Title: Diel vertical migration of the Southern Ocean euphausiid, *Euphausia triacantha*, and its metabolic response to consequent short-term temperature changes.

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Abstract

We investigated the effect of short-term temperature change on the respiration rate of *Euphausia triacantha*, a common component of the Southern Ocean zooplankton and a prominent vertical migrator. We found respiration to vary in response to size, with a value of 0.84 for the scaling coefficient, *b*. When scaled to *b*, respiration varied strongly in response to transitory temperature change, ranging from 0.37 to 1.65 µl O_2 mg DW^{-b} h^{-1} between 0.17 and 4.74 ºC, resulting in a Q_{10} of 3.6. This Q_{10} is higher than found by other studies examining the short term respiration response of euphausiids, including those taking a multi-species

perspective. This indicates that *E. triacantha* shows little compensation during short-term exposure to temperatures normally encountered during their migration. Furthermore, it shows that there is a distinct metabolic cost to diel vertical migration (DVM) when substantive changes in temperature are encountered over the course of the transit. This temporal variability in respiration rate has important implications for how community respiration is estimated, and for our understanding of the behaviour of DVM. Our results also have particular relevance to estimating the flux and sequestration of respiratory products, such as dissolved carbon dioxide (CO_2), to and within the ocean interior.

Keywords: Respiration, temperature coefficient (Q_{10}), Diel Vertical Migration (DVM), *Euphausia triacantha*, Southern Ocean, Scotia Sea, elemental composition

1. Introduction

Diel migrant zooplankton have been considered agents of active carbon flux for well over four decades, although early attention was focussed solely on the contribution made from faecal pellets (Angel 1986). Longhurst (1990) additionally considered the role of respiration, suggesting that the respiratory carbon flux from diel migrants could be considerable, and range from 13-53% of estimated particulate carbon flux sinking across the pycnocline. This process becomes particularly important when it is assumed that the majority of carbon being respired is consumed at the surface, thus expediting the transport of respired CO_2 into the comparatively unmixed waters of the deeper ocean. The question has remained an important one with Ariza et al. (2015) estimating that respiratory flux from migrators alone could account for the equivalent of 23-71% of the gravitational flux of carbon measured at 150 m depth. This is underscored by the suggestion that the mesopelagic migrant pump may be the greatest contributor to carbon sequestration of all biological and physical carbon pumps (Boyd et al. 2019).

The question of how metabolism varies as a response to changing physical and chemical conditions as zooplankton migrate within the water column remains poorly parameterised. For organisms where aerobic respiration is the primary means of supplying the oxygen required for cellular metabolism, its measurement is most commonly achieved by quantifying oxygen consumption (Lampert 1984). Certain relationships are commonly upheld when considering rates of metabolism, the first being that respiration rate increases as a function of size, described by the relationship $R = aW^b$, where the scaling exponent b is generally between 0.7-0.9 (Hernández-León & Ikeda 2005a). The second is that respiration rate is strongly regulated by temperature. Activation energy (E_a) is fundamental to defining the thermodynamic relationship between temperature and reaction rate (Clarke 2017) and is described according to the Arrhenius relationship, $k = Ae^{-Ea/RT}$ where k is the rate constant, Ea is the activation energy, R

is the ideal gas constant (8.314 J mol^{-1} K^{-1}) and T is the temperature in Kelvin. In physiological studies, this is often described as the van't Hoff rule, with reference to the Q_{10} coefficient, a measure used to describe the sensitivity to temperature of a species' respiration rate, defined as the increase in rate with a 10 °C rise in temperature.

Many studies have addressed the role of temperature on zooplankton respiration through global or interspecific relationships (e.g. Teal & Carey 1967, Ikeda 1970, Ivleva 1980, Ikeda 2013). However, with some notable exceptions such as *Euphausia superba*, *E. pacifica* and *Meganyctiphanes norvegica* (e.g. Small & Hebard 1967, Hirche 1983, Torres & Childress 1983, Ikeda & Kirkwood 1989, Opalinski 1991, Saborowski et al. 2000), detailed studies of individual species, and particularly their response in relation to DVM, are still relatively few.

Euphausia triacantha (Holt & Tattersall 1906) is a common, predominantly sub-Antarctic, constituent of the Southern Ocean zooplankton with a circumpolar distribution. It generally ranges from south of the Antarctic Convergence to the northerly limits of the East Wind and Weddell Drifts (Mauchline & Fisher 1969, Kirkwood 1982), although it has been found as far south as 66 ° 08 ' S in the waters off the Antarctic Peninsula (Piatkowski 1985). It is a large species of euphausiid, with adults ranging from 24 to 41 mm, and has a lifespan of up to three years (Baker 1959, Siegel 1987). *Euphausia triacantha* does not swarm, yet it is the most abundant euphausiid on the north-west shelf of South Georgia, contributing 6% to overall nekton biomass (Piatkowski et al. 1994). It is also one of the most important contributors to abundance and biomass in the Polar Frontal Zone and replaces *E. superba* as the dominant euphausiid in that region (Pakhomov & McQuaid 1996). In addition, *E. triacantha* displays a pronounced diel vertical migration (DVM). It has been recorded at depths of up to 750 m during the day and in surface waters at night (Baker 1959), thus experiencing temperatures across the most thermally variable parts of the water column within its natural habitat.

Due to its extensive migratory behaviour and high levels of abundance, *E. triacantha* has the potential to be a significant contributor to the active flux of carbon. Whilst clearly able to cross sharp thermal gradients, the effect of temperature on the respiration rate of *E. triacantha* remains unknown. The purpose of this study is twofold: first, to describe the distribution and diel migratory behaviour of *E. triacantha* in the Scotia Sea using abundance data obtained from cruises as part of the DISCOVERY 2010 programme. Second, to investigate the respiration rate of *E. triacantha* over the range of temperatures it is likely to experience *in situ*, determining its variation in response to the time-course of temperature changes experienced during DVM, based on experiments conducted on two experimental cruises.

2. Materials and methods

2.1. Distribution, abundance and environmental conditions of *Euphausia triacantha*

Day and night time MOCNESS (Multiple Opening and Closing Net with Environmental Sensing System, 1 m^2 mouth opening, 330 μm mesh) net samples were collected at four stations encompassing Antarctic and Polar Frontal zones in the Scotia Sea, Southern Ocean, on one summer (JR177) and one autumn (JR200) cruise (see Table S1). Day and night were defined as before and after apparent sunset respectively. These cruises took place in December to February 2007/08 and March to April 2009 respectively, on the RRS James Clark Ross. They were part of the multidisciplinary DISCOVERY 2010 sampling programme, an objective of which was the collection of depth-discrete zooplankton abundance data at repeat locations during different seasons, across three consecutive years, therefore having excellent geographical, vertical and seasonal coverage. The campaign is described in detail in Tarling et al. (2012). Hereafter, the summer and autumn DISCOVERY cruises will be referred to as JR177 (2008) and JR200 (2009), respectively. Nets were sequentially closed at 125 m depth intervals from 1000 m to the surface: 1000 - 875 m, 875 - 750 m, 750 - 625 m, 625 - 500 m, 500 - 375 m, 375 - 250 m, 250 - 125 m and 125 - 0 m. The stations sampled were R1 (an ice-

influenced station), C3 (an open water, oligotrophic region), P2 (a putative oligotrophic region, upstream of the South Georgia & South Sandwich Island (SGSSI) archipelago) and P3 (a naturally iron-fertilised and highly productive region, downstream of the archipelago) (Figure 1). Samples were preserved in buffered formalin and were subsequently sorted and analysed for species counts at the home laboratory.

[Insert Figure 1]

Where counts were obtained from a split, they were multiplied by the split to give a count for the whole sample. The whole sample count was converted to abundance m^{-2} by dividing by the volume of water filtered by the net (m^3) and multiplying by the sampled depth interval (125 m). Flow rate data were taken from the flowmeter attached to the MOCNESS where possible. Where this failed (<25% nets), the volume filtered was calculated by plotting duration of individual net haul against volume filtered from the observed flowmeter readings.

The vertical movement of *Euphausia triacantha* between day and night, based on the abundance data obtained on JR177 (2008) and JR200 (2009), was assessed by calculating the weighted mean depth (WMD, m) of individuals during the day and at night, according to Equation 1:

$$WMD\ (m) = \frac{\sum(n_i \times z_i)}{N} \qquad \text{Equation 1}$$

where n_i is the number of the concentration of individuals (inds. m^{-2}) in each net horizon i, z_i is the mid-depth (m) of each net horizon i, and N is the total number of depth horizons sampled. The significance of the change in WMD, between day and night, was tested statistically using a paired Wilcoxon signed-rank test.

Temperature data for the same cruises were obtained by deploying a Sea-Bird Scientific SBE 9Plus Conductivity Temperature Depth (CTD) profiler with a dual SBE 3Plus temperature

sensor (Sea-Bird Scientific, Bellevue, Washington). Data were averaged for every 2 m from the surface to 1000 m.

2.2. On-board respiration experiments

Oxygen consumption experiments were conducted on board RRS James Clark Ross during two Southern Ocean research cruises which took place 5-7 years later in the austral spring: JR304, in November to December 2014; and JR15002, in November to December 2015. Hereafter, these cruises will be referred to as JR304 (2014) and JR15002 (2015) (see Table S2).

Oxygen measurements were taken using a PreSens (Regensburg, Germany) Fibox 4 fibre-optic oxygen optode with temperature sensor Pt100 and PSt3 sensor spots (Presens GmbH 2014), a device based on the principle of dynamic luminescence quenching by molecular oxygen. During JR304 (2014), experiments were carried out on manufacturer calibrated (to 0% and 100% saturated water) sensor spots. Prior to JR15002 (2015), all sensor spots were user calibrated following manufacturer's instructions. Briefly, the 0% saturation point was achieved by adding a sodium sulphite and cobalt nitrate mixture to 0.22 µm filtered seawater (FSW) and shaking the bottles vigorously. 100% oxygen saturated water was produced by half filling bottles with 0.22 µm FSW, shaking the bottles vigorously, removing the stoppers and allowing them to equilibrate to temperature and atmospheric O_2 concentrations. Calibration was carried out at ~3.5 °C. Bottles were prepared at least 48 hours prior to calibration using the prescribed routine on the PreSens device. The 100% saturation calibrations were repeated on board prior to first use, as per the manufacturer's recommendation. Subsequent on-board recalibration was not necessary as the number of measurement points did not exceed the re-calibration threshold.

To account for differences in calibration procedure and to allow both years to be compared, raw phase data from JR304 (2014) were retrospectively calibrated with the calibration constants from JR15002 (2015). This followed advice from the manufacturer and employed

the PreSens Oxygen Calculator v3.0.0.0 and PreSens Oxygen Calculator Software Instruction Manual V3.0.0 designed for this purpose (Presens GmbH 2016).

2.3. Incubator set-up

Incubations for the oxygen consumption experiments were conducted in the cold room (set to ~4 ºC) on the RRS James Clark Ross, in tanks designed to simulate the temperature range (0.2 – 4.7 ºC) experienced by *E. triacantha* during their DVM within the area of study. Experiments involved the incubation of euphausiids concurrently at two temperatures. The low temperature experiments (T1) were conducted at temperatures of 1.26 ± 0.70 °C (JR304 (2014)) and 1.58 ± 0.17 °C (JR15002 (2015)) and the high temperature experiments (T2) at 3.08 ± 0.29 °C (JR304 (2014)) and 4.66 ± 0.07 °C (JR15002 (2015)) (Table 1). Incubations took place in the dark with the exception of the few minutes when measurements were taken. The incubator setup was modified between JR304 (2014) and JR15002 (2015) to provide improved temperature control. Specifically, this meant that, in JR15002 (2015), the two temperatures were achieved in separate tanks as opposed to a graded tank in JR304 (2014).

During JR304 (2014), a purpose-built incubator (Spartel Temperature Gradient Incubator) was used. This had a C-400 circulator unit at the warm end and an FC-500 in-line cooler unit and C-85D circulator unit at the cold end, with temperature at each end controlled by ethylene-glycol anti-freeze being circulated through the end blocks of the incubator.

In JR15002 (2015), two separate thermostatically-controlled incubation tanks were used. This comprised a chiller unit (Julabo FL300 chiller) and a thermocirculator heating unit with cooling coil for each tank (ED Heating Immersion Circulator and Julabo Cooling Coil). The chiller unit worked against the heater unit to achieve the desired temperatures. The chiller unit was filled with ethylene-glycol anti-freeze, which was circulated through tubing sequentially connected to the cooling coils fitted to each thermocirculator.

The experimental setup is illustrated in Figure 2.

2.4. Animal capture and experiment set-up

Healthy specimens of *E. triacantha* were selected from three RMT8 (8 m^2 mouth opening, 5 mm mesh) and five MOCNESS (1 m^2 mouth opening, 330 µm mesh) net catches during JR304 (2014) and two RMT8 (specification as above) net catches during JR15002 (2015), all of which were in the northern Scotia Sea (see Figure 1). Temperature data were obtained from deployment of the CTD (details given in Section 2.1). All animals in a given incubation originated from the same net catch. Details of the nets that animals were obtained from are given in Supplementary Table S2. Animals were deemed to be healthy if they were translucent, swimming actively and had no visible signs of damage. Animals were gently rinsed twice in 0.22 µm FSW and transferred to incubation bottles.

On JR304 (2014), one animal was incubated in each 60 ml bottle. On JR15002 (2015), four or five animals were incubated in 250 ml bottles, depending on the numbers of healthy animals at the time of the experiment. The change in vessel size was due to the change in design of experimental incubators, whilst maintaining approximately the same volume of water per animal between years. In an experiment on *E. superba*, the size of experimental vessel was found not to impact the measured metabolic rate, thus rates obtained in different sized vessels were considered comparable (Opalinski 1991). A drop of unfiltered seawater, equivalent to between 2-5 ml, from the same container as the euphausiids, was placed into control bottles to model the amount of unfiltered seawater added to the incubation bottles while transferring the animals.

Details of experiments are given in Table 1.

[Insert Figure 2]

Experimental bottles were topped up with FSW, maintained at experimental temperatures for at least an hour before incubations and stoppered, taking care to remove all air bubbles. At least 2 hours elapsed between capture and introduction to the experimental bottles, during which time animals were maintained in water at the temperature of the experiment. Approximately a further 30 minutes elapsed between experiment set-up and the first measurement. Readings were taken every one to four hours throughout the incubation period, with between three and seven measurements made during the ~2 to 28 hour duration of the eight experiments.

Whilst taking readings, the animals were inspected for general health and activity i.e., that the animals responded to the gentle rotation of bottles with active swimming and were displaying the same colour, shape and translucency as at the start of the incubation, or had visible signs of deterioration. Bottles were subject to the natural movement of the ship and gently rotated before measurement to ensure water was well mixed. The oxygen saturation of the water in the bottles was also carefully monitored. The critical concentration of oxygen, below which respiration rates of marine animals markedly decline, is considered to be ~50% (Ikeda 1970) although this varies between species. In this study, measured oxygen saturation (% air saturation (a.s.)) at the end of incubations was always >70%.

Bottles containing dead or deteriorating animals were discarded. Incubations of bottles containing healthy animals were completed after at least three readings. At the end of the incubation, the animals were removed from the incubation bottles and frozen immediately at -80 °C. The length of incubations (generally between 2 and 6 hours) was a compromise between being long enough to obtain at least three reliable readings following a period of acclimation, and the space and time requirements to prepare for the next net haul and experiment setup. INC3 was the exception since there was no immediate pressure on equipment. In general, mortality was low (<10%) and showed no association with temperature.

[Insert Table 1]

2.5. Determination of length, weight and elemental content

Frozen experimental specimens were thawed and length (L, mm) was measured, taken from the front of the rostrum to the tip of the telson. Wet weight (WW, g) was determined immediately after the measurement of body length, within two minutes of thawing, in pre-weighed weighing boats. Animals were quickly dabbed on absorbent paper to remove excess water. Animals were dried at 65 °C for 48 hours and re-weighed for dry weight (DW, g).

After dry weight measurements, animals from JR15002 (2015) were homogenised using a ceramic pestle and mortar and transferred to tin capsules for elemental (C and N) analysis and weighed. Analysis was carried out with a CE440 Elemental Analyser (Exeter Analytical (UK) Limited, Coventry, United Kingdom). Malfunction of the elemental analyser prevented the same analysis being carried out on animals from JR304 (2014).

2.6. Respiration data treatment and statistical analysis

Oxygen (O_2) consumption per bottle (µmol l^{-1}) was calculated by subtracting the mean O_2 consumption of control bottles from that of experimental bottles. Values were converted from µmol l^{-1} to µl l^{-1} by multiplying by 22.391 (ICES, 2018). Oxygen consumption (µl O_2) ind^{-1} h^{-1} was calculated by dividing by duration (h) of experiment and number of individuals per bottle, and adjusting for bottle volume.

To examine the effect of body size and temperature on the respiration rate of *E. triacantha*, body weight (in mg dry weight (DW), carbon (C) and nitrogen (N)) and temperature were included as variables in a multiple regression, following the model described by (Ikeda 1985). Since C and N were only experimentally determined for JR15002 (2015) animals, the regressions between DW, and C/N given in Figure 4 were applied to the DW for animals incubated during JR304 (2014) to obtain a full set of C and N values for the multiple regression.

In the multiple regression we performed, three coefficients resulted: *a0* = constant, *a1* = body weight, and *a2* = temperature (Table 3). Since respiration rate tends to scale with body size according to the relationship $R = a * W^b$, the body weight constant, *a1*, thus determined the scaling exponent, *b*. Similarly, the sensitivity of an animal's respiration rate to increases in temperature can be assessed by calculation of the Q_{10}, following the van't Hoff rule (Equation 2).

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)} \quad \text{Equation 2}$$

where T_1 is the lower temperature, T_2 is the higher temperature and R_1 and R_2 are the respiration rates at T_1 and T_2 respectively.

Using a rearrangement of the formula as set out in Equation 3 (see Ikeda 1985), the temperature constant, *a2*, can be used to calculate the Q_{10} for *E. triacantha*.

$$a_2 = \frac{lnQ_{10}}{10} \quad \text{Equation 3}$$

Length-weight coefficients were determined by log transforming all data and regressing WW, DW, C and N against length with a linear fit according to the relationship y = a + b*x. The relationship between DW and C or N content; and DW and the C:N ratio was examined by fitting the data (from JR15002 (2015) alone, see section 3.2) to the same linear model.

Statistics were carried out in SigmaPlot V.13.0.0.83 (Systat Software Inc.) and RStudio (V.3.6.2 (R Core R Development Core Team 2019).

3. Results

3.1. Distribution and environment of *Euphausia triacantha*

Specimens of *Euphausia triacantha* were found throughout the water column at three stations across the Scotia Sea from the sub-Antarctic to Antarctic zones, both in the summer (JR177 (2008)) and autumn (JR200 (2009)) abundance cruises, where surface temperatures varied from <1 to 5 °C (Figure 3). This included C3 (the open water oligotrophic region south of the Sub-Antarctic Circumpolar Current Front (SACCF)), P2 (upstream of SGSSI and putatively oligotrophic), and P3 (the naturally iron fertilised area downstream of SGSSI). None were found at the ice-influenced station, R1. Across the 1000 m water column, summer abundances ranged from 3 inds. m^{-2} at C3 to 18 inds. m^{-2} at P3, and autumn abundances ranged from 1 ind. m^{-2} at C3 to 46 inds. m^{-2} at P3. The animals occurred throughout the water column, from the surface to as deep as 1,000 m, at all stations in both seasons. They also displayed clear evidence of diurnal vertical migration, with a total absence of animals in the top 250 to 375 m at C3 and P2 during the day, but occupation of these layers during the night in both seasons. At P3, animals were found between 125 and 250 m during the day in summer, followed by an ascent by the majority of the population to the surface at night. In autumn, a bimodal distribution was apparent and, although a small number of animals were found in the surface during the day, this markedly increased at night. The weighted mean depth (WMD) of animals during day and night (Table 2) illustrated the consistency of DVM by *E. triacantha*, with shallower night-time compared to daytime depths in every sample. During daytime, the WMD of *E. triacantha* ranged from 232 to 500 m in summer and 438 to 531 m in autumn, whilst at night, WMDs were 80 to 349 m in summer, and 81 to 339 m in autumn. A Wilcoxon signed-rank test between paired day and night samples confirmed the WMD (m) during the day to be significantly deeper than during night ($n = 6$; $p = 0.0156$). Across their vertical migratory ranges, the temperatures experienced by *E. triacantha* during JR177 (2008) and JR200 (2009) ranged from -1.5 to 2.1 °C at the most southerly station, and from 0.4 to 5.0 °C at the most northerly, representing a temperature range of 6.5 °C. The strongest temperature gradient experienced during a typical

DVM was across the thermocline, between ~60 to 100 m, where the mean temperature change was 3.5 °C (range 2.5 °C to 4.5 °C). Temperatures during experimental years, JR304 (2014) and JR15002 (2015), were similar, ranging from -1.4 to >3 °C over the same geographical area, representing a range of >4.4 °C.

[Insert Table 2]

[Insert Figure 3]

3.2. Morphometric and elemental analysis

A total of 159 animals obtained from the experimental cruises, JR304 (2014) and JR15002 (2015) in austral spring, were measured and weighed. Animals ranged in length (L) from 20.0 to 39.4 mm. This likely represents animals from at least two age cohorts, the sub-adult population (<24 mm) and the adult population (>24 mm) (Baker 1959, Siegel 1987). Wet weights (WW) ranged from 60.31 to 427.32 mg and dry weights (DW) ranged from 12.49 to 96.01 mg. A significant relationship between L and weight was found ($P < 0.0001$) (see Figure 4, panel A and Supplementary Table S3).

A total of 90 animals were analysed for elemental composition, in addition to length and weight measurement. The plots are shown in Figure 4, panels B and C, and regression results are given in Supplementary Table S4. C content ranged from 37.9% to 45.0% and N content ranged from 8.8% to 10.1%. The mean C:N ratio was 4.39 (ranging from 3.93 to 4.90). Significant relationships between C and N were found for both measures of weight. The relative proportions and hence the C:N ratio varied positively, weakly but significantly ($P < 0.0001$) with DW.

[Insert Figure 4]

3.3. Effect of size and temperature on respiration

Over our experimental temperature range of 0.17 to 4.74 °C, individual respiration rates ranged from 5.83 to 38.06 µl O_2 ind^{-1} h^{-1}. Multiple regression confirmed a significant effect of all measures of weight (mg DW, C or N), and temperature, on individual respiration (Table 3). The body weight coefficient, or scaling exponent, *b*, was 0.84 for mg DW, ranging from 0.81 (mg C) to 0.87 (mg N). The temperature coefficient yielded a Q_{10} of 3.6 for all measures of weight (Table 3). Body weight contributed most to the variance in individual respiration rate, and temperature contributed a significant additional component (Figure 5).

[Insert Figure 5].

When standardised to body weight using the *b* value of 0.84, respiration rates over the same temperatures ranged from 0.37 to 1.65 µl O_2 mg DW^{-b} h^{-1}, decreasing with increasing DW and increasing with temperature.

[Insert Table 3]

4. Discussion

4.1. Distribution and abundance of *Euphausia triacantha*

Analysis of depth-discrete zooplankton samples taken from across the Scotia Sea during the abundance cruises, JR177 (2008) and JR200 (2009), showed *Euphausia triacantha* to be abundant (up to 46 individuals m^{-2}) and distributed over a wide latitudinal gradient in both summer and autumn. This included the colder waters south of the Southern Antarctic Circumpolar Current Front (SACCF) to the warmer, more productive region of the north Scotia Sea, north of the front. Abundances were substantially greater north of the SACCF in both summer and autumn, ranging from 1 to 3 inds. m^{-2} south of the SACCF, to between 8 and 46 inds. m^{-2} in the north. *Euphausia triacantha* also displayed a prominent diel vertical migration (DVM), with deeper weighted mean depths in daytime compared to night-time demonstrative of a clear movement of the population towards the surface at all stations and in

both seasons. This confirms previous observations of the species exhibiting a wide vertical and latitudinal distribution, and a consistent absence of *E. triacantha* in surface waters during the day compared with their presence at night (Baker 1959). Temperatures experienced by the animals over this DVM varied with location and season, reaching up to 6.5 °C, with the most rapid and substantial changes occurring in the 50 to 100 m layer and corresponding to the depth the euphausiids would likely cross during their upward and downward migrations. This justified our study into the effect of short-term temperature change on the respiration rate of *E. triacantha*, and the temperature range examined during this study.

4.2. Response of respiration rate to size and temperature

To consider the effect of temperature on *E. triacantha*, we used a multiple regression model that incorporated both body size and temperature variables concurrently. The respiration of *E. triacantha* was found to be significantly dependent on both variables. The majority of the variance was explained by body mass, with a scaling exponent of 0.84, and a significant additional component was explained by temperature, with a Q_{10} of 3.6. Despite the suggestion that pelagic animals may be more likely to scale isometrically i.e. with ratio of 1 (Small & Hebard 1967, Glazier 2006), the scaling coefficient obtained confirmed that the respiration of *E. triacantha* scales allometrically with size. Nevertheless, the value of 0.84 was greater than the three quarter power rule commonly used to describe the relationship between respiration and body weight (Kleiber 1932), and greater than the coefficient obtained in a global analysis of 24 species of euphausiid (Ikeda 2013). It was, however, closer to the value of 0.81 obtained for adult and juvenile *E. pacifica* (Ross 1982) where an increase in the value of the coefficient with age was observed. Considering that the present study on *E. triacantha* also incorporated both adults and juveniles, our result may support the suggestion of Ross (1982) that an increasing slope could reflect a metabolic change that occurs at maturation.

When considering the response of biological rate processes to temperature, a Q_{10} of between 2 and 3 is typical (Schmidt-Nielsen 1997) essentially producing an exponential curve. This relationship has been found to hold across a wide spectrum of marine zooplankton, including euphausiids (e.g. Paranjape 1967, Small & Hebard 1967, Teal & Carey 1967, Ivleva 1980, Ikeda 2013). It is also consistent with previous studies that have found the respiration rate of northern krill, *M. norvegica* (Saborowski et al. 2002), and mixed Arctic zooplankton (Alcaraz et al. 2013), to rise exponentially as experimental temperature increased, and appears independent of experimental technique. In Saborowski et al. (2002), the same pattern was observed from measurements made with both a Clark-type electrode and the Winkler method, the only difference being a lower rate in the Winkler experiments due to reduced swimming activity; whilst Alcaraz et al. (2013) used optodes from the same manufacturer as in the present study.

Although a Q_{10} of between 1 and 4 may be considered within the 'normal' range within the geographical region of our study (Clarke & Peck 1991), higher values may be indicative of some sort of stress (Hirche 1984), too short an acclimation period (Ivleva 1973) or active rather than routine metabolism being measured (Conover 1978). In our study, the majority of experimental animals were taken from the northern Scotia Sea where the average ambient temperature below the thermocline was 1.8 ºC, in contrast to 0.06 ºC in the southern Scotia Sea. Saborowski et al. (2002) found different populations of *M. norvegica* to compensate for the temperature of their habitat, displaying the same respiration rate whether residing at 4, 8 or 12 ºC. Given the acute nature of our experiments, it may be that rates measured at the lowest end of the temperature spectrum are lower than might be expected of animals taken directly from that environment and that, with time, they may have compensated for this by elevating their metabolism (Clarke 1983). Another feature of the Q_{10}, is that it is inversely related to the temperature range over which it is calculated, and to temperature itself (Clarke 2017), with

lower temperatures and smaller ranges yielding higher values. Indeed, in his study of seven polar pelagic zooplankton species, Hirche (1984) found the Q_{10} to be systematically lower when calculated over 5-10 ºC (Q_{10} of 2.6 to 5.2) compared to 0-5 ºC (2.8 to 5.6).

Nevertheless, our Q_{10} of 3.6 is higher than the average of 2.8 obtained during intraspecific studies on euphausiids (Paranjape 1967, Small & Hebard 1967, Teal & Carey 1967, Hirche 1984, Iguchi & Ikeda 1995), and substantially higher than the Q_{10} of 1.7 determined by Ikeda (2013) in his global, interspecific compilation of 24 euphausiid species. This suggests that *E. triacantha* has not developed a mechanism to compensate for short-term temperature changes such as it was exposed to in this study, and that over the course of its migration it does not adjust its rate of respiration. This therefore represents a metabolic cost to the animal which we hypothesise is balanced by food intake upon reaching surface waters. We further hypothesise that this lack of compensation may be attributable to the migratory regime of *E. triacantha*. Specifically, the vertical distribution profiles of *E. triacantha* in Fig. 2 consistently show a relatively deep spread of animals throughout the water column at night, even though the bulk population moves upwards. Additionally, a bimodal distribution is apparent in at least three of the profiles (P2 in summer; C3 and P3 in autumn). This suggests that migration of the population is asynchronous (Pearre 1979) and that the whole population does not carry out full DVM every day. The implication of this for our study is that, if DVM is not as ubiquitous as currently assumed, and the animals do not cross a sharp thermal boundary every day, the cost to the animal is lower, and there is consequently little advantage to the development of a temperature compensation mechanism. The combination of elevated Q_{10} and insight into vertical distribution may therefore shed new light on the migratory behaviour of *E. triacantha,* with potential implications for biogeochemical fluxes.

4.3. High variability in respiration rate across the temperature spectrum

Notwithstanding the general trend of increasing respiration rate with temperature, we also observed individual-level variability across the temperature spectrum measured. This may be influenced by a number of factors, including locomotive activity, feeding, developmental stage, sex, injury or container crowding, and chemical or physical factors (e.g. Hernández-León & Ikeda 2005b). Some variability may also be attributable to measurement at temperatures ~2 °C different to that at which the instrument was calibrated. In our study, oxygen saturation was not found to be limiting, and the effect of pressure has been found to be low or negligible, especially at low temperatures (Teal & Carey 1967, King & Packard 1975, Torres & Childress 1983, Childress & Thuesen 1993, Thuesen et al. 1998). Thus, the determination of respiration rates of deeper-dwelling zooplankton at surface pressures is deemed experimentally sound (Childress & Thuesen 1993, Hernández-León & Ikeda 2005a). No obvious effect of injury was observed on any of the animals that were alive at the end of the experiment, and there was no evidence of a container effect in either year. Regarding activity, the distinction between basal, routine and active metabolism is important (Harris et al. 2000). Whilst not possible to quantify the effects of activity in the current study, activity levels of individuals were qualitatively noted throughout experiments, and most exhibited a mixture of periods of inactivity interspersed with bouts of active swimming. This likely represents routine metabolism, thus is consistent and comparable with most studies on euphausiid respiration where measurement in containers constrains full movement (Clarke & Morris 1983, Ikeda 2013, Tarling 2020). As a result, the rates presented here are likely conservative estimates and do not take into account the full range of locomotive activity an animal is likely to exhibit in the environment.

Another consideration is light, which may exert an influence on respiration rate through its control on circadian rhythm (e.g. Mortola 2004, Teschke et al. 2011). Light was largely controlled for in this study, with all animals subjected to the same experimental conditions, so it was assumed that any variability observed in a given experiment was individual rather than

due to variability in circadian phase. However, since the timing of individual experiments varied (see Table S2), differences in phase of photoperiod may have led to variability in respiration rate between experiments. Feeding, or Specific Dynamic Action (SDA) (Secor 2009), may also affect respiration rates, with polar ectotherms compensating for a lower post-prandial peak with a longer period of metabolic elevation (Peck 1998, Whiteley et al. 2001). Whilst we cannot exclude the possibility that some animals had fed more recently than others, the influence of SDA was minimised with a period of acclimation, and any risk of feeding in the net is likely to be outweighed by capture stress expediting gut passage. Unfortunately, the effects of SDA in epipelagic crustaceans, particularly euphausiids, are poorly understood, although one study did conclude that increases in post-prandial oxygen consumption were the effect of SDA, with the authors suggesting that the metabolism of wild krill may be 1.6 times that of their non-feeding counterparts (Ikeda & Dixon 1984).

Finally, variability between individuals may be introduced as a result of differences in developmental or reproductive state. Elevated rates have been observed in moulting specimens of *E. pacifica* (Paranjape 1967) and *E. triacantha* (Ikeda & Mitchell 1982), and are associated with gametogenesis (Lasker 1966, Clarke 1980). Since spawning in *E. triacantha* is thought to occur between October and November (Dzik & Jazdzewski 1978), it was considered that any females would have been spent before our period of study, and no moulting specimens were observed. However, we observed an increasing C:N ratio with size which may indicate greater lipid stores of larger animals (e.g. Post et al. 2007, Logan et al. 2008), or ovarian maturation in females. In their study on *E. superba*, Ikeda and Mitchell (1982) found the lowest weight-specific respiration rates for gravid krill, so values at the lowest extreme of our ranges could represent those of females with maturing ovaries.

4.4. Comparison of respiration rate with other euphausiids

To contextualise our findings, we compared rates presented in this study with literature values from euphausiids of a similar size and lifestyle, normalised to the scaling coefficient $b = 0.84$ (Figure 6). The relationship we obtained is almost identical to that obtained for *M. norvegica* (Saborowski et al. 2002), and close to rates observed for *E. crystallorophias* (Ikeda & Fay 1981) and *E. triacantha* (Ikeda & Mitchell 1982, Torres et al. 1994). *Meganyctiphanes norvegica* and *E. crystallorophias* are omnivorous species of a comparable size so may represent a good analogue for *E. triacantha*. *Meganyctiphanes norvegica* performs DVM and has been found to exhibit elevated rates of respiration during migration (Saborowski et al. 2000), and recent work suggests *E. crystallorophias* may also perform shallow DVM (Conroy et al. 2020). We also compared our results to the global-bathymetric model proposed by Ikeda (2013) in which body mass, temperature and depth of occurrence were used in an attempt to describe the global respiration of euphausiids (Figure 6). We assumed an average size for *E. triacantha* of 34.2 mg DW and a median depth of occurrence of 370 m based on our own data, and calculated rates across a 5 °C temperature range using the Ikeda (2013) empirical model. The modelled curve was flatter and predicted a smaller increase in respiration rate with temperature than the regression we obtained. Torres et al. (1994) also considered depth, estimating that the metabolism of Antarctic micronekton at 1000 m is a third of that at the surface. However, our comparison of rates suggests that seasonality may be of greater influence than depth, and this may be especially true for a migrating organism such as *E. triacantha,* whose depth of occurrence at the time of sampling may not be representative of the depth at which it most commonly resides (e.g. Teal & Carey 1967). In copepods, higher rates in spring compared to winter have been linked to food availability, nutritional condition and metabolic slowdown during diapause (Conover 1959, Marshall & Orr 1966, Båmstedt 1979, Castellani & Altunbaş 2014). The effects of seasonality on euphausiids are less well-studied, although Torres et al. (1994) confirmed lower metabolic rates in *E. superba* over winter. More recent

work suggests that reduced feeding (Meyer et al. 2010) and changes to the local light regime (Meyer 2012, Tremblay et al. 2014, Piccolin et al. 2018) may drive seasonal metabolic cycles in *E. superba*. Although *E. triacantha* exhibits less seasonality in growth than *E. superba* (Siegel 1987), a study by Donnelly et al. (2004) obtained substantially higher respiration rates during a summer ice-edge bloom than similar experiments conducted during winter (Torres et al. 1994), however data were insufficient to enable a statistical comparison. The rates obtained in the present study, which was carried out in spring, were more comparable to the winter-time rates of Torres et al. (1994) but still substantially lower than those of Donnelly et al. (2004), suggesting that there may be a seasonal component to the metabolism of *E. triacantha* that merits further investigation. However, our experiments took place in late-spring in the northern Scotia Sea, where day lengths were long (~17 hours) and diatom blooms were encountered in both years. Since this was not associated with a rate as high as that observed by Donnelly et al. (2004), factors such as population structure, availability of alternative food sources, timing of the bloom and nutritional state at bloom onset may also be important. It is also worth noting that the average seasonal temperature change (~2 ºC in the vicinity of South Georgia, Whitehouse et al. (2008)) is low in comparison to the gradient experienced during DVM (up to 4.5 ºC), a factor that may also contribute to the steeper curve that we observed (Figure 6).

Finally, the difference between the steeper slope obtained in the present study and that predicted from Ikeda (2013)'s model may be attributable to intra- versus inter-specific responses. It is common for a lower Q_{10} to be obtained when comparing between species that have *adapted* to a temperature over evolutionary time, than for individuals within a species where the response being measured is *acclimation* to a short-term temperature change (Ikeda 2013). We therefore suggest that the value of such a global model lies in describing general relationships across contrasting temperatures and habitats but it is not able to predict the full

physiological response elicited by an organism such as *E. triacantha* which is subject to substantial depth and temperature changes over the course of a diel vertical migration.

As we suggest, the strong response of *E. triacantha* to temperature indicates a lack of compensation, or adjustment, for substantive temperature changes experienced over its migration, which we propose may represent a metabolic cost to migration. This may reflect asynchronous or partial DVM, with the implication being that a compensatory response has not been developed due to animals not being engaged in extensive DVM on a daily basis. This not only challenges our assumptions of the ubiquity of DVM, but also has important implications for biogeochemical cycling in the Southern Ocean. Animals that undertake deep DVMs, such as euphausiids, have significant potential to pump carbon into deeper layers of the ocean, bypassing the mixed layer and isolating carbon from the atmosphere over climatically significant timescales (Longhurst & Harrison 1988, Steinberg & Landry 2017, Cavan et al. 2019). How much time migrators spend above or below the thermocline is therefore an important quantity to resolve if we are to generate reliable estimations of active CO_2 flux (Conroy et al. 2020), and a better understanding of DVM in euphausiids is fundamental to this (Cavan et al. 2019). For example, a high respiration rate in warmer surface waters may result in increased amounts of dissolved carbon dioxide (CO_2) being respired during periodic excursions into the mixed layer. This may however, be more than offset by substantially longer durations spent at depth, where carbon consumed at the surface is respired below the thermocline, under a scenario whereby an animal only migrates a fraction of the time. A clearer understanding of the behaviour and ubiquity of DVM in *E. triacantha* is therefore of critical importance in estimating its contribution to the active flux of carbon.

[Insert Figure 6]

4.5. Concluding remarks

This analysis of the distribution, migration and metabolic rate of *E. triacantha* is the most comprehensive such study to date. It is also the first to consider the potential effect of temperature changes experienced during DVM on the respiration of this important euphausiid. Over our experimental temperature range of 0.17 to 4.74 °C, respiration ranged from 0.37 to 1.65 µl O_2 mg DW^{-b} h^{-1}. Despite *E. triacantha* being found in environments with wider ranging temperatures than the ~4.5 °C range they were exposed to in this study, our results suggest that it does not compensate for the short-term exposure to such temperature change, perhaps indicating that DVM is not so ubiquitous a behaviour as commonly assumed. We hypothesise that diel vertical migration in *E. triacantha* represents a metabolic cost that is offset by exposure to prey. Comparison with literature-derived rates suggest that *E. triacantha* has a wide metabolic scope, which may be seasonally-influenced, thus our results represent a conservative estimate of average spring-time rates. We conclude that global relationships of respiration to body size and temperature may not be sufficient to understand the full range of physiological and interspecific variability in how organisms respond to temperature changes over short time scales such as occurs during DVM. Such interspecific variations in behaviour and physiology may have important implications for how community respiration is estimated, with associated implications for our understanding of biogeochemical cycling. Our results are of particular relevance for constructing accurate estimates of the flux and sequestration of respiratory carbon to the ocean interior.

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Tables

Table 1: Details of the experiments carried out on *Euphausia triacantha* during JR304 (2014) and JR15002 (2015). T1 and T2 refer respectively to the mean and standard deviations of the low and high temperatures experienced by animals during each experiment. Animals were exposed either to T1 or to T2 for the duration of the experiment. 'Animals at start' refers to the number of animals exposed to each temperature. 'Animals at end' refers to the number of healthy animals remaining at the end of each experiment. INC and EXP refer to the names that experiments were given in respective cruises.

Cruise	Month/ Year	Exp #	Duration (h)	T1 (°C)	T2 (°C)	Animals per bottle	Animals at start	Animals at end	Mean animal length (mm)
JR304 (2014)	11/14	INC3	27.9	0.80 ± 0.12	3.28 ± 0.15	1	6	3	24.7 ± 0.5
JR304 (2014)	12/14	INC4	4	1.20 ± 0.13	2.92 ± 0.06	1	12	10	25.4 ± 1.3
JR304 (2014)	12/14	INC5	6.4	0.20 ± 0.68	3.35 ± 0.16	1	12	11	24.5 ± 1.2
JR304 (2014)	12/14	INC6	3.7	1.81 ± 0.04	3.14 ± 0.01	1	12	11	31.5 ± 3.1
JR304 (2014)	12/14	INC7	6.2	1.56 ± 0.10	2.57 ± 0.05	1	12	9	30.4 ± 3.3
JR304 (2014)	12/14	INC8	4	2.20 ± 0.05	3.19 ± 0.12	1	12	12	28.8 ± 4.3
JR15002 (2015)	12/15	EXP1	2.1	1.43 ± 0.00	4.66 ± 0.00	5	50	50	27.0 ± 3.1
JR15002 (2015)	12/15	EXP2	2.2	1.74 ± 0.11	4.73 ± 0.08	4	40	40	27.7 ± 2.6

Table 2: Weighted mean depth (WMD, m) of *Euphausia triacantha* for pairs of nets deployed during summer, JR177 (2008) and autumn, JR200 (2009) abundance cruises. Nets were deployed at stations C3, P2 and P3, during the DISCOVERY 2010 programme. Calculations are based on abundances (inds. m^{-2}) and depth profiles presented in Figure 3 following Equation 1. For each pair of nets, the shallower depth is highlighted by bold text.

DISCOVERY 2010 sampling station	**Summer: JR177 (2008)**		**Autumn: JR200 (2009)**	
	Day	**Night**	**Day**	**Night**
C3	500	**173**	438	**339**
P2	404	**349**	531	**81**
P3	232	**80**	460	**172**

Table 3: Multiple regression statistics for *Euphausia triacantha* O_2 consumption (y = O_2 ind^{-1} h^{-1}) as a function of log weight (lnX1, mg DW, C and N) and temperature (X2, °C), following Ikeda (1985). The coefficients are: *a0* = constant, *a1* = DW and *a2* = temperature. R^2_{adj} = adjusted R^2, SE = standard error and DF = degrees of freedom.

lnY (µl O_2 consumption)	**Mass (mg)**	***a0***	***a1***	***a2***	**R^2_{adj}**	**Q_{10}**	**SE**	**DF**
R ind^{-1} h^{-1}	DW	-0.64226 *	0.84255 ***	0.12846 ***	0.677	3.6	0.271	73
R ind^{-1} h^{-1}	C	0.17957	0.80857 ***	0.12776 ***	0.678	3.6	0.271	73
R ind^{-1} h^{-1}	N	1.31137 ***	0.86891 ***	0.12773 ***	0.679	3.6	0.270	73

Model form $lnY = a0 + a1 lnX_1 + a2 X_2$
* P < 0.05; *** P < 0.001

Figures

Figure 1: Map showing the sampling locations for the distribution and abundance of *Euphausia triacantha*, based on the DISCOVERY 2010 abundance cruises: JR177 (2008) and JR200 (2009); and for the collection of animals for respiration experiments: JR304 (2014), prefixed with INC- and JR15002 (2015), prefaced with EXP-. The position of the fronts are, from north: Polar Front (solid), Sub-Antarctic Circumpolar Current Front (dashed) and Southern Boundary (dotted) based on Park and Durand (2019).

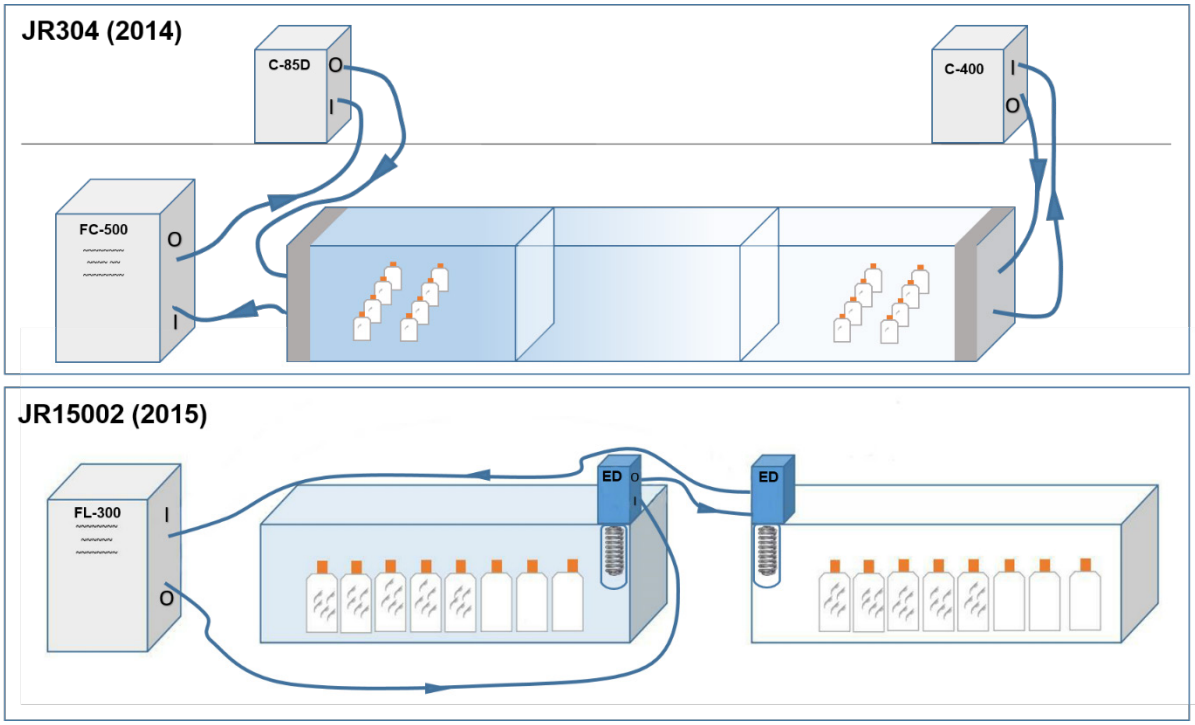

Figure 2: Schematic showing the setup of the *Euphausia triacantha* incubation experiments in Cruise 1 (JR304) and Cruise 2 (JR15002) (not to scale). The main blocks depict the incubation units containing incubation bottles. The boxes on the left of each image (FC-500 and FL-300) depict the chiller units. The smaller boxes (C-85D and C-400 in JR304 (2014); and ED in JR15002 (2015)) depict the thermocirculators. The chillers and circulators are connected to one another via the blue tubing containing ethylene glycol antifreeze, with arrows showing the direction of flow. I = In; O = Out.

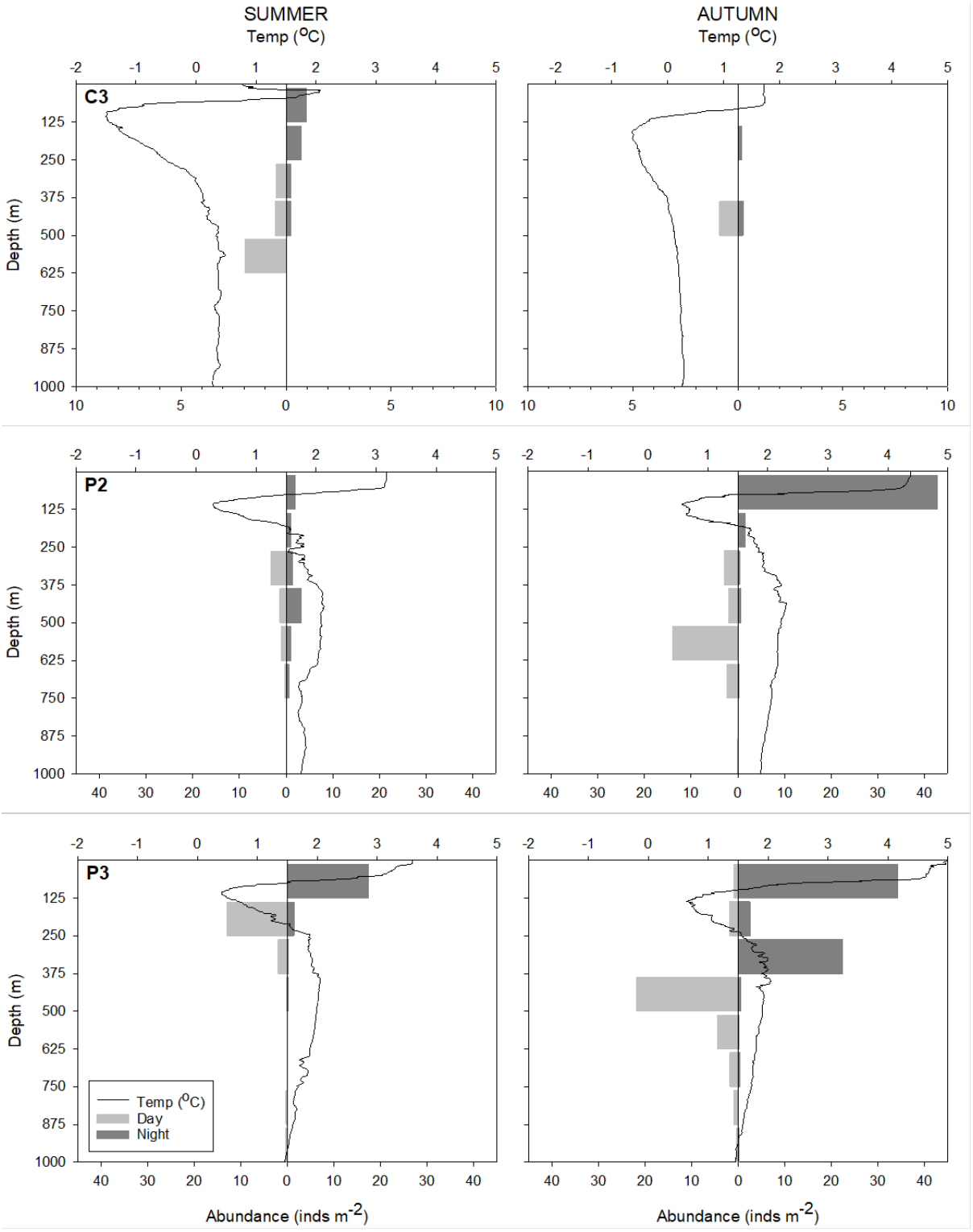

Figure 3: Plots showing the day and night time abundances (individuals m^{-2}, bottom axis) of *Euphausia triacantha* from the DISCOVERY 2010 sampling programme during summer (JR177 (2008)) and autumn (JR200 (2009)) at C3 (top), P2 (middle) and P3 (bottom). No *E. triacantha* were recorded at R1 in the years sampled. Temperature data recorded from the CTD

at each station at the same time are overlain (top axis). Note the different scale for the abundance axis at C3.

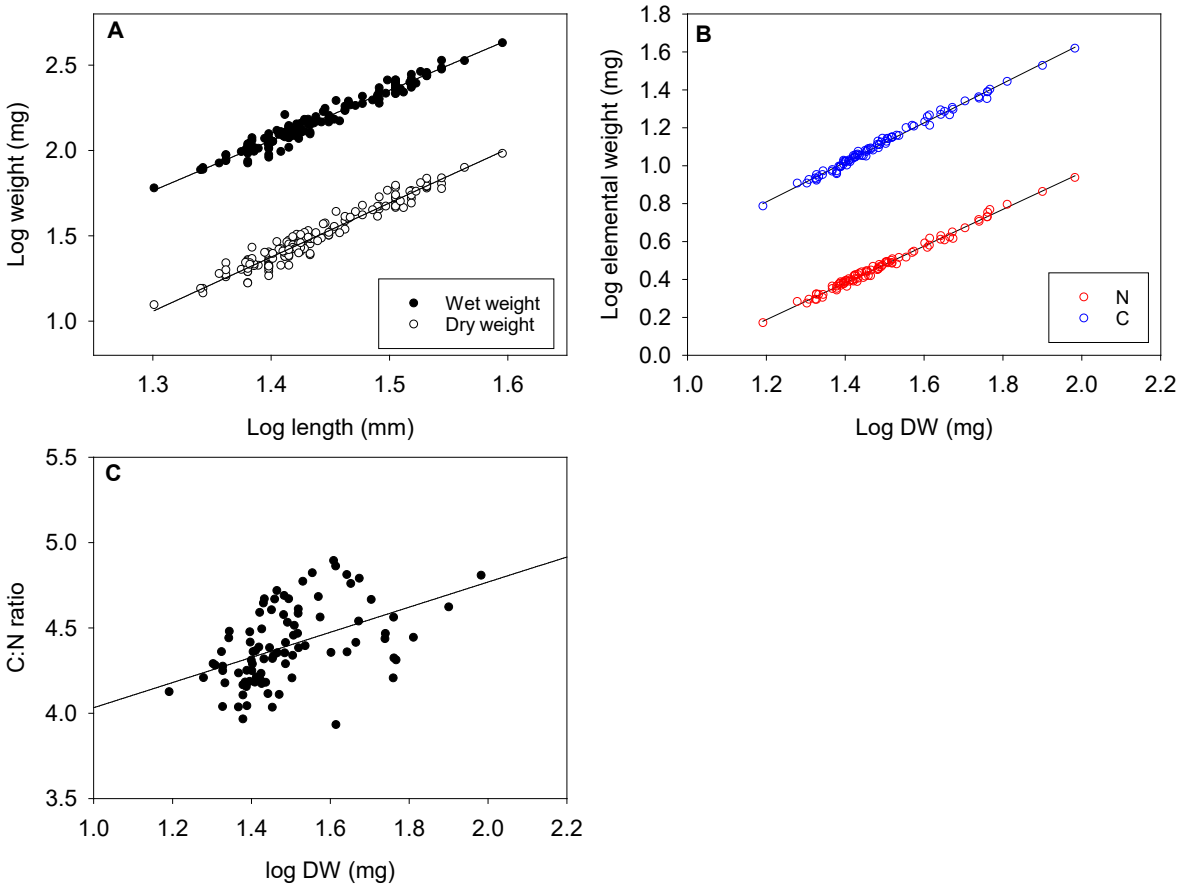

Figure 4: Plots showing the relationships between A) log length (L) and log wet/ dry weight (WW/DW) (DW = 3.1723*L - 3.0656, WW = 2.9432 * L - 2.0619, B) log dry weight (DW) and log carbon (C)/ nitrogen (N) content (C = 1.0424 * DW - 0.4416; N = 0.9703 * DW - 0.9763), and C) log DW and C:N ratio (C:N = 0.7364* DW + 3.2960) for *Euphausia triacantha*. All significant at $P < 0.001$.

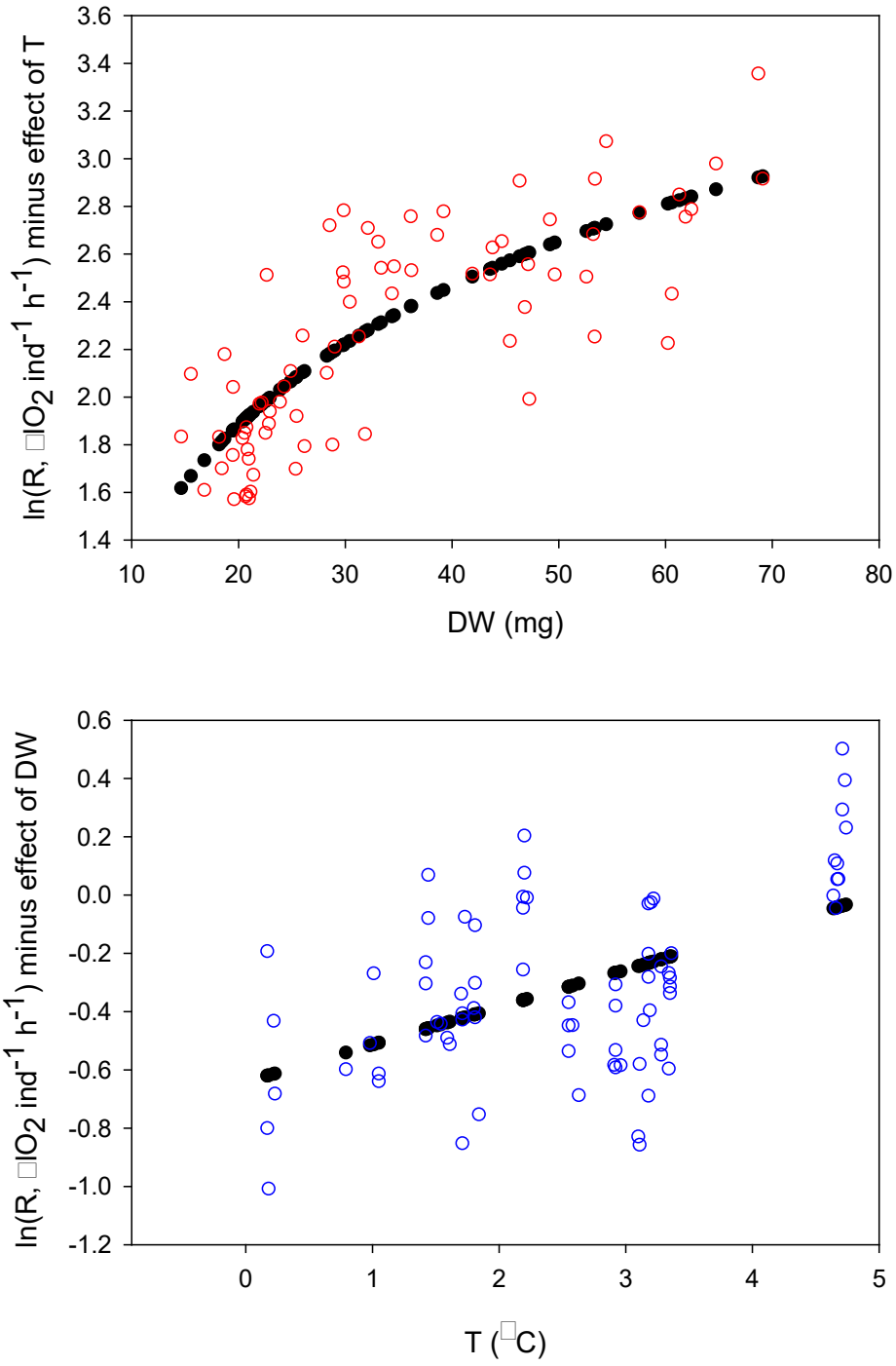

Figure 5: Plot showing the predicted and derived respiration rate (lnR, µl O_2 ind^{-1} h^{-1}) of *Euphausia triacantha* in response to dry weight (DW, mg), minus the effect of temperature (top panel); and in response to temperature (T, ºC), minus the effect of DW (bottom panel). Predicted values (black dots) were calculated from the regression coefficients in Table 3 following a rearrangement of the regression equation. Derived values (red, top; and blue,

bottom) were calculated by subtracting the predicted values of temperature or DW, respectively, from the measured respiration rate (ind^{-1} h^{-1}).

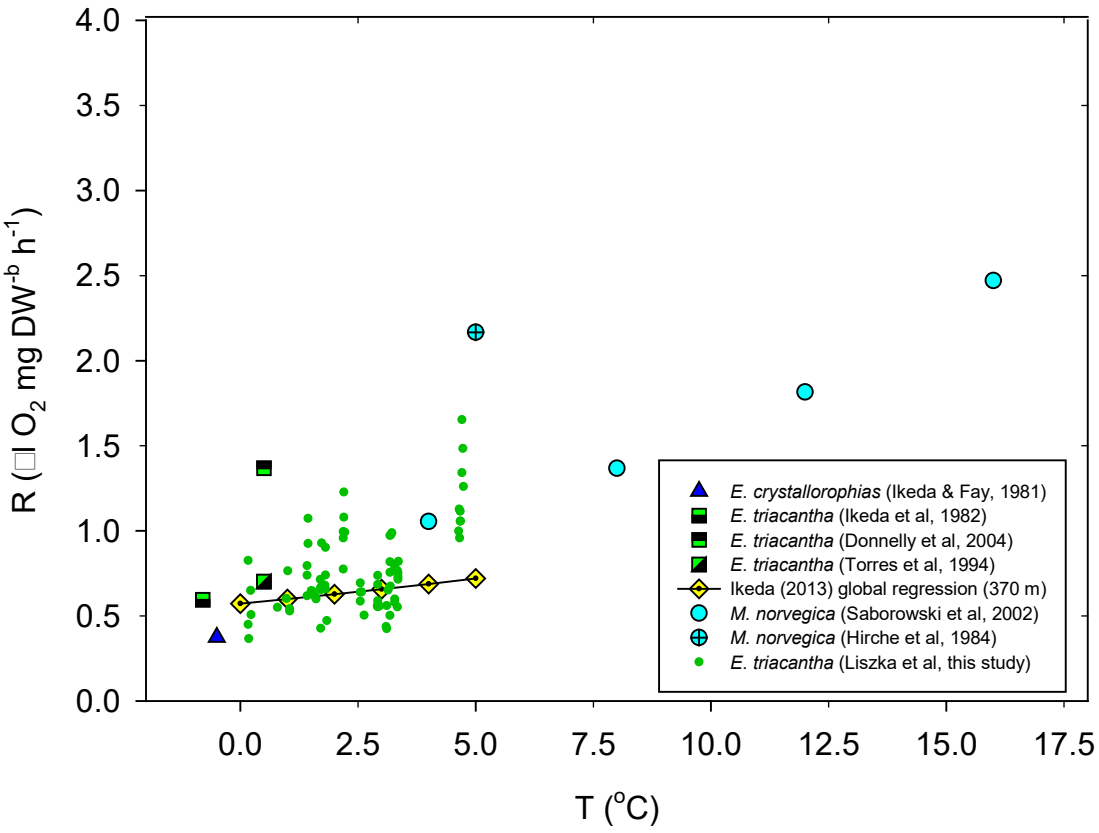

Figure 6: Published respiration rates (converted to µl O_2 mg DW^{-b} h^{-1} using the *b* coefficient determined in this study) for *Euphausia triacantha* and comparable species of euphausiid, across temperatures ranging from -0.8 to 16 °C. Where rates for a range of body weight were provided, the rate plotted is the mean. The line joining the yellow diamonds is calculated following Ikeda (2013)'s global regression, $lnY = a_0 + a_1 lnX_1 + a_2 X_2 + a_3 lnX_3$, where X_1 was DW (mg), X_2 was temperature (°C) and X_3 was depth (m). Values used from this study were average dry mass for euphausiids (34.21 mg DW), with temperatures ranging from 0 °C to 5 °C, and a median depth of 370 m.