Seasonal change in acclimatised respiration rate of *Temora longicornis*

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Abstract:

We investigated the seasonal changes in the respiration rate (R) of adult *Temora longicornis* (Müller) acclimatised to *in situ* conditions over 1 yr. Mean (± 1 SE) R varied from 50.5 ± 2.8 nl O₂ ind.⁻¹ h⁻¹ in December to 73.2 ± 3.53 nl O₂ ind.⁻¹ h⁻¹ in August for copepods of mean body dry weight (DW) of 33.5 ± 1.1 and 26.3 ± 0.95 µg, respectively. Males represented ~8% of the total measurements, and their respiration did not differ significantly from that of females. R scaled isometrically with dry weight (DW), i.e. the allometric exponent of the power function ($R = aDW^b$) did not differ significantly from unity ($b = 0.83$ to 1.35). The relationship between *in situ* weight-specific respiration rate (R_{sp}) and temperature (T) was described by a sigmoid trend with Q_{10} ranging from 1 to 2.88 (mean 1.57 to 1.89). In contrast, the acclimated and acutely measured R_{sp} of copepods maintained under optimal feeding conditions in the laboratory increased exponentially with temperature and were characterised by higher mean Q_{10} of 2.05 and 2.41, respectively. Acclimatised $\ln R$ increased significantly with $\ln DW$, T , $\ln$ chlorophyll *a* ($\ln Chl$) and $\ln$ egg production rate ($\ln EPR$). Our results indicate that seasonal changes in *T. longicornis* respiration rate are not simply determined by body mass and temperature but also reflect copepod nutritional and reproductive condition. We argue that predictive ecological models using fixed thermal coefficient values may overestimate copepod respiration, particularly under ambient conditions limiting growth and reproduction. Our findings have important implications for the calculation of carbon flow in marine food-webs and for understanding how zooplankton physiology responds to changes in global temperature.

Keywords: *Temora longicornis*; Respiration; Acclimatisation; Acute rate; Temperature; Reproduction

Introduction

Respiration is a fundamental property of organisms and ultimately it 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 copepod, as in poikilotherm 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 and Lawton 1970) both of which can represent a substantial energetic cost to an organism (Parry 1983, Kiørboe 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 effect 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 et al. 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 thermodynamics 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

99 rates (i.e. M-T curves) of animals maintained under optimal feeding conditions (Peters 1986).
100 In nature, however, copepod can often be food limited (Hirst & Bunker 2003) and under such
101 circumstances respiration rate is reduced and does not respond to temperature changes in the
102 same way of that of well fed organisms (Mayzaud 1976, Thor 2003). Hence, Cossins &
103 Bowler (1987) have argued that eco-physiological studies should measure acclimatised
104 metabolic rates as these are ecologically meaningful. Acclimatised rates refer to the
105 physiological rates of poikilotherms measured at *in situ* conditions. In contrast acclimated rates
106 refers to the rates of poikilotherms maintained in the laboratory at a given temperature whereas
107 “acute” rates refers to measurements made on poikilotherms maintained in the laboratory and
108 exposed to a sudden change in ambient conditions i.e. generally temperature (Cossins & Bowler
109 1987).

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

119 The small copepod *Temora longicornis* often dominates the spring and early summer
120 zooplankton communities of coastal temperate waters of the North Atlantic (Peterson 1985,
121 Fransz et al. 1991, Castellani & Lucas 2003). In its environment this species experiences a wide
122 fluctuation in temperature and feeding conditions over the seasonal cycle (Castellani & Altunbaş
123 2006). Because of its reduced ability to store body lipid, *T. longicornis* closely depends on its
124 food supply to meet basal energy requirements let alone to grow and reproduce (Clarke & Walsh
125 1993, Kreibich et al. 2008). Such physiological trait makes this species an ideal model organism
126 to study how environmental change affects the respiration rate of small neritic copepods. Hence,
127 the aim of this study was to investigate how the acclimatised respiration rate of adult copepod *T.*
128 *longicornis* varies in relation to seasonal changes in body mass, temperature, salinity, prey
129 availability and reproduction. In addition, we explore whether temperature acclimatised
130 respiration rates differ from acclimated and acutely measured rate of copepod maintained in the
131 laboratory under high food concentration.

Material and methods

Sampling

Zooplankton was 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 ml - 750 ml, filtered onto GF/F filters, extracted in neutralised 90 % acetone solution for 24 hrs 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 (Nielsen & Kiørboe 1994) stored into 100 ml dark glass bottles in the dark at 4°C and analysed by the Utermöhl (1958) technique within one month.

Copepod maintenance prior to respiration rate experiments

Adults *T. longicornis* were sorted from the catch within one hour of collection in a walk-in cold room set at *in situ* temperatures between 5°C and 17.5°C. The copepods were used for one of the three following experiments: to investigate 1) seasonal changes in acclimatised respiration rate, 2) the respiration rate of copepod 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 individuals 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 day at their original *in situ* temperature. The respiration rates of copepod 2) acclimated and 3) acutely exposed were measured over the temperature range of 4°C - 20°C and these copepods were maintained in the laboratory on *ad libitum* diet of the cultured flagellate alga *Rhinomonas reticulata* (Novarino 1991) for a week 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 to 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, Kiørboe et al. 1985, Secor 2009). Thus, freshly caught copepods were placed in filtered seawater and their respiration rate measured continuously until 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 approximately 10 hours after capture and that such respiration rate remained stable at $\sim 1.7 \text{ nl } \mu\text{g DW}^{-1} \text{ hr}^{-1}$ over at least the following 24 hours (Figure 1). Hence, a minimum time of 10 hours since the start of fasting was allowed to elapse prior to all respiration rate measurements carried out in the three 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 *T. longicornis* between April 1996 and April 1997. Here respiration rate corresponds to routine rate, that is, the oxygen consumed over time by fasting copepod 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 (Kanwisher 1959). The pO₂-electrode was fitted to the base of a closed transparent glass micro-respirometer chamber of 100 µl to 150 µl in volume to allow regular monitoring of animal activity and behaviour. The temperature inside the respirometer was kept constant within $\pm 0.2 \text{ }^{\circ}\text{C}$ by a thermostat through a re-circulating 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 graphical 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 0-100% 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. $0.5 - 3 \times 10^{-10}$ 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 hour maximum.

Copepod dry weight vs length relationship

At the end of each experiment, the prosome length of copepod was measured with an eye-piece graticule under a dissecting microscope (Wild M5). Lengths were converted to body mass from the regression $\ln DW = 2.79 \ln PL - 15.9$ ($r = 0.92$, $p < 0.001$, d.f. = 29), constructed using copepod 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 20-30 individuals of similar prosome length ($\pm 20 \mu\text{m}$) 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 and Ikeda 1984).

Copepod egg production rate

Measurements of egg production rates (EPR) of *T. longicornis* were carried out at *in situ* conditions concomitantly to respiration rate measurements as described by Castellani &

Altunbas (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 sea water pre-screened through a 53 µm mesh. The dishes were kept for 24 hrs 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 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 24 hours incubation period.

Calculation of the thermal coefficients Q_{10} and activation energy (E_a)

The relationship between the seasonal changes respiration rate of *T. 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):

$$Q_{10} = \left(\frac{K_1}{K_2} \right)^{\frac{10}{(t_1 - t_2)}} \quad (\text{Eq. 1})$$

where K_1 and K_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 temperature, expressed in degrees Kelvin (K) against the respiration rates:

$$k = A e^{-\frac{E_a}{RT}} \quad (\text{Eq. 2})$$

where k is the reaction velocity constant, A is the frequency factor constant, e is the natural base of the logarithms (i.e. 2.718..), T is the absolute temperature in K, R is the gas constant (i.e. 8.31 J mole⁻¹ K⁻¹) and E_a (i.e. in J mol⁻¹) is the activation energy. The change in reaction velocity with temperature can be predicted from the proportionality constant E_a of an integrated form of the Arrhenius equation, that is:

$$\ln\left(\frac{k_2}{k_1}\right) = \frac{E_a}{R\left(\frac{1}{T_1} - \frac{1}{T_2}\right)} \quad (\text{Eq. 3})$$

where k_1 and k_2 are the reaction velocities constant at temperatures T_1 and T_2 in K. The Arrhenius plot of $\ln k$ against the reciprocal of the absolute temperature gives a straight line of slope equal to E_a/R from which E_a , the activation energy, can be calculated as shown in Eq. 4:

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

RESULTS

Environmental conditions and microplankton composition

The sampling area is characterised by shallow water depth between 1m 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 Figure 2 summarises the seasonal changes in ambient variables at the sampling site already reported by Castellani & Altunbaş (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 (Figure 2, a). Salinity changed little reaching maxima values of 34.1 ppt in summer and minima of 31.7 ppt in autumn (Table 1), during the highest local annual rainfall (i.e. 110-126 mm month⁻¹, Royal Meteorological Society, Weather Log 1996-1997).

The early spring increase of Chl from low winter concentration of 0.5 - 0.9 $\mu\text{g l}^{-1}$ to ~ 3 - 8 $\mu\text{g l}^{-1}$ (Figure 2, b) 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.*-*Rhizosolenia delicatula* during which Chl increased to ~ 16 - 26 $\mu\text{g l}^{-1}$. The spring bloom was followed by euglenoids and cryptomonas-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 $\mu\text{g l}^{-1}$ to 8 $\mu\text{g l}^{-1}$. Ciliate biomass, mostly belonging to the genus *Strombidium*, also peaked between spring early-summer and it was correlated with Chl concentration ($r = 0.67$, $p < 0.05$, d.f. = 33). Dinoflagellates, on the other hand, peaked in summer after the Chl *maxima* (Castellani & Altunbaş 2006).

Seasonal variation of copepod respiration with biotic and abiotic variables

T. longicornis respiration rates began to increase between January and April, reached a maximum in August (R, mean $\pm$ SE, 83.2 ± 7.6 nlO₂ cop⁻¹ hr⁻¹) and a lower secondary peak in October-November before it declined to the annual minimum in December (R, mean $\pm$ SE, 40.4 ± 5.7 nlO₂ cop⁻¹ hr⁻¹) (Figure 2, c). In contrast copepod dry weight (DW, mean $\pm$ SE) ranged between 19.6 ± 1.1 μ g in September and 42.9 ± 3.7 μ g in February (Figure 2, c & d). The seasonal change in egg production rate (EPR) at the study site has already been reported in detail by Castellani & Altunbaş (2006). Briefly *T. longicornis* produced eggs all year round. A mean ($\pm$ SE) EPR maximum of 48 ± 7 egg female⁻¹d⁻¹ occurred in spring-early summer during the phytoplankton bloom whereas a minimum of 1.3 ± 0.5 eggs fem⁻¹d⁻¹ was recorded in autumn-winter (Figure 2,e). *In situ* copepod respiration rate followed the variation in DW, Chl, EPR and T (Figure 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 °C to 17.5 °C and 8 °C to 13 °C (i.e. the temperature range within which respiration rate increased linearly with temperature, see Figure 4). The normalised β -coefficient from the multiple regression analysis, over both temperature intervals, indicated that, ln DW ($\beta = 0.6$ -0.5) explained the largest proportion of variability in copepod respiration rates followed by temperature ($\beta = 0.4$ -0.5), ln Chl ($\beta = 0.2$) and ln EPR ($\beta = 0.1$) whereas salinity was not significant. Table 2 also shows that the goodness of fit of the regression is higher for the 8 °C to 13 °C temperature interval where respiration rate increase linearly with temperature.

Relationship between respiration and body dry weight

Copepod respiration rate increased with DW with the largest copepods showing the widest range in rates (Figure 3). Table 3 summarises the relationship between respiration rate and body DW of *T. 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 ANCOVA (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 for each individual month and a positive trend in respiration rate with temperature was evident (Figure 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} , $\text{nLO}_2 \mu\text{gDW}^{-1} \text{hr}^{-1}$) with temperature was characterised by a sigmoid pattern (Figure 4); respiration rate remained at its lowest between 5°C and 7°C from December to April, it increased between 8°C and 13°C from April to May and reached a maximum plateau between 15°C to 17.5°C from June to August. The pattern of respiration rate 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 was found to fit statistically (i.e. $r^2 = 93\%$, See Figure 4) better than the linear regression model (i.e. $r^2 = 86\%$). The multiple regression model shown in Table 2 predicts that the highest rate of increase in the acclimatised respiration of *T. longicornis* occurs between 8°C and 13°C (i.e. $Q_{10} = 2.01\text{-}2.88$). In contrast, above and below this temperature range respiration rate remains virtually constant (i.e. $Q_{10} = 1$). Overall, the change in respiration rate 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 2 b) shows the activation energy (i.e. E_a) values calculated from the Arrhenius equation (i.e. Eq. 4). E_a varied from 30.5 KJ mol^{-1} to 42.9 KJ mol^{-1} (or 0.31 eV - 0.45 eV) over the whole *in situ* temperature range reaching the highest values between 47.7 KJ mol^{-1} to 71.7 KJ mol^{-1} (or 0.49 eV - 0.74 eV) when calculated over the 8°C to 13°C temperature range.

Relationship between respiration with Chl and EPR

Figure 5 and Figure 6 show the pattern of increase in respiration rate (i.e. R) with Chl and EPR. Both relationships were characterised by an asymptotic trend with R reaching a maximum around $5 \mu\text{g Chl L}^{-1}$ and $15 \text{ egg fem}^{-1} \text{day}^{-1}$. These results suggest that R reaches its maximum values during the phytoplankton bloom i.e. when feeding conditions for copepods are most favourable (Figure 2 b). Moreover, the relatively constant R (i.e. energy consumption) for EPR increasing from ~ 15 to $50 \text{ eggs fem}^{-1} \text{d}^{-1}$ 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 about 8 % of the total copepod in the population sampled during the present study and they were smaller than females. As a result the mean weight specific respiration rate of *T. longicornis* males ($1.67 \pm 0.12 \text{ nlO}_2 \mu\text{gDW}^{-1} \text{ hr}^{-1}$) was on average lower than that of females ($2.27 \pm 0.048 \text{ nlO}_2 \mu\text{gDW}^{-1} \text{ hr}^{-1}$). However, comparison of copepod of similar DW measured the *in situ* temperature of 6.5 °C, showed that the respiration rate of male did not differ significantly from that of the female (Table 4).

Discussion

Seasonal variation of copepod respiration rate

We investigated the seasonal variation in the respiration rate of *T. longicornis* acclimatised to field conditions, during one year study, by measuring individually 299 adults over the full range of copepod dry weight (DW), egg production rates (EPR), ambient temperature (T), salinity and chlorophyll-a (Chl) 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 (Figure 2). Results from multiple regression analysis showed that seasonal changes in Ln R were significantly related to Ln DW, 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 which 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 (Berner 1962, Marshall & Orr 1966, Raymont 1959, Conover 1959, 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.

The 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 (Table 2, Table 3 and Figure 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 *T. longicornis* from Southampton waters. Using the data published by Marshall & Orr (1966), we calculated a weight exponent ($\pm$ 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 copepod (Mauchline 1998, Ikeda et al. 2000).

Although, it is generally assumed that metabolic rate increases as the body mass to the power of $\frac{2}{3}$ (i.e. 0.66, proportionality to body surface) or $\frac{3}{4}$ (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, Kolokotronis et al. 2010, Glazier 2010, Agutter & Tuszynski 2011). West et al. (1997) have argued that the $\frac{3}{4}$ 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 $\frac{3}{4}$ -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; Kolotrones et al. 2010; Agutter and Tuszynski 2011). Kolotrones 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 adopt a molecular-cellular perspective can be used to predict the large variations in body scaling exponents and 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 reproductive rates in response to high levels of mortality in open water.

The relationship between respiration rate and temperature

The respiration rate of *T. longicornis* increased with temperature (Figure 4) similar to what 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 16-17°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 (Figure 7) measured over a similar temperature range (Figure 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 copepod acclimatised to environmental conditions, previous authors measured groups of copepod, 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 *T. longicornis* at 10°C over 40-50 hrs incubation between the end of March and mid-July. Le Ruyet-Person et al. (1975) measured single fasting copepod acclimatised to 16-17 °C during 3-6 hrs incubation in July. In contrast, Raymont (1959) and Conover (1959) measured copepod, maintained in the laboratory between 5°C and 10°C on cultured phytoplankton, at 15°C and 20°C respectively, over 3 hrs 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 (i.e. acute M-T 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 between 5°C to 15°C degrees requires between ~ 2 to 6 days, respectively, before respiration stabilises to the new rate, intermediate between the original and the acute rate (Castellani & Altunbaş, in preparation). 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 i.e. considering *in situ* temperatures ranging from 4°C 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 copepod over a short time (i.e. 3 hrs) following temperature increases of up to $\sim 15^{\circ}\text{C}$, probably corresponded to acute respiration rates (Table 5).

In our study, the respiration rate of *T. longicornis* acclimatised to field temperatures between 5°C and 17.5°C followed a sigmoid pattern with Q_{10} ranging between 1 and 2.88 depending on the temperature interval (mean Q_{10} ranging between 1.56 and 1.88, Table 2 and Figure 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°C and 20°C and by a higher mean Q_{10} of 3.09 (Figure 8). The Q_{10} 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 $Q_{10} > 2$ are generally estimated from acute measurements of copepods maintained in the laboratory on high microplankton concentration (Cossins & Bowler 1987, Ikeda et al. 2001, Castellani et al. 2005). For instance, using data by Gauld & Raymont (1953) and our own data (Figure 9-a) on the acute respiration rates of fasting *T. longicornis* maintained on *at libitum* microalgal diet, we calculated mean Q_{10} values of 2.4 for the temperature interval 4°C 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 by an 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 copepod (Berner 1962, Marshall & Orr 1966, Ikeda et al. 2001, this study Table 5) to avoid the added effect of Specific Dynamic Action (i.e. 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 *T. longicornis* takes approx. 10 hrs to decrease to the routine rate after copepods are deprived of food (Figure 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 (Figure 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 a temperature fluctuating environment (Gaudy 1972, 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 respiration rate) unless it is required to produce work and unless there is sufficient fuel 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 (Figure 2, b & 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 maximum (Table 2, Figure 2 & Figure 4). Similarly, Ikeda et al (2001) reported Q_{10} between 1.8 and 2.1 for the respiration rate of fasting copepod species acclimatised between -1°C and 30°C calculated only from measurements made during spring-summer when feeding conditions were most favourable.

A comparison of acclimatised respiration rate, from the present study, with acclimated and acute respiration rate suggests that the response of *T. longicornis* metabolism to temperature depends on both exposure time and nutritional conditions (Figure 9). The respiration of field acclimatised copepod 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 the lowest ($Q_{10} = 1.56-1.88$). The acclimated respiration of laboratory maintained copepod increased exponentially but the rate of change was intermediate ($Q_{10} = 2.1$). In acutely measured copepod the response was also exponential but the rate of increase was the highest ($Q_{10} = 2.4$). However, Figure 9-a shows a substantial overlap between the three 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 Two-sample T-test statistic with equal variances at each of the common temperature. 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 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 concentration had already increased above winter level to reach $\sim 1 \mu\text{g L}^{-1}$ due to a mixed diatom bloom and this promoted an increase in copepod EPR (mean $\pm$ SE, 16 ± 9.1 eggs $\text{fem}^{-1} \text{day}^{-1}$; range 0 – 61 eggs $\text{fem}^{-1} \text{day}^{-1}$). Diatoms are known to support higher egg production rates in *T. longicornis* compared to other phytoplankton diets (Dam and Lopes 2003, Jonasdottir 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 copepod maintained on a high flagellate diet in the laboratory and associated lower reproductive rates (see also next section). In support of our argument, Figure 10 shows a sigmoid trend in the weight specific reproduction rate of *T. longicornis* (measured during the same study and published in Castellani and Altunbas 2006) with temperature which is very similar to the acclimatised respiration rate trend presented in Figure 4 and Figure 9-a. Furthermore, the logistic equation we fitted to the acclimatised respiration rate data in Figure 4 and Figure 9-a 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 (Figure 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 copepods species which store large body

lipid reserves or those who experience large temperature changes over a short time scale through, for instance, diel vertical migration.

The effect of food sources and reproduction on respiration

The positive significant relationship we found between *T. longicornis* respiration rates and ambient Chl 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*, *Calanus helgolandicus* and *Calanus 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 *T. 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 already after 24 h in starving *T. longicornis* (Clarke & Walsh 1993). The increase in respiration rate of fed copepod, 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 (Kiørboe 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 protein recycling (e.g. enzymes). However, the recycling of enzymes is probably not constant and it 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 quality 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 copepod 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

appears to be the result of a higher level of protein turnover and/or anabolism supported by the constant high prey supply in the laboratory (Figure 9).

In our study, the seasonal change in *T. longicornis* respiration rate was also significantly related to EPR. Coupling between egg production and respiration rate has been reported in *A. tonsa* (Kiørboe et al. 1985, Thor 2003) and Conover (1962) observed that ripe *C. hyperboreus* females had higher respiratory rate than immature or spent ones. In adult female copepod anabolic processes are mainly linked to the cost of reproduction (e.g. in the copepod *Acartia tonsa*, Kiørboe 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. Such result is not surprising since copepod respiration combines different energetic costs besides 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 egg (Tester & Turner 1990). In addition, in our study we measured respiration on fasting copepods and EPR on copepod that had been feeding before incubation (see material and methods). Furthermore, the asymptotic relationship, shown in Figure 6, between respiration and EPR indicates an increase in reproductive efficiency in spring that might have resulted from an increase in food quality (e.g. the increase in nitrogen rich ciliate during the spring bloom). Results of a recent laboratory study (Nobili et al. in preparation) showing a decrease in the reproductive cost of *T. longicornis* with increase in the N:P ratio of the diet would support our field observation. The similarity in the pattern of EPR and respiration rate with temperature we shown in Figure 4 and Figure 10 we already discussed in the previous section also support 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 *T. longicornis* scaled isometrically (i.e. $b = 1$) with metabolism similarly to what commonly reported in planktonic animals (Glazier 2006). Such value is significantly different from the coefficient $b = 3/4$ predicted by the Metabolic Theory of Ecology (MTE, Gilloly et al. 2001, Brown et al. 2004). Our and past studies have also shown that under natural conditions the Q_{10} of copepod physiological rates are often characterised by Q_{10} values < 2 (Present study, Ikeda 1985, Ikeda et al. 2000, Hirst and Bunker 2003). In contrast, the physiological responses to temperature of zooplankton experiencing optimal feeding conditions either in the field or in the laboratory and measured over unrealistic and sudden temperature changes seem to be characterised by $Q_{10} > 2$ (i.e. acute measurements, present study, Peters 1986, Cossins & Bowler 1987, Hirche 1987). Moreover, our study has also shown that under acclimatised and acclimated conditions the physiology of copepods is characterised by lower Q_{10} than in copepods exposed acutely to temperature changes. It is not unreasonable to assume that in the marine environment the metabolism of most copepods will have sufficient time to acclimatise to changes in ambient temperature since here seasonal thermal fluctuations are relatively small, compared to the terrestrial environment, due to the higher heat capacity of seawater. Most copepod species experience food scarcity and variable prey quality over large part of the year all of which could affect their metabolism and reproductive rates. In this respect, our results have shown that the goodness of fit of the regression improved when we included Chl and EPR in addition to DW and temperature (Table 2). Moreover, the value of the Q_{10} differed substantially depending not only on the temperature interval considered but also on the variables included in the regression analysis, whereas the body mass exponent was never significantly different from unity. Modelling studies generally use relatively high values of Q_{10} to estimate the effect of temperature change on metabolic rates of zooplankton; Interestingly Stock & Dunne (2010) have observed that the report of high Q_{10} values by several high profile zooplankton studies (e.g. Huntley & Lopez (1992) report a Q_{10} of 3 for copepod generation times and Rose & Caron (2007) report Q_{10} of 2.4 and 3.75 for bacterivorous and herbivorous protists respectively), has prompted many modelers to adopt a Q_{10} of 3 despite substantial observational evidence that zooplankton rates (including growth) exhibit Q_{10} values of 2 or below (Hirst and Bunker 2003, Ikeda 1985, Ikeda et al. 2000). The activation energy (E_a) we calculated from the slopes of the Arrhenius equation for copepod acclimatised between 5 °C and 17.5 °C ranged between 31 to 43 KJ mol⁻¹ or 0.31 to 0.45 eV (i.e. 1 eV = 96.49 kJ mol⁻¹)

(Figure 9-b & Table 2b). These values are well below the E_a range 0.60-0.70 eV and the mean E_a values between 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°C - 13°C, that is during the phytoplankton bloom when copepods were exposed to good feeding conditions and actively reproducing (see section above “the 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 of not only 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 *T. 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 (Ikeda 1974, 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 1996) and that $\mu\text{g carbon catabolized} = 0.8 \times \mu\text{l O}_2 \text{ day}^{-1} \text{ respired} \times 12 \mu\text{g C } \mu\text{mol}^{-1} / 22.4 \mu\text{l } \mu\text{mol}^{-1}$, the daily carbon requirement by a *T. longicornis* of 1000 μm in length, estimated from our multiple regression model (Table 2), would vary from ~5 % and 10 % of body carbon at 5°C and 17°C respectively. However, Table 2 also shows that using DW and T only, results in copepod C consumption being almost 3 times higher than that estimated taking into account Chl and EPR. The discrepancy in energetic cost is stronger during the most productive time of the year when copepods are actively reproducing (Table2).

Summary & Conclusions

Using one of the most comprehensive data sets on acclimatised copepod respiration rate, our study has shown that *in situ* seasonal changes in *T. 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 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_a we measured in our studies compared to those reported in the literature also suggests that metabolic rates of acclimatised copepods may be less responsive to temperature changes than it has been inferred so far from thermal coefficients calculated from acutely measured rates of copepods maintained in the laboratory on high food ration. The value of the thermal coefficient also differed according to the type of variable used in the regression model. Therefore, using high and fixed coefficients values may lead to overestimate 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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Figure 1: Time course of changes in the weight specific respiration rate (R_{sp} , nl O₂ μg DW⁻¹ hr⁻¹) of fasting *T. longicornis*. Time 0 hours is the time at which copepod were deprived of food.

Figure 2: Seasonal changes in a) temperature (T, °C), b) chlorophyll-a (Chl, $\mu\text{g L}^{-1}$), c) respiration rate ($\text{nlO}_2 \text{ cop}^{-1} \text{ hr}^{-1}$), d) dry weigh (DW, μg) and e) egg production rate (EPR, egg $\text{female}^{-1} \text{ d}^{-1}$).

a)

b)

c)

d)

975 e)

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Figure 3: Scatter plot of ln-transformed respiration rate ($\ln R$, $\text{nLO}_2 \text{ cop}^{-1} \text{ hr}^{-1}$) of *T. longicornis* versus copepod dry weigh ($\ln DW$, μg). The lines represent fitted values for each temperature obtained from the multiple regression between $\ln R$, $\ln DW$ and temperature (T , $^{\circ}\text{C}$) which is used as a proxy to differentiate between the monthly experimental trials (Table 3).

Figure 4: Scatter plot of weight specific respiration rate (R_{sp} , nlO₂ µg DW⁻¹ hr⁻¹) of *T. longicornis* versus temperature (T, °C). The Q_{10} values calculated from the regressions in Table 2 for the temperature intervals 5 °C to 7°C, 8 °C to 13 °C and 15°C to 17.5 °C are also shown. The continuous line represents the logistic function fitted to the data.

Figure 5: Scatter plot of the respiration rate (ln R, nlO₂ cop⁻¹ hr⁻¹) of *T. longicornis* versus chlorophyll-a (Chl, µg L⁻¹).

1008 **Figure 6:** Scatter plot of the respiration rate (ln R, nlO₂ cop⁻¹ hr⁻¹) of *T. longicornis* versus egg
1009 production rate (EPR, egg female⁻¹ d⁻¹).

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Figure 7: Scatter plot comparing of the respiration rate ($\ln R$, $\text{nlO}_2 \text{ cop}^{-1} \text{ hr}^{-1}$) of *T. longicornis* versus prosome length (PL, μm) obtained by different field studies (See Table 5)

Figure 8: Scatter plot comparing of the respiration rate ($\ln R$, $\text{nlO}_2 \text{ cop}^{-1} \text{ hr}^{-1}$) of *T. longicornis* versus temperature (T , $^{\circ}\text{C}$) obtained by different field studies (See Table 5). ($\ln R$, $\text{nlO}_2 \text{ cop}^{-1} \text{ hr}^{-1}$).

Figure 9: Scatter plot of acclimatised, acclimated and acute mean ($\pm$ SE) weight specific respiration rates (R_{sp} , nlO₂ μ gDW hr⁻¹) of *T. longicornis* versus a) temperature (°C) and b) the inverse of the absolute temperature (1/ K) i.e. the Arrhenius plot. Lines represent the fits from the regressions models.

Figure 9: continue

b)

Figure 10: Scatter plot of weight specific egg production rate (EPR_{sp} , d^{-1}) of *T. longicornis* versus *in situ* temperature ($^{\circ}C$).

1051 **Table 1:** Sampling dates for *T. longicornis* used for the respiration rates experiments and corresponding environmental variables

Sampling Date	T (°C)	Salinity	Chla (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

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1054 **Table 2:** Multiple regression analysis between ln-transformed *T. longicornis* respiration rate (ln R, nlO₂ cop⁻¹ hr⁻¹) with copepod body dry weight (ln DW, µg) *in situ*
1055 temperature (T, °C), chlorophyll-a (ln Chl, µg l⁻¹) and egg production rate (ln (EPR+1), egg fem⁻¹ d⁻¹). The thermal coefficient Q₁₀ and the % of body carbon requirement
1056 calculated from the equation models is also shown. Note that the F test for all regression models is statistically significant at 1% level. The *, **, and *** symbols indicate
1057 statistical significance of the variables at the 10%, 5% and 1% levels respectively.
1058 a)

Regression Model	ln DW	T	ln Chl	(EPR+1)	Intercept	N	r ²	F-value	Q ₁₀	% body-C
Temperature range: 5 °C- 17.5 °C										5 °C 10 °C 17.5 °C
1) ln R = 1.00 ln DW + 0.06 T + 0.03	1.00 *** (0.056)	0.06 *** (0.005)			0.03 (0.218)	299	47.6	180.94	1.89	4.46 6.02 9.16
2) ln R = 0.96 ln DW + 0.06 T + 0.05 ln Chl + 0.21	0.96 *** (0.058)	0.06 *** (0.005)	0.05 *** (0.014)		0.21 (0.225)	299	49.6	124.40	1.75	4.51 7.25 10.25
3) ln R = 0.87 ln DW + 0.05 T + 0.05 ln Chl + 0.04 ln (EPR+1) + 0.56	0.87 *** (0.099)	0.05 *** (0.007)	0.05 ** (0.020)	0.04 ** (0.017)	0.56 (0.377)	188	43.9	36.79	1.57	4.75 7.62 9.90
Temperature range: 8 - 13 °C										
4) ln R = 1.04 ln DW + 0.11 T - 0.50	1.04 *** (0.067)	0.11 *** (0.010)			-0.50 * (0.272)	189	53.2	146.81	2.88	18.2
5) ln R = 1.00 ln DW + 0.09 T + 0.05 ln Chl - 0.25	1.00 *** (0.071)	0.09 *** (0.011)	0.05 *** (0.017)		-0.25 (0.290)	189	54.7	99.33	2.39	7.07
6) ln R = 0.83 ln DW + 0.07 T + 0.06 ln Chl + 0.03 ln (EPR+1) + 0.46	0.83 *** (0.119)	0.07 *** (0.014)	0.06 ** (0.025)	0.03 (0.021)	0.46 (0.474)	121	52.5	32.35	2.01	6.83

1073 b)

	ln DW	1/K	ln Chl	(EPR+1)	Intercept	N	r ²	F-value	Ea	
Temperature range: 5 °C- 17.5 °C									KJ mol-1	e V
7) ln R= 0.99 Ln DW - 5163/KT + 18.9	0.99 *** (0.055)	-5163 *** (385.64)			18.9 1.28	299	47.8	180.16	42.93	0.45
8) ln R= 0.95 Ln DW - 4585/KT + 0.05 ln Chl+16.9	0.95 *** 0.058	-4585 *** 395.83	0.045 *** 0.014		16.89 1.31	299	49.5	122.25	38.12	0.39
9) ln R= 0.85 Ln DW - 3670/KT + 0.05 ln Chl+0.03 ln (EPR+1) +14.01	0.85 *** 0.099	-3670 *** 570	0.046 ** 0.0198	0.034 ** 0.0169	14.01 1.83	188	35.8	43.7	30.51	0.31
Temperature range: 8 - 13 °C										
10) ln R= 1.01 Ln DW - 8627/KT + 31.1	1.01 *** 0.067	-8627 *** 812			31.09 2.83	189	53.6	144.02	71.72	0.74
11) ln R= 0.99 Ln DW - 7259/KT + 0.04 ln Chl + 26.3	0.989 *** 0.071	-7259 *** 912	0.041 ** 0.0177		26.3 3.15	189	54.6	96.9	60.35	0.63
12) ln R= 0.81 Ln DW - 5740/KT + 0.05 ln Chl + 0.03 ln (EPR+1) + 21.48	0.81 *** 0.11	-5740 *** 1182	0.054 ** 0.025	0.026 0.021	21.48 4.02	121	31.66	52.3	47.72	0.49

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Table 3: Summary of regression analysis between *T. longicornis* ln-transformed respiration rate (ln R, nlO₂cop⁻¹hr⁻¹) and body dry weight (ln DW, µg). 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. The *, **, and *** symbols indicate statistical significance of the regression coefficients at the 10%, 5% and 1% levels respectively.

Date	T	N	PL Mean (SD)	DW Mean (SD)	R Mean (SD)	Intercept Coff (SE)	Slope Coff (SE)	r ² (%)	F-value
February 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
December 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
April 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
November 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
June 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
June 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
October 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
September 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
August 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

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1085 **Table 4:** Result of the Two-sample T-test statistic comparing the mean ($\pm$ SE) respiration rate (R, in $\text{nLO}_2 \text{ cop}^{-1} \text{ hr}^{-1}$) and weight specific respiration rate (R_{sp} , $\text{nLO}_2 \mu\text{gDW}^{-1} \text{ hr}^{-1}$) of individual (N) females and males *T. longicornis* of similar prosome length (PL, μm) and dry weight (DW, μg) at 6.5 °C.
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Gender	N	PL	DW	R	R _{sp}
Female	13	1060 (± 10.4)	34.4 (± 0.93)	65.9 (± 4.4)	1.91 (± 0.11)
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

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1090 **Table 5:** Summary of *T. longicornis* respiration rates (R, nlO₂ cop⁻¹hr⁻¹) measured during either field¹ or laboratory experiments². Prosome length
1091 (PL, µm), dry weigh (DW, µg), length of time copepods were fasting prior to experiment (FT, hrs), respirometer volume (RV, ml), number of
1092 copepods incubated (Cop, N), experimental temperature (ET, °C), acclimation temperature (AT, °C).

PL (µm)	DW (µg)	FT (hr)	RV (ml)	Cop (N)	ET (°C)	AT (°C)	R (nlO ₂ cop ⁻¹ hr ⁻¹)	Method [Incubation Time]	Source
720 - 1340	12-66 ^(a)	9-12	0.15	1	5-17.5	5-17.5 [Jan-Dec]	13-150 [66]	Micro-electrode [1 hr]	¹ Present Study, Menai Strait, UK
973	27 ^(a)	24	0.07	1	16-17	16-17 [July]	87 – 186 [121] ^(c)	Clark-electrode [2-6 hrs]	¹ Le Ruyet-Person et al (1975), Roscoff, France
594-968	7-27 ^(a)	24	7 - 35	1-10	10	- [April- Sept]	16 - 47 [32.1]	Winkler [1-5 days]	¹ Berner (1962), Milliport, UK ^(b)
722-1139	12-42 ^(a)	Over night	30 - 40	5 - 10	10	- [March- July]	24-143 [80]	Winkler [40-50 hrs]	¹ Marshall & Orr (1966), Milliport, UK ^(b)
859 - 1040	19-33 ^(a)	- ^(d)	5	29 - 50	15	7-8 [April – May]	116-142	Barcroft-Dixon Manometer [3 hrs]	¹ Raymont (1959) Harvard, USA
804-1082	16-36 ^(a)	-	5	50	20	5-10 [Feb- June]	98 - 183 [130]	Barcroft-Dixon Manometer [3 hrs]	¹ Conover (1959), Southampton, UK
700-1030	11-32 ^(a)	-	5	30-62	10-20	-	62 - 253	Barcroft-Dixon Manometer [3-4 hrs]	² Gauld & Raymont (1953), Southampton, UK

1093 (a) Dry weight estimated from the equation $\ln DW = -15.9 + 2.79 \ln PL$ for *T. longicornis* (Castellani & Altunbaş, 2006)

1094 (b) Use of antibiotics streptomycin (5 mg l⁻¹) and chloromycetin (5 mg l⁻¹)

1095 (c) Measurements standardised to copepod of 1 mm in prosome length.

1096 (d) Kept in lab for min. 24 hrs before experiment.

1097 (e) Author method supplemented with information from Razouls (1971)

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