (n) | ET (°C) | AT (°C) | R (nl O$_2$ ind.$^{-1}$ h$^{-1}$) | Method (incubation time) | Source                      |
|--------|--------|----|---------|--------|--------|--------|-------------------------------|--------------------------|---------------------------|
| 720−1340 | 12−66  | 9−12 | 0.15    | 1      | 5−17.5 | 5−17.5 | 13−150 | Micro-electrode (1 h)         | Present study, Menai Strait, UK$^c$ |
| 973     | 27     | 24  | 0.07    | 1      | 16−17  | 16−17  | 87−186 | Clark-electrode (2−6 h)       | Le Ruyet-Person et al. (1975), Roscoff, France$^c$ |
| 594−968 | 7−27   | 24  | 7−35    | 1−10   | 10     | –      | 16−47  | Winkler (1−5 d)               | Berner (1962), Milliport, UK$^{c,d}$ |
| 722−1139| 12−42  | Over-night | 30−40  | 5−10   | 10     | –      | 24−143 | Winkler (40−50 h)             | Marshall & Orr (1966), Milliport, UK$^{c,d}$ |
| 859−1040| 19−33  | Fed$^a$ | 5      | 29−50  | 15     | 7−8    | 116−142| Barcroft-Dixon manometer (3 h) | Raymont (1959), Harvard, USA$^c$ |
| 804−1082| 16−36  | Over-night$^f$ | 5      | 50     | 20     | 5−10   | 98−183 | Barcroft-Dixon manometer (3 h) | Conover (1959), Southampton, UK$^c$ |
| 700−1030| 11−32  | –   | 5       | 30−62  | 10−20  | –      | 62−253 | Barcroft-Dixon manometer (3−4 h) | Gauld & Raymont (1953), Southampton, UK$^a$ |

$^a$Kept in lab overnight or up to 2 days feeding on cultured phytoplankton before experiment; $^b$measurements standardised to copepod of 1 mm PL; $^c$field experiment; $^d$Use of antibiotics streptomycin (5 mg l$^{-1}$) and chloromycetin (5 mg l$^{-1}$); $^e$laboratory experiment

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Table 5. Summary of *Temora longicornis* respiration rates ($R$) measured during either field or laboratory experiments, given as ranges. PL: prosome length, DW: dry weight, FT: length of time copepods fasted prior to experiment, RV: respirometer volume, Cop: number of copepods incubated, ET: experimental temperature, and AT: acclimation temperature. Dry weight estimated from the equation ln DW= −15.9 + 2.79 ln PL for *T. longicornis* (Castellani & Altunbaş 2006). –: no data available
Mar Ecol Prog Ser 500: 83–101, 2014

T. longicornis at 10°C over 40 to 50 h incubations between the end of March and mid-July. Le Ruyet-Person et al. (1975) measured single fasting copepods acclimatised to 16 to 17°C during 3 to 6 h of incubation in July. In contrast, Raymont (1959) and Conover (1959) measured copepods maintained in the laboratory between 5 and 10°C on cultured phytoplankton at 15 and 20°C, respectively, over 3 h experiments between February and June (Table 5). Exposing a poikilotherm to a sudden temperature change results in a shift in the physiological rate lasting minutes to hours, known as the acute rate (Cossins & Bowler 1987). This rate, which is used to construct the acutely measured metabolism-temperature curve (Prosser & Brown 1961) is generally followed by a new, steady or acclimated state, which is gradually acquired some hours to days after the temperature change (Pretch 1958). In T. longicornis, a temperature shift of 5 and 15°C requires ~2 and 6 d, respectively, before respiration stabilises to the new rate, intermediate between the original and the acute rate (C. Castellani & Y. Altunbaş pers. comm.). Therefore, it is likely that the long incubation times used by Berner (1962) and by Marshall & Orr (1966) enabled copepod respiration to acclimate to their 10°C experimental temperature following a shift of at most 6°C, considering in situ temperatures ranging from 4 to 16°C for their study area. The copepods measured by Le Ruyet-Person et al. (1975) were also acclimatised, and therefore, their data coincide with our measurements. In contrast, the high respiration rates reported by Raymont (1959) and Conover (1959), who measured copepods over a short time (i.e. 3 h) following temperature increases of up to ~15°C, probably corresponded to acute respiration rates (Table 5).

In our study, the respiration rate of Temora longicornis acclimatised to field temperatures between 5 and 17.5°C followed a sigmoid pattern with Q10 values ranging between 1 and 2.88 depending on the temperature interval (mean Q10 ranging between 1.56 and 1.88; Table 2, Fig. 4). In contrast, the relationship we obtained from a compilation of published field data for T. longicornis was characterised by an exponential increase in respiration between 10 and 20°C and by a higher mean Q10 of 3.09 (Fig. 8). The Q10 reported for the respiration rates of marine copepod usually range between 2 and 4, although values above and below this range have also been reported (Hirche 1987, Mauchline 1998, Gaudy & Thibault-Botha 2007). It is noteworthy, however, that Q10 > 2 are generally estimated from acute measurements of copepods maintained in the laboratory on high microplankton concentrations (Cossins & Bowler 1987, Ikeda et al. 2001, Castellani et al. 2005). For instance, using data from Gauld & Raymont (1953) and our own data (Fig. 9a) on the acute respiration rates of fasting T. longicornis maintained on an ad libitum microalgal diet, we calculated mean Q10 values of 2.4 for the temperature interval 4 to 20°C. As mentioned above, the acutely measured respiration rate is a transient and short-lived shock response of an organism exposed to a sudden and often unrealistic change in temperature (e.g. the 15°C temperature shift applied by Raymont 1959 and Conover 1959), and as such, it represents a distortion of the ‘natural’ respiration rate. In contrast, the acclimatised respiration rate we measured in the present study displays the ecologically meaningful relationship between metabolism and the ambient conditions experienced.
Castellani & Altunbaş: Acclimatised respiration rate of *Temora longicornis*

by the organism (Cossins & Bowler 1987). Hence, the high $Q_{10}$ we obtained pooling published field data on *T. longicornis* was probably the result of the acute measurements by Raymont (1959) and Conover (1959).

Respiration is typically measured on fasting copepods (Berner 1962, Marshall & Orr 1966, Ikeda et al. 2001, present study Table 5) to avoid the added effect of SDA (Kiørboe et al. 1985, Thor 2000, Secor 2009). However, the copepods measured by Raymont (1959) and Conover (1959) were maintained in the laboratory on cultured microalgae, and it is unclear whether they had been fasting prior to experiment. Since the respiration rate of *Temora longicornis* takes ~10 h to decrease to the routine rate after copepods are deprived of food (Fig. 1), it is possible that the higher respiration rates measured by Raymont (1959) and Conover (1959) were also partly due to the effect of SDA.

The low $Q_{10}$ of 1 we measured in the present study during winter and late summer shows that over that time of the year, copepod metabolism did not change with temperature (Fig. 4). Values of $Q_{10} < 2$ have been generally interpreted as the result of homeostasis, resulting from seasonal adjustments in enzymes concentration and type (Somero & Hochachka 1971), and thought to confer a metabolic advantage to organisms living in an environment with fluctuating temperature (Gaudy 1973, Gaudy & Thibault-Botha 2007). However, Clarke (1993) has argued that attempting to explain seasonal variation in oxygen consumption as a direct response of metabolic rate to temperature is simplistic because respiration represents a cost to an organism, i.e. ATP demand for physiological processes such as growth, reproduction and locomotion besides the maintenance of basic bodily functions. Organisms are adapted to minimise energetic costs, and therefore, their cells will not synthesize ATP (i.e. will not increase $R$) unless it is required to produce work and unless they have sufficient energy to do so. Hence, Clarke (1993) has proposed that seasonal changes in the oxygen consumption of poikilotherms could reflect changes in their growth and reproductive rates. Interestingly, we obtained the lowest rate of increase in respiration (i.e. $Q_{10} = 1$) during winter and late summer when both prey concentration and copepod reproductive rates were lower (Fig. 2b,e). In contrast, we measured the highest $Q_{10}$ (between 2.01 and 2.88) during spring–early summer at a time when *in situ* feeding conditions for the copepods were optimal and reproductive activity was maximal (Table 2, Figs. 2 & 4). Similarly, Ikeda et al. (2001) reported $Q_{10}$ values between 1.8 and 2.1 for the respiration rate of fasting copepod species acclimatised between −1 and 30°C calculated only from measurements made during spring–summer when feeding conditions were most favourable.

A comparison of acclimatised respiration rates, from the present study, with acclimated and acute respiration rates suggests that the response of *Temora longicornis* metabolism to temperature depends on both exposure time and nutritional conditions (Fig. 9). The respiration of field-acclimatised copepods, which experience a lower and more variable availability/quality of prey, was the most variable (i.e. sigmoid trend) and the mean rate of change was the lowest ($Q_{10} = 1.56$ to 1.88). The acclimated respiration of laboratory maintained copepods increased exponentially, but the rate of change was
intermediate ($Q_{10} = 2.1$). In acutely measured copepods, the response was also exponential, but the rate of increase was the highest ($Q_{10} = 2.4$). However, Fig. 9a shows a substantial overlap between the 3 data sets, particularly in the middle of the temperature range. The logarithmic transformation did not linearise the sigmoidal pattern of the field-acclimatised respiration rates, and therefore, a statistical comparison between the slopes of the relationships was not possible. Nevertheless, we compared the data sets using a Two-sample t-test with equal variances at each of the common temperatures. Our results show that the respiration rates of field-acclimatised copepods was significantly higher at 5°C ($df = 40, t = 2.54, p = 0.014$) and significantly lower at 17.5°C ($df = 35, t = −3.31, p = 0.0021$) compared to that of acutely measured copepods. Similarly, field-acclimatised respiration rates were significantly lower than laboratory-acclimated rates at 17.5°C ($df = 34, t = −2.33, p = 0.025$), but at 5°C they were significantly higher than acclimated rates only at 10% ($df = 37, t = 1.48, p = 0.0734$). There were no significant differences between the respiration rates of acute and laboratory-acclimated rates. The higher respiration rates we measured for acclimatised copepods at 5°C may be the result of not only temperature acclimatisation but also of the favourable feeding conditions encountered by the copepods in the field at the beginning of February and the associated high reproductive rates; at this time, Chl had already increased above winter level to reach ~1 μg l$^{-1}$ due to a mixed diatom bloom, and this promoted an increase in copepod EPR (mean ± SE: 16 ± 9.1 eggs female$^{-1}$ d$^{-1}$; range: 0 to 61 eggs female$^{-1}$ d$^{-1}$). Diatoms are known to support higher EPR in $T. longicornis$ compared to other phytoplankton diets (Dam & Lopes 2003, Jónasdóttir et al. 2009), including the flagellate diets we fed to the copepods in our acclimated and acute respiration rates experiments. Similarly, the lower respiration rates we measured for the copepods from the field at 17.5°C may be the result of poorer feeding conditions (i.e. Noctiluca spp. bloom and the absence of diatoms) in the field, compared to copepods maintained on a high flagellate diet in the laboratory, and associated lower reproductive rates (see also next section). In support of our argument, Fig. 10 shows a sigmoid trend in the EPR$_{sp}$ of $T. longicornis$ (measured during the same study and published by Castellani & Altunbaş 2006) with temperature which is very similar to the acclimatised respiration rate trend presented in Fig. 4 and Fig. 9a. Furthermore, the logistic equation we fitted to the acclimatised respiration rate data in Figs. 4 & 9a is very often used to describe the growth of organisms, and hence, our results suggest that the seasonal trend in in situ respiration rate we measured largely reflects female copepod growth rate, i.e. their reproductive rate (Fig. 10). In summary, our findings suggest that respiration rates measurements carried out under acute temperature exposure are not representative of field-acclimatised copepod respiration rates, particularly at the extreme of the temperature range and depending on the nutritional conditions of the copepods. It is worth noting, however, that our findings may not apply to copepod species that store large body lipid reserves or those who experience large temperature changes over a short time scale through, for instance, diel vertical migration.

**Effect of food sources and reproduction on respiration**

The positive significant relationship we found between $Temora longicornis$ respiration rates and ambient Chl levels suggests that seasonal changes in nutritional conditions were an important determinant of copepod metabolic rates (Table 2). Our results support the observation made by Conover (1959) that the spring increase in the respiration rate of $T. longicornis$ might ‘be geared in some way to its food supply’. Similarly, other studies have reported higher respiration rates for $Calanus finmarchicus$, $C. helgolandicus$ and $C. hyperboreus$ during the phytoplankton bloom in spring and summer compared to rates in winter (Marshall & Orr 1958, Conover & Corner 1968, Butler et al. 1970).
Aerobic metabolism is tightly coupled with feeding, particularly in small copepods such as Temora longicornis that do not store large lipid reserves (Clarke & Walsh 1993, Kreibich et al. 2008). For instance, citrate synthase, which is an important metabolic key enzyme of the tricarboxylic acid cycle, decreases after only 24 h in starving T. longicornis (Clarke & Walsh 1993). The increase in respiration rate of fed copepods, i.e. SDA, is largely related to protein biosynthesis (i.e. to growth and reproduction) and protein metabolism rather than to the mechanical filtering and ingestion of the food (Kierboe et al. 1985, Thor 2000, Clarke & Fraser 2004, Secor 2009). However, protein synthesis is also a key component of basal metabolic rate (e.g. protein turnover, Clarke & Fraser 2004). Basal metabolic rate represents, in fact, the continuous cost the organism must meet to repair cell damage (e.g. protein turnover) and to conduct protein recycling (e.g. enzymes). However, the recycling of enzymes is probably not constant and is associated with the rate of growth, which depends on the type and quantity of substrate metabolised (Flynn 2005, Hochachka & Somero 2002). Thus, the ‘basal rate’ of a fasting poikilotherm can be expected to change according to the growth rate of the organism. The respiration rate of a starved copepod will also depend on the level and type of substrate metabolised (i.e. either protein or lipids) and its body reserves. The copepods we collected in the field were feeding on different concentrations and qualities of food sources, i.e. either lower or higher compared to the monospecific algal diet we fed to the copepods maintained in the laboratory. Since both the acclimatised and acclimated copepods in our study were fasted for the same length of time prior to measurement, the higher respiration rates we recorded in acclimated copepods at 17.5°C compared to those acclimatised in the field appear to be the result of a higher level of protein turnover and/or anabolism supported by the constant high prey supply in the laboratory (Fig. 9).

In our study, the seasonal change in Temora longicornis respiration rate was also significantly related to EPR. Coupling between egg production and respiration rate has been reported in Acartia tonsa (Kierboe et al. 1985, Thor 2003), and Conover (1962) observed that ripe Calanus hyperboreus females had higher respiratory rates than immature or spent ones. In adult female copepods, anabolic processes are mainly linked to the cost of reproduction (e.g. in the copepod A. tonsa, Kierboe et al. 1985). Several authors have also suggested that body size, food availability and temperature often indirectly influence metabolic rates through their effects on growth rates, rather than directly (Parry 1983; see Clarke 1993 for a critical review of this topic). For instance, Parry (1983) showed that the ‘cost of growth’ could account for up to 80% of ectotherm metabolism, and he interpreted seasonal changes in respiration rates of marine poikilotherms as a reflection of changes in synthetic activity rather than simply the result of a mechanistic response of metabolism to temperature changes. Indeed, the protein content (hence the synthetic activity) of T. longicornis fluctuates over the year as a result of changes in the nutritional conditions experienced by the copepods (Helland et al. 2003). In our study, however, EPR explained the lowest proportion of variability in respiration compared to other variables. This result is not surprising since copepod respiration combines different energetic costs in addition to that of producing eggs. It is also difficult to equate rapid changes in EPR with metabolism because of the lag time between the formation (i.e. demand for ATP) and the release of eggs (Tester & Turner 1990). In addition, in our study, we measured respiration on fasting copepods and EPR on copepods that had been feeding before incubation (see ‘Materials and methods’). Furthermore, the asymptotic relationship between respiration and EPR (Fig. 6) indicates an increase in reproductive efficiency in spring that might have resulted from an increase in food quality (e.g. the increase in N-rich ciliates during the spring bloom). The results of a recent laboratory study (R. Nobili et al. pers. comm.) showing a decrease in the reproductive cost of T. longicornis with increase in the N:P ratio of the diet support our field observation. The similarity in the pattern of EPR and respiration rate with temperature shown in Fig. 4 and Fig. 10, as already discussed in the previous section, also supports our view that seasonal changes in metabolic rates were driven by seasonal changes in copepod growth rates. Overall, our results suggest that reproductive activity (i.e. anabolic processes) modulated by food availability and possibly quality also contributed to the observed seasonal changes in the respiration rate of T. longicornis.

Metabolic coefficients and the importance of acclimatised rates for predictive models

Using the correct metabolic coefficient values is critical to estimate energy flow and secondary production. Our study has shown that the body mass exponent of Temora longicornis scaled isometrically
between 5 and 17.5°C ranged between 31 and 43 kJ mol⁻¹, or 0.31 to 0.45 eV (i.e. 1 eV = 96.49 kJ mol⁻¹) (Fig. 9b, Table 2b). These values are well below the $E_a$ range of 0.60 to 0.70 eV and the mean $E_a$ values of 0.63 eV (excluding endotherms) predicted by the MTE (Brown et al. 2004). We recorded the highest $E_a$ (0.61 eV) only in the temperature interval 8 to 13°C, that is, during the phytoplankton bloom when copepods were exposed to good feeding conditions and actively reproducing (see 'Relationship between respiration rate and temperature'). Thus, our results indicate that during the largest part of the year, the respiration rate of T. longicornis would be well below the rates predicted by the MTE equation. This result has important implications for the interpretation of the response of plankton to environmental change and for predictive ecological modelling. In summary, the relationships between physiological rates and ecologically relevant parameters such as temperature and body size, upon which global predictive models rely, are incomplete and subject to significant methodological artefacts. Additional detailed studies of acclimatised in situ rates as a function not only of temperature and body size but also of other key parameters such as the nutritional conditions, reproductive and growth rates and the genetic ‘make-up’ of the organism are essential to improving these relationships and reducing uncertainties in predicted ecosystem responses to environmental change.

How much carbon is required by T. longicornis to support routine metabolism?

Our results provide information about individual variability of Temora longicornis respiratory response and energy demand. Metabolic studies based on the O:N ratio have indicated that the main substrate catabolized in small calanoid copepods, such as T. longicornis, is protein (Bamstedt 1988, Thor 2000) because these species usually do not store lipids and carbohydrates in large quantities (Evjemo & Olsen 1997, Helland et al. 2003). Thus, considering a respiratory quotient $RQ = 0.8$ for protein catabolism (Schmidt-Nielsen 1991) and that $\mu g$ carbon catabolized = $0.8 \times \mu l O_2 d^{-1}$ respired $\times (12 \; \mu g \; C \; \mu mol^{-1}/22.4 \; \mu l \; \mu mol^{-1})$, the daily carbon requirement by a T. longicornis of 1000 $\mu m$ in length, estimated from our multiple regression model (Table 2), would vary between ~5 and 10% of body carbon at 5 and 17°C, respectively. However, Table 2 also shows that using DW and T only results in an estimated copepod C consumption almost 3-fold higher than that esti-
CONCLUSIONS

Using one of the most comprehensive data sets on acclimatised copepod respiration rate, our study has shown that in situ seasonal changes in Temora longicornis metabolism are the result not only of body mass and temperature but also of copepod nutritional and reproductive conditions. Thus, the constancy of metabolic rate with temperature increase that we observed in winter and late summer appears to be the result of reduced anabolic processes due to food limitation rather than simply a seasonal physiological adjustment to temperature, i.e. homeostasis. The lower values of $Q_{10}$ and $E$, we measured in our studies compared to those reported in the literature also suggest that metabolic rates of acclimatised copepods may be less responsive to temperature changes than has been inferred so far from thermal coefficients calculated from acutely measured rates of copepods maintained in the laboratory on high food rations. The value of the thermal coefficient also differed according to the type of variable used in the regression model. Therefore, using high and fixed coefficient values may lead to overestimation or misrepresentation of copepod respiration, particularly under ambient conditions limiting growth and reproduction. Our results have important implications for ecological models aiming to predict energy flow in marine food-webs and to determine the impact of climate change on copepod metabolic rates.

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LITERATURE CITED

Agutter PS, Tuszynski JA (2011) Analytic theories of allometric scaling. J Exp Biol 214:1055–1062

Bamstedt U (1988) Ecological significance of individual variability in copepod bioenergetics. Hydrobiologia 167/168: 43–59

Berner A (1962) Feeding and respiration in the copepod Temora longicornis (Muller). J Mar Biol Assoc UK 42: 625–640

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Towards a metabolic theory of ecology. Ecology 85: 1771–1789

Butler EI, Corner EDS, Marshall SM (1970) On the nutrition and metabolism of zooplankton. VIII. Seasonal survey of nitrogen and phosphorus excretion by Calanus in the Clyde Sea area. J Mar Biol Assoc UK 50:525–566

Castellani C, Altunbaş Y (2006) Factors controlling the temporal dynamics of egg production in the copepod Temora longicornis. Mar Ecol Prog Ser 308:143–153

Castellani C, Lucas IAN (2003) Seasonal variation in egg morphology and hatching success in the calanoid copepods Temora longicornis, Acartia clausi and Centropages hamatus. J Plankton Res 25:527–537

Castellani C, Robinson C, Smith T, Lampitt RS (2005) Temperature affects respiration rate of Oithona similis. Mar Ecol Prog Ser 283:129–135

Clarke A (1993) Seasonal acclimatization and latitudinal compensation in metabolism: Do they exist? Funct Ecol 7: 139–149

Clarke A, Fraser KPP (2004) Why does metabolism scale with temperature? Funct Ecol 18:243–251

Clarke ME, Walsh PJ (1993) Effect of nutritional status on citrate synthase activity in Acartia tonsa and Temora longicornis. Limnol Oceanogr 38:414–418

Conover RJ (1959) Regional and seasonal variation in the respiratory rate of marine copepods. Limnol Oceanogr 4:259–268

Conover RJ (1962) Metabolism and growth in Calanus hyperboreus in relation to its life cycle. J Cons Int Explor Mer 153:190–197

Conover RJ, Corner EDS (1968) Respiration and nitrogen excretion by some zooplankton in relation to their life cycles. J Mar Biol Assoc UK 48:49–75

Cossins AR, Bowler K (1987) Temperature biology of animals. Chapman & Hall, London

Dam HG (2013) Evolutionary adaptation of marine zooplankton to global change. Annu Rev Mar Sci 5:349–370

Dam HG, Lopes RM (2003) Omnivory in the calanoid copepod Temora longicornis: feeding, egg production and egg hatching rates. J Exp Mar Biol Ecol 282:119–137

Downs CJ, Hayes JP, Tracy CR (2008) Scaling metabolic rate with body mass and inverse body temperature: a test of the Arrhenius fractal supply model. Functional Ecology 22:239–244

Eden P (Ed) (1997) Weather Log 1996-1997. Royal Meteorological Society, Reading

Enquist BJ, Economou EP, Huxman TE, Allen AP, Ignace DD, Gillooly GF (2003) Scaling metabolism from organisms to ecosystems. Nature 423:639–642

Ejvemo JO, Olsen Y (1997) Lipid and fatty acid content in cultivated live feed organisms compared to marine copepods. Hydrobiologia 358:159–162

Flynn KJ (2005) Incorporating plankton respiration in models of aquatic ecosystem function. In: del Giorgio PA et al. (eds) Respiration in aquatic ecosystems. Oxford University Press, Oxford, p 248–266

Frasnch HG, Colebrook JM, Gamble JC, Krause M (1991) The zooplankton of the North Sea. Neth J Sea Res 28:1–52

Gaudry R (1973) Les variations saisonnières de la respiration chez quatre espèces de copepods pelagiens du golfe de Marseille. Neth J Sea Res 7:267–279 (in French)
Gaudy R, Thibault-Botha D (2007) Metabolism of Centropages typicus in the Mediterranean Sea and the North Atlantic Ocean. Prog Oceanogr 72:151–163

Gauld DT, Raymont JEG (1953) The respiration of some planktonic copepods II. The effect of temperature. J Mar Biol Assoc UK 31:447–460

Gillooly JF, Brown JH, West GB, Charnov EL (2001) Effects of size and temperature on metabolic rate. Science 293:2248–2251

Glazier DS (2006) The 3/4-power law is not universal: evolution of isometric, ontogenetic metabolic scaling in pelagic animals. Bioscience 56:325–332

Glazier DS (2009) Activity affects intraspecific body-size scaling of metabolic rate in ectothermic animals. J Comp Physiol B 179:821–828

Glazier DS (2010) A unifying explanation for diverse metabolic scaling in animals and plants. Biol Rev Camb Philos Soc 85:111–138

Green EJ, Carritt DE (1967) New tables for oxygen saturation

Hirche HJ (1987) Temperature and plankton. II. Effect on respiration and swimming activity in copepods from the Greenland Sea. Mar Biol 94:375–356

Hirst AG, Bunker AJ (2003) Growth of marine planktonic copepods: global rates and patterns in relation to chlorophyll a, temperature and body weight. Limnol Oceanogr 48:1988–2010

Ikeda T, Norimatsu H, Somero GN (2002) Biochemical adaptation: mechanisms and process in physiological evolution. Oxford University Press, New York, NY

Huntley ME, Lopez M (1992) Temperature-dependent production of marine copepods: a global synthesis. The American Naturalist 140:201–242

Ikeda T (1985) Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. Mar Biol 85:1–12

Ikeda T, Torres JJ, Hernandez-Leon S, Geiger SP (2000) Metabolism. In: Harris RP, Wiebe PH, Lenz J, Skjoldal HR, Huntley M (eds) Ices zooplankton methodology manual. Academic Press, San Diego, CA, p 453–532

Ikeda T, Kanno Y, Ozaki Y, Shinada A (2001) Metabolic rates of epipelagic marine copepods as a function of body mass and temperature. Mar Biol 139:587–596

Jönasdóttir SH, Visser AW, Jespersen C (2009) Assessing the role of food quality in the production and hatching of Temora longicornis eggs. Mar Ecol Prog Ser 382:139–150

Kanwisher JW (1959) Polarographic oxygen electrode. Limnol Oceanogr 4:210–217

Kierboe T, Nielsen TG (1994) Regulation of zooplankton biomass and production in a temperate coastal ecosystem. 1. copepods. Limnol Oceanogr 39:493–507

Kierboe T, Mohlenberg F, Hamburger K (1985) Bioenergetics of the planktonic copepod Acartia tonsa: relation between feeding, egg production and respiration and composition of specific dynamic action. Mar Ecol Prog Ser 26:85–97

Kleiber M (1961) The fire of life: an introduction to animal energetics. Wiley, New York, NY

Kolokotrones T, Savage V, Deeds EJ, Fontana W (2010) Curvature in metabolic scaling. Nature 464:753–756

Kreibich T, Saborowski R, Hagen W, Niehoff B (2008) Short-term variation of nutritive and metabolic parameters in Temora longicornis females (Crustacea, Copepoda) as a response to diet shift and starvation. Helgel Mar Res 62:241–249

Le Ruyet-Person J, Razouls C, Razouls S (1975) Biologie compare entre especes vicariantes et communes de copepods dans un ecosystems neritique en Mediterranee et en Manche. Vie Milieu 25:283–312 (in French with English abstract)

Marshall SM, Orr AP (1958) On the biology of Calanus finmarchicus. X. Seasonal changes in oxygen consumption. J Mar Biol Assoc UK 37:459–472

Marshall SM, Orr AP (1966) Respiration and feeding in some small copepods. J Mar Biol Assoc UK 46:513–530

Marshall SM, Nicholls AG, Orr AP (1935) On the biology of Calanus finmarchicus. Part VI. Oxygen consumption in relation to environmental conditions. J Mar Biol Assoc UK 20:1–27

Mauchline J (1998) The biology of calanoid copepods. Academic Press, San Diego, CA

Mayzaud P (1976) Respiration and nitrogen excretion of zooplankton. IV. The influence of starvation on the metabolism and the biochemical composition of some species. Mar Biol 37:47–58

Mc Neill S, Lawton JH (1970) Annual production and respiration in animal populations. Nature 225:472–474

Novarino G (1991) Observations on Rhinomonas reticulata comb. nov. and R. reticulata var. nov. (Cryptophyceae) with comments on genera Pyrenomonas and Rhodo-monas. Nord J Bot 11:243–252

Olomscheck D, Hofmann M, Worm B, Schellnhuber HJ (2013) Decomposing the effects of ocean warming on chlorophyll a concentrations into physically and biologically driven contributions. Environ Res Lett 8:014043

Omoti M, Ikeda T (1984) Methods in marine zooplankton ecology. John Wiley & Sons, New York, NY

Parry GD (1983) The influence of the cost of growth on ectotherm metabolism. J Theor Biol 101:453–477

Peters RH (1986) The ecological implication of body size. Cambridge University Press, Cambridge

Peterson WT (1985) Abundance, age structure and in situ egg production rates of the copepod Temora longicornis in Long Island Sound, New York. Bull Mar Sci 37:726–738

Petch H (1958) Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. In: Profssor CL (ed) Physiological adaptation. American Physiological Society, Washington, DC, p 50–78

Prosser CL, Brown FA (1961) Comparative animal physiology, 2nd edn. WB Saunders, Philadelphia, PA

Raymont JEG (1959) The respiration of some planktonic copepods. III. The oxygen requirements of some American species. Limnol Oceanogr 4:479–491

Razouls S (1971) Variation annuelles du metabolisme respiratoire de deux copepods pelagiques: Temora stylifera et Centropages typicus a Banyuls-sur-mer (Golfe du Lion). Vie Milieu 22:95–112 (in French)

Rose JM, Caron DA (2007) Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. Limnol Oceanogr 52:886–895

Schmidt-Nielsen K (1991) Animal physiology, adaptation and environment, 4th edn. Cambridge University Press, Cambridge

Secor SM (2009) Specific dynamic action: a review of the...
postprandial metabolic response. J Comp Physiol B 179: 1–56

Somero GN (2012) The physiology of global change: linking patterns to mechanisms. Annu Rev Mar Sci 4:39–61

Somero GN, Hochachka PW (1971) Biochemical adaptation to the environment. Am Zool 11:159–167

Stock C, Dunne J (2010) Controls on the ratio of mesozooplankton production to primary production in marine ecosystems. Deep-Sea Res I 57:95–112

Stock C, Dunne J (2010) Controls on the ratio of mesozooplankton production to primary production in marine ecosystems. Deep-Sea Res I 57:95–112

Tester PA, Turner RE (1990) How long does it take copepods to make eggs? J Exp Mar Biol Ecol 141:169–182

Tett P (1987) Plankton. In: Baker J, Wolff WJ (eds) Biological survey of estuaries and coasts. Cambridge University Press, Cambridge, p 280–341

Thor P (2000) Relationship between specific dynamic action and protein deposition in calanoid copepods. J Exp Mar Biol Ecol 245:171–182

Thor P (2003) Elevated respiration rates of the neritic copepod Acartia tonsa during recovery from starvation. J Exp Mar Biol Ecol 283:133–143

Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton Methodik. Mitt Int Ver Theor Angew Limnol 9:1–38

West GB, Brown JH, Enquist BJ (1997) A general model for the origin of allometric scaling laws in biology. Science 276:122–126