Ammonium excretion and oxygen respiration of tropical copepods and euphausiids exposed to oxygen minimum zone conditions

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Abstract

Calanoid copepods and euphausiids are key components of marine zooplankton communities worldwide. Most euphausiids and several copepod species perform diel vertical migrations (DVMs) that contribute to the export of particulate and dissolved matter to midwater depths. In vast areas of the global ocean, and in particular in the eastern tropical Atlantic and Pacific, the daytime distribution depth of many migrating organisms corresponds to the core of the oxygen minimum zone (OMZ). At depth, the animals experience reduced temperature and oxygen partial pressure (pO_2) and an increased carbon dioxide partial pressure (pCO_2) compared to their near-surface nighttime habitat. Although it is well known that low oxygen levels can inhibit respiratory activity, the respiration response of tropical copepods and euphausiids to relevant pCO_2, pO_2 and temperature conditions remains poorly parameterized. Further, the regulation of ammonium excretion at OMZ conditions is generally not well understood. It was recently estimated that DVM-mediated ammonium supply could fuel bacterial anaerobic ammonium oxidation – a major loss process for fixed nitrogen in the ocean considerably. These estimates were based on the implicit assumption that hypoxia or anoxia in combination with hypercapnia (elevated pCO_2) does not result in a downregulation of ammonium excretion. We exposed calanoid copepods from the Eastern Tropical North Atlantic (ETNA; *Undinula vulgaris* and *Pleuromamma abdominalis*) and euphausiids from the Eastern Tropical South Pacific (ETSP; *Euphausia mucronata*) and the ETNA (*Euphausia gibboides*) to different temperatures, carbon dioxide and oxygen levels to study their survival, respiration and excretion rates at these conditions. An increase in temperature by 10˚C led to an approximately 2-fold increase of the respiration and excretion rates of *U. vulgaris* ($Q10_{respiration}$ = 1.4; $Q10_{NH4\text{-}excretion}$ = 1.6)*, P. abdominalis* ($Q10_{respiration}$ = 2.0; $Q10_{NH4\text{-}excretion}$ = 2.4) and *E. gibboides* ($Q10_{respiration}$ = 2.0; $Q10_{NH4\text{-}excretion}$ = 2.4; *E. mucronata* not tested). Exposure to differing carbon dioxide levels had

no overall significant impact on the respiration or excretion rates. Species from the ETNA were less tolerant to low oxygen levels than *E. mucronata* from the ETSP, which survived exposure to anoxia at 13˚C. Respiration and excretion rates were reduced upon exposure to low oxygen levels, albeit at different species-specific levels. Reduction of the excretion and respiration rates in ETNA species occurred at a pO_2 of 0.6 (*P. abdominalis*) and 2.4 kPa (*U. vulgaris* and *E. gibboides*) at OMZ temperatures. Such low oxygen levels are normally not encountered by these species in the ETNA. *E. mucronata* however regularly migrates into the strongly hypoxic to anoxic core of the ETSP OMZ. Exposure to low oxygen levels led to a strong reduction of respiration and ammonium excretion in *E. mucronata* ($p_{\text{crit respiration}}$ = 0.6, $p_{\text{crit NH4 excretion}}$ = 0.73). A drastic reduction of respiratory activity was also observed by other authors for euphausiids, squat lobsters and calanoid copepods, but was not yet accounted for when calculating DVM-mediated active fluxes into the ETSP OMZ. Current estimates of DVM-mediated active export of carbon and nitrogen into the ETSP OMZ are therefore likely too high and future efforts to calculate these export rates should take the physiological responses of migratory species to OMZ conditions into account.

1. Introduction

Many zooplankton and nekton organisms feed in the ocean's surface layer during the night and migrate to beneath the photic zone during daytime, mainly to avoid visual predation (Lampert, 1989). These so-called diel vertical migrations (DVMs) mediate the active flux of particulate and dissolved organic and inorganic matter from the surface layer to midwater depths (Steinberg et al., 2000; 2002), as the zooplankton and nekton organisms respire, excrete, defecate and die at depth. The DVM mediated active flux is

an important aspect of marine biogeochemical cycles and can be as high as the passive flux via sinking particles (Putzeys, 2013). The active flux to a certain depth depends on the migrating biomass to that depth and on the respiration, excretion and defecation activity of the migrating organisms, as well as their mortality rate at the respective depth. Here we focus on the regulation of respiration and excretion rates via environmental factors. Environmental temperature (Ikeda, 2014), oxygen (Ekau et al., 2010; Seibel, 2011), and carbon dioxide levels (e.g. Rosa and Seibel, 2008; Maas et al., 2012a) modulate the activity of migrating organisms, with temperature being the best understood factor. In general, a 10˚C temperature decrease results in an approximately 2-fold reduction of metabolic activity (Ikeda, 2014). Likewise, hypoxia ultimately leads to reduced metabolic rates (Ekau et al., 2010; Seibel, 2011), whereas elevated pCO_2 can increase or decrease metabolic rates (e.g. Rosa and Seibel, 2008; Thomsen and Melzner 2010, Kroeker et al., 2010; Maas et al., 2012a). The vertical gradients of temperature, oxygen and carbon dioxide are very pronounced at the eastern margins of tropical oceans. Here, migrating organisms encounter relatively high temperatures, high oxygen and low carbon dioxide levels at the surface, but relatively low temperatures, low oxygen and high carbon dioxide levels in the oxygen minimum zone (OMZ) at midwater depth (Karstensen et al., 2008; Paulmier et al., 2011).

OMZs in tropical oceans result from restrained mixing due to strong thermal stratification and sluggish circulation that result in a reduced oxygen supply and a comparatively high supply of particulate organic matter from the surface layer that is respired at midwater depth (Brandt et al., 2015). OMZs structure the pelagic habitat and influence the distribution and activity of marine organisms, as many organisms cannot survive at low environmental oxygen concentrations (e.g. Ekau et al., 2010). In the Eastern Tropical South Pacific (ETSP), the upper boundary of the oxycline is the single most critical factor structuring the habitat of most zooplankton organisms (Semenova et al., 1982;

Escribano et al., 2009). The ETSP OMZ features severely hypoxic to anoxic conditions, anoxia being detected at about 100 to 300 m depth and oxygen levels between 0 and 5 μmol kg^{-1} (~0 to about 0.4 kPa) at about 30 to 100 m depth (Thamdrup et al., 2012). In contrast, oxygen concentrations in the Eastern Tropical North Atlantic (ETNA) seldom fall below 40 μmol kg^{-1} (~ 3.4 kPa at 12˚C, Karstensen et al., 2008), but transient severely hypoxic to anoxic conditions have been observed in mesoscale eddies (Karstensen et al., 2015). OMZs have expanded in the recent past and a further expansion, mainly due to global warming, is expected (Stramma et al., 2008; Keeling et al., 2010). An expansion of OMZs will have far-reaching consequences for marine tropical ecosystems. For example it results in the compression of the habitat of billfishes (Stramma et al., 2012), but also in the extension of areas where fixed nitrogen is lost from the ocean (Kalvelage et al., 2011).

Many organisms have developed special adaptations like an enhanced oxygen uptake capacity to thrive at particularly low oxygen levels of less than 5 kPa (~ 40 μmol kg^{-1}) oxygen (Childress and Seibel, 1998, Seibel 2011). The abundance and biomass of zooplankton and nekton permanently inhabiting extreme OMZs is rather low (e.g. Wishner et al., 1998; Auel and Verheye, 2007; Escribano et al., 2009), but the migrating zooplankton and nekton biomass can be very high even in these regions. Most zooplankton and nekton organisms can regulate their oxygen uptake rate over a wide range of oxygen concentrations, but this regulatory ability breaks down at a certain critical oxygen concentration. The point at which the aerobic metabolism can no longer be maintained independent of the environmental oxygen partial pressure (pO_2) is called the critical oxygen partial pressure p_{crit} (e.g. Seibel, 2011). The p_{crit} is species specific and varies with habitat oxygen concentrations (e.g. Childress & Seibel, 1998; Richards, 2011) and temperature (Deutsch et al. 2015). Many species inhabiting the OMZ have evolved significantly lower p_{crit} values than non-OMZ inhabiting species (Childress &

Seibel, 1998) and can survive extended periods of time at oxygen levels below their p_{crit} or even at anoxia (Childress 1975, Kiko et al., 2015). Migrations into waters with oxygen levels below the p_{crit} should result in a metabolic suppression that reduces the amount of oxygen respired and carbon dioxide excreted. This reduction needs to be taken into account when calculating the active flux of respiratory carbon into the OMZ and when estimating the biological oxygen consumption within the OMZ.

The impact of hypoxia on other metabolic processes is not described as well as that on respiration. Its impact on the excretion of nitrogenous compounds is of particular interest, as pelagic primary productivity is primarily dependent on dissolved inorganic nitrogen (e.g. Hauss et al., 2012; Meyer et al., 2015). The active removal of fixed nitrogen from the euphotic zone to midwater depths is therefore an important aspect of DVMs (Steinberg et al., 2002). Furthermore, the release of ammonium in the OMZ by diel vertical migrators could possibly fuel bacterial anaerobic ammonium oxidation (anammox) – a process that removes fixed nitrogen from the ocean (Bianchi et al., 2014). Several studies have found a reduction in ammonium excretion in response to long-term (days to weeks) exposure to hypoxic and anoxic conditions for benthic and pelagic crustaceans (e.g.; Hagerman et al., 1990; Hagerman and Szaniawska, 1994; Rosas et al., 1999). Cass and Daly (2014) on the other hand found both enhanced and reduced rates of nitrogen excretion in response to short-term (few hours) exposure to mild hypoxia (initial O_2 concentration 36 to 78 µmol O_2 kg^{-1}; initial pO_2 2.7 to 6 kPa) in different calanoid copepod species and report that excretion rates at severe hypoxia (initial O_2 concentration 4 to 17 µmol O_2 kg^{-1}; initial pO_2 0.3 to 1.3 kPa) were too low to be measured. Svetlichny et al. (1998) showed for the Black Sea calanoid copepod *Calanus euxinus* that short-term exposure to mild hypoxia (26 µmol O_2 kg^{-1}; 1.9 kPa) did not affect the ammonium excretion rate, whereas it resulted in a downregulation of respiration. Maas et al. (2012b) found no reduction of ammonium excretion in three

thecosomate pteropod species when animals were exposed to initial oxygen concentrations of 31.5 ± 8 µmol O_2 kg^{-1} (2.5 ± 0.6 kPa). Kiko et al. (2015) observed a very strong downregulation of ammonium excretion in the squat lobster *Pleuroncodes monodon* upon exposure to anoxia and calculated a p_{crit} for ammonium excretion of 0.5 kPa (6.1 µmol O_2 kg^{-1} at 13°C). In general, it still remains unclear how nitrogen excretion is regulated in response to low environmental oxygen concentrations in diel vertical migrators.

It is also not clear how the increase in pCO_2 that coincides with a decrease in pO_2 impacts the metabolic activity of zooplankton and nekton organisms and if interactive effects exist. Previous studies to analyze the impact of OMZ conditions on zooplankton metabolic activity often let the animals respire the oxygen in the test bottle (e.g. Childress, 1975; Donnelly and Torres, 1988), which would result in elevated pCO_2 levels consistent with the rise in pCO_2 levels in the OMZ. However, this approach could also lead to the build-up of potentially harmful amounts of metabolic endproducts. In other studies, pure nitrogen or nitrogen/oxygen mixes were used to adjust oxygen levels (e.g. Svetlichny et al., 1998; Trübenbach et al., 2013; Cass and Daly, 2014). The latter approach leads to the removal of carbon dioxide from the incubation water and therefore to a pCO_2 reduction and a pH increase, non-representative of OMZ conditions (Melzner et al., 2013). In other studies, the pCO_2 was adjusted to OMZ-conditions, but not the pO_2 (e.g. Maas et al., 2012a), or both pO_2 and pCO_2 were adjusted, but interactive effects were not tested (Kiko et al., 2015). It is therefore necessary to develop procedures to test metabolic activity at realistic pO_2 and pCO_2 levels.

We here established a method to mimic the OMZ conditions (temperature, pO_2 and pCO_2) of the ETNA and ETSP using premixed $N_2/O_2/CO_2$ gas mixes and tested the hypothesis that metabolic activity in copepods and euphausiids is changed under these incubation conditions compared to the incubation conditions realized when only N_2/O_2

gas mixes are used. Furthermore, we tested the hypothesis that exposure to OMZ conditions not only reduces the amount of oxygen respired, but also the amount of ammonium excreted. To test these hypotheses, we measured respiration and ammonium excretion rates of migrating and non-migrating copepods from the ETNA (*Pleuromamma* spp. and *Undinula vulgaris*, respectively) and krill species from the ETSP OMZ (*Euphausia mucronata*) and the ETNA OMZ (*Euphausia gibboides*) at different oxygen levels, carbon dioxide levels and temperatures to characterize their metabolic response. Calanoid copepods from the *Pleuromamma* genus are ubiquitous members of the migrating zooplankton community of the tropical Atlantic (Steinberg et al., 2000; Auel and Verheye, 2007), whereas *Undinula vulgaris* is an ubiquitous non-migrating epipelagic calanoid copepod of the tropical oceans (Chahsarvar-Archard and Razouls, 1982; Razouls et al., 2005 - 2015). *Euphausia gibboides* is found regularly at low latitudes in the Atlantic and Pacific (Siegel, 2015), and *E. mucronata* is the dominant euphausiid in the ETSP (Antezana, 2009; 2010). Both euphausiid species conduct diel vertical migrations into the OMZ. Our work should help to better parameterize ammonium excretion and respiration rates of crustacean zooplankton and nekton in OMZ regions and aims to provide a base for revising model formulations of DVM-mediated export in OMZ regions.

2. Material and Methods

2.1 Animal collection and maintenance

Sampling was conducted during RV *Maria S. Merian* cruise MSM22 (ETNA; 24.10.2012 to 23.11.2012) and RV *Meteor* cruises M93 (ETSP; 6.2.2013 to 4.3.2013) and M97 (ETNA; 25.5.2013 to 28.6.2013; Fig 1). Zooplankton was collected using a Hydrobios Multinet Midi (0.25 m^2 mouth opening, 200 µm mesh size, 5 nets), a Hydrobios Multinet Maxi (0.5 m^2 mouth opening, 330 µm mesh size, 9 nets), a WP-2 net (0.26 m^2 mouth

opening, 200 µm mesh size), a MOCNESS (1 m^2 mouth opening, 2 mm mesh size) or a CalCOFI-Net (0.78 m^2 mouth opening, 500 µm mesh size). All specimens used for experiments were caught in the upper 400 m of the water column and only animals appearing unharmed and fit were used for experiments. Specimens were sorted, identified and transferred into aquaria with filtered, well-oxygenated seawater immediately after the catch and maintained for 1 to 13 hours prior to physiological experiments at the respective experimental temperature. Only adult euphausiids and adult female copepods were used for the experiments. OMZ temperatures during MSM22 and M97 ranged from 5.5 to 13.6˚C at 300 to 600 m depth and from 13 to 29˚C in the upper 100 m. Temperature at 200 to 300 m depth during M93 ranged from 10.2 to 13.3˚C. Maintenance and physiological experiments were therefore conducted in darkness in temperature-controlled incubators at 11, 13 or 23˚C (±1˚). Animals were not fed before or during experiments.

Stomach fullness and coloration of the mid gut gland were routinely categorized in *E. gibboides*. Possible scores of the mid gut gland coloration were 0-5, indicating transparent through green to dark brown coloration (Morris et al. 1983).

2.2 Incubation conditions

Respiration and ammonium excretion rate measurements (both in µmol h^{-1} gDW^{-1}) at varying oxygen concentrations were conducted simultaneously in 12 to 60 mL gas-tight glass bottles. These were equipped with oxygen microsensors (ø 3 mm, PreSens Precision Sensing GmbH, Regensburg, Germany) attached to the inner wall of the bottles to monitor oxygen concentrations non-invasively. Read-out of oxygen concentrations was conducted using multi-channel fiber optic oxygen transmitters (Oxy-4 and Oxy-10 mini, PreSens Precision Sensing GmbH, Regensburg, Germany) that were connected via optical fibers to the outside of the bottles directly above the oxygen

microsensor spots. Calibration of the oxygen microsensors was conducted at the beginning of each cruise or when a different incubation temperature was set with a Na_2SO_3-solution (0% oxygen) and aerated seawater (100% air saturation) at the respective measurement temperature. Oxygen concentration was calculated from air saturation according to the PreSens manual. All other oxygen unit conversions were conducted using the R-package AquaEnv (Hofmann et al., 2010) and R scripts obtained from Andreas F. Hofmann. Measurements were started at pre-adjusted oxygen and carbon dioxide levels. For this, seawater stocks with adjusted pO_2 and pCO_2 were prepared by equilibrating 3 to 4 L of filtered (0.2 µm Whatman GFF filter) and UV – sterilized (Aqua Cristal UV C 5 Watt, JBL GmbH & Co. KG, Neuhofen, Germany) water with premixed gases (certified gas mixtures from Air Liquide) for 4 hours at the respective experimental temperature. pCO_2 levels were chosen to mimic the environmental pCO_2 in the ETSP OMZ or the ETNA OMZ. pCO_2 levels for the respective area were calculated from data published by the World Ocean Circulation Experiment (WOCE 2002; ETSP: data from the upper 150 m between 14.74˚S to 16.38˚S and 75.25˚W to 76.93˚W; ETNA: data from the upper 400 m between 4.5˚S to 11˚N and 7˚W to 26˚W) using CO2sys_v2.1 (Pierrot et al., 2006). Furthermore, gas mixes with different levels of oxygen, but without CO_2 were used to test the effects of this experimental manipulation. The detailed composition of the premixed gases used is described in Table 1. Antibiotics (25 mg L^{-1} ampicillin and 25 mg L^{-1} streptomycin) were added to the stocks after equilibration to inhibit microbial activity.

The salinity and pH of the prepared water was measured immediately on board with a handheld multiparameter meter Multi 350i equipped with a Sentix 41 pH and a ConOx conductivity probe (WTW). Calibration of the pH-probe was conducted daily prior to the measurements using 7.000 and 10.012 pH IUPAC standard buffers (Radiometer analytical) and the conductivity probe was calibrated at the beginning of each cruise

using 0.01 mol L^{-1} KCl. 250 mL of the prepared water were collected in gas-tight glas bottles and fixed with 100 µL saturated mercury chloride solution for later measurements of total alkalinity (A_T) and dissolved inorganic carbon (C_T) in the home laboratory. C_T was measured using an AIRICA system (Marianda, Kiel, Germany) via a LI-COR 7000 infrared CO_2/H_2O analyser. A_T was measured in duplicates via potentiometric titration with an automated titrator (Titroline 7000, SI Analytics, Germany) using 50 ml of sample and 0.05 M HCl. C_T was measured for samples from cruise MSM22, whereas A_T was measured for samples from cruises M93 and M97, due to a breakdown of the C_T measurement system. A_T and C_T were measured against certified reference material provided by Andrew Dickson of Scripps Institution of Oceanography (http://andrew.ucsd.edu/co2qc/). The pCO_2 established in the different incubation trials was calculated with CO2sys_v2.1, using experimental temperature, water salinity, pH_{NBS} and C_T or A_T as input.

2.3 Excretion and respiration measurement

Experimental runs to simultaneously measure the ammonium excretion and respiration rates were conducted with 11 to 15 trial incubations (1 or 2 animals per incubation bottle and three different treatment levels) and three animal-free control incubations (one per experimental treatment). During each run, experimental treatments comprised 100% air saturation as well as one reduced air saturation level with and without CO_2. A typical run for example comprised three replicates plus one animal-free control at 100% air saturation, four replicates plus one animal-free control at 50% air saturation plus CO_2 and four replicates plus one animal-free control at 50% air saturation minus CO_2. To start the incubations, the incubation bottles were filled with the respective prepared water and the animals were added immediately. This handling took a maximum of 5 min for each animal and about 30 min for all replicates. Subsequently, the respective

incubation bottle was immediately transferred to the incubator. Oxygen concentrations in the incubation bottles were recorded every 5 min using the fiber-optic microsensor system and the recording of data for the determination of respiration rates was started immediately after all animals were transferred. Respiration rates were calculated from the slope of oxygen decrease over selected time intervals. Chosen time intervals were 20 to 105 min long. No respiration rate was calculated for the first 20 to 60 min after animal transfer to avoid the impact of enhanced activity of the animal or changes in the bottle water temperature during initial handling on the respiration rates and oxygen readings. Respiration rates were obtained over a maximum incubation time of 16 hours and slopes were linear at normoxia to mild hypoxia. Respiration rates in animal-free control bottles were used to correct for microbial activity. These rates were < 2% of animal respiration rates at normoxia.

Animals were incubated for 2 to 10 h for the measurement of ammonium excretion rates. Ammonium concentration was determined fluorimetrically (Holmes et al., 1999). Ammonium excretion was calculated as the concentration difference between incubation and animal-free control bottles. Some specimens died during the respiration and excretion rate measurements, as indicated by a cessation of respiration. No excretion rate measurements were conducted in this case, but the oxygen level at which the animal died was noted.

Euphausia mucronata survived exposure to anoxia, enabling the measurement of ammonium excretion rates under severely hypoxic to anoxic conditions. These measurements were started after 2-5 hours when animals had respired all oxygen from the incubation bottles. Thereafter, a 1 mL water sample was taken from the 12-13 mL bottles used to determine the ammonium concentration at the onset of the trial. The withdrawn water was replaced with 0% oxygen water prepared using pure nitrogen gas. Oxygen levels slightly increased during this procedure, but never rose above ~ 3% air

saturation. This small amount of oxygen was in most cases respired after about 30 min, but in some cases anoxia was not reached until the end of the trial. After an incubation period of 2.5 hours at severe hypoxia to anoxia, a second sample for the ammonium concentration measurement was taken. The ammonium excretion rate was calculated as the difference between the first and second measurement and the oxygen concentration was calculated as the mean of the initial and end oxygen concentrations. All individuals used in the described experiments were subsequently frozen at -80°C and dried at 50°C for 72 hours and weighed to determine their dry weight. Oxygen consumption and ammonium excretion rates (R, µmol h^{-1} gDW^{-1}) were standardized to an average dry weight (DW in mg) of 0.1 g applying a scaling coefficient b of -0.25 (Moloney and Field, 1989) as

$$R_{std} = R * \left(\frac{0.1}{weight} \right)^{-0.25} \quad (1).$$

The rates presented should be considered routine metabolic rates, as activity was not monitored and animals were not fed (Prosser 1961).

2.4 Statistical analysis

General linear models (GLM) with pO_2 as continuous variable and pCO_2 (two levels) and temperature (for all except *E. mucronata*, two levels) as categorical predictor were used on log-transformed respiration and excretion rate data for each species separately to explore the overall effect of experimental conditions on the metabolic response.

Pairwise t-tests were employed to compare the respiration and excretion rates of the two pCO_2-treatments maintained under similar temperature and pO_2 conditions. For these tests, the mean respiration rate obtained at a given experimental condition (starting pO_2 value and experimental temperature) for a replicate was determined. E.g. if an

experimental run with an animal was started at 50% airsaturation, stopped at 20% airsaturation and lasted 8 hours, four determinations of the respiration rate were available for this replicate and the mean of these respiration rates was determined. To analyze the effects of different oxygen levels on the respiratory activity, the four determinations of the respiration rates obtained at different oxygen levels were used separately for subsequent analysis.

Where possible, metabolic rates were modelled as a function of pO_2 using nonlinear regressions with the python module lmfit. If hypoxia was found to have lethal effects at a given temperature, the metabolic data was transformed prior to fitting the power function by subtracting the lowest observed lethal pO_2 from the abscissa data. A power function with a y-intercept of zero

$$R_{std,O2} = a * pO_2^b \qquad (2)$$

was fitted to the standardized (according to equation (1)) and transformed respiration rate data as the respiration rate at 0 kPa oxygen or that of dead individuals is zero by definition. A power function with y-intercept

$$R_{std,NH4} = a * pO_2^b + c \quad (3)$$

was fitted to the standardized and transformed ammonium excretion rate data.

To calculate the p_{crit}, the standardized and transformed respiration or excretion rates were normalized by dividing each respiration or excretion rate by the highest observed respiration or excretion rate, respectively. Power functions as described above were fitted to these standardized, transformed and normalized rates. The respiration or excretion p_{crit} was then calculated according to Marshall et al., (2013) as

$$p_{crit} = \left(\frac{0.065}{a * b} \right)^{\left(\frac{1}{1-b}\right)} \qquad (4)$$

where a and b are the respective factors obtained from fitting equation (2) or (3) to the standardized and normalized

The fitted functions and the calculated p_{crit} were back-transformed to the original scaling of the abscissa.

3. Results

3.1 Impact of pCO_2-levels on respiration and excretion

We prepared incubation water using both O_2/N_2-mixes (CO_2-minus) and $O_2/N_2/CO_2$-mixes (CO_2-plus) to test if differences in pH and pCO_2 in the incubation water lead to significant differences in the metabolic activity of copepods and euphausiids from the ETNA and ETSP. $O_2/N_2/CO_2$-mixes representative of the respective regions were used. The use of O_2/N_2-mixes led to an artificial reduction of the pCO_2 and an artificial increase in pH compared to untreated seawater (pO_2 ~ 21 kPa; Fig. 2). Differences to the respective simulated OMZ conditions were even larger and increased with decreasing oxygen levels (Fig. 2). The use of $O_2/N_2/CO_2$-mixes resulted in pH and pCO_2 levels that were very close to the environmental target conditions for the ETNA OMZ, whereas pCO_2 levels were slightly above those of the ETSP OMZ.

In the general linear model (GLM) with the mean pO_2 as continuous and pCO_2 and - if applicable - temperature as categorical predictors, applied to each species separately, no significant overall effects of the CO_2-level on the respiration or excretion rates were detected ($p > 0.1$), while temperature and pO_2 were significant (univariate $p<0.0001$ for respiration, $p<0.05$ for excretion, all species) predictors of metabolic activity. Respiration

whole model adjusted r^2 was 0.63, 0.36, 0.41 and 0.37 for *E. gibboides*, *E. mucronata*, *Pleuromamma abdominalis* and *Undinula vulgaris*, respectively and ammonium excretion whole model adjusted r^2 was 0.30, 0.31, 0.84 and 0.77 for *E. gibboides*, *E. mucronata*, *P. abdominalis* and *U. vulgaris*, respectively.

In the pairwise comparison, respiration and ammonium excretion rates of animals incubated in CO_2-plus waters were not found to be significantly different from those incubated in CO_2-minus waters at most oxygen and CO_2 levels analyzed (t-tests, p-value > 0.05; Fig. 3 and Fig. 4) However, in *Euphausia mucronata* incubated at 33% air saturation at 13˚C, the CO_2-plus treatment resulted in a significant 1.35-fold increase ($p<0.05$) in respiration. No effect was observed in this species at 10% air saturation and no consistent effects were observed in any other species. Likewise, the CO_2-plus treatment resulted in a significant 1.6-fold increase ($p<0.05$) in ammonium excretion in *P. abdominalis* at 33% air saturation and 11˚C, but no effects were observed in this species at any other condition tested or in any other species. To estimate the sensitivity of our analysis we artificially increased the respiration rates at CO_2-plus or CO_2-minus conditions stepwise by a factor of 0.1 to determine the fold-changes at which more than 50% of the tests become significant. This was the case at a 1.2-fold increase for the respiration rate tests (7 of 14 tests significant) and a 1.5-fold increase for the excretion rate tests (6 of 11 tests significant). As no consistent significant effects of our CO_2-treatment were found, we combined data from pCO_2-plus and pCO_2-minus incubations to further characterize the effects of temperature and oxygen levels on metabolic activity.

3.2 Temperature and oxygen-dependence of respiration and hypoxia tolerance

Respiration rates at normoxia (trial start conditions 100% airsaturation) were always significantly lower at 11 or 13˚C than at 23˚C in *Undinula vulgaris*, *Pleuromamma abdominalis* and *Euphausia gibboides* (ttest, $p < 0.05$). The Q_{10} temperature coefficient

of respiration at normoxia (pO_2 > 15 kPa, Temp. = 11-23°C) was 1.4 for *U. vulgaris*, and 2.0 for *P. abdominalis* and *E. gibboides* (Table 3). Standardized individual respiration rates and fitted power functions are shown in the upper panels of Fig. 5 and 6. Respiratory p_{crit} of *E. gibboides* and *P. abdominalis* were 2.4 kPa (at 13˚C) and 0.6 kPa (at 11˚C), respectively (Fig. 5 and Fig. 6, Table 3). As no data on respiratory activity below 1.9 kPa was obtained for *P. abdominalis*, the p_{crit} likely lies between the calculated p_{crit} of 0.6 and 1.9 kPa. In the case of *E. gibboides* the respiratory p_{crit} was almost identical with the mean lethal pO_2 of 2.5 kPa (SD = 1.4, n = 20) at 13˚ C. The mean lethal pO_2 of *U. vulgaris* was 2.7 kPa (SD = 0.3, n = 6) at 11˚ C. No mortality was observed for *P. abdominalis* down to 1.9 kPa at 11˚C. *E. mucronata* survived several hours at anoxia at 13˚C, but survival time at anoxia was not systematically determined. The respiratory p_{crit} of *E. mucronata* was found to be 0.6 kPa at 13˚C. Data from Teal and Carey (1967) supplement our observations on the respiratory rates of *E. mucronata*. From their Fig. 2, an approximate p_{crit} of 2 to 4 kPa at 20˚C can be deduced. In all species that we tested, the respiratory p_{crit} was higher at 23˚C than at 11 or 13˚C (Table 3). At 23°C, the mean lethal pO_2 was 6.6 kPa in *U. vulgaris* (SD = 0.7 ,n = 6), 6.3 in *P. abdominalis* (SD = 1.0, n = 2) and 7.5 in *E. gibboides* (SD = 1.9, n = 20). Respiration rates calculated according to Ikeda (2014) for 0.1 g copepods or euphausiids coincided well with the standardized respiration rates observed at normoxia in all species (Fig. 5, Fig. 6).

3.3 Ammonium excretion rates

The Q_{10} temperature coefficient of ammonium excretion at normoxia (trial start conditions 100% airsaturation) was 1.6 for *Undinula vulgaris*, and 2.3 for both *Pleuromamma abdominalis* and *Euphausia gibboides.* It was possible to fit power functions to most ammonium excretion data (Fig. 5 and 6, Table 3) except for *U. vulgaris*

at 23˚C and for *E. gibboides* at 13 and 23 ˚C. For the latter species and experimental conditions students t-tests grouped by initial oxygen concentrations revealed no statistically significant changes in excretion rates with decreasing oxygen levels, compared to the excretion rates at normoxia. The ammonium excretion p_{crit} was very similar when compared to the respiratory p_{crit} in all species observed and ammonium excretion at anoxia was drastically downregulated in *E. mucronata*. Mean (± SD) standardized ammonium excretion at severe hypoxia (pO_2 < 0.5 kPa) was significantly reduced by a factor of 5.9 compared to ammonium excretion (0.65 ± 0.33 vs. 3.82 ± 2.54 µmol h^{-1} gDW^{-1}) under normoxia (15 - 22 kPa pO_2, t-test, p < 0.01) in *E. mucronata*.

Ammonium excretion rates calculated according to Ikeda (2014) for 0.1 g copepods or euphausiids coincided well with the excretion rates observed at normoxia in almost all species (Fig. 5, Fig. 6). Only the excretion rates observed in *E. gibboides* were lower than predicted. Stomachs of *E. gibboides* were mostly half-full and midgut coloration ranged between 1 and 2 in this species.

4. Discussion

Empirical models exist to predict copepod (Ikeda, 2014) and euphausiid (Tremblay et al., 2014; Ikeda, 2014) respiration and excretion rates, but these models do not include pO_2 or pCO_2 as environmental factors. We here developed an approach to determine copepod and euphausiid respiration and excretion rates at temperatures, and oxygen and carbon dioxide levels consistent with those found in the ETNA and ETSP OMZ. We furthermore tested whether respiration and excretion rates are altered when the oxygen, but not the carbon dioxide level is experimentally adjusted to represent OMZ conditions compared to the scenario when both levels are adjusted according to OMZ conditions.

4.1 Impact of pCO_2-levels on respiration and excretion

As expected, the use of CO_2-minus gas mixes resulted in a removal of CO_2 from the incubation water, mirrored in a pH increase and a pCO_2 decrease. The use of CO_2-plus gas mixes allowed a more realistic simulation of OMZ conditions. pH, A_T and C_T data were available for the Peruvian OMZ (WOCE 2002) and we calculated the CO_2-plus gas mix composition based on the available pH and C_T data. pCO_2 calculated from A_T and C_T is lower and more realistic, as these two parameters can be estimated more reliably. We therefore now suggest the use of the gas mixes listed in Table 2 to experimentally establish Peruvian OMZ pO_2 and pCO_2 levels.

Realistic adjustment of the carbonate system did not result in consistent, statistically significant changes in the respiration or ammonium excretion rate in tropical Atlantic copepod or euphausiid species, or the Pacific *Euphausia mucronata,* compared to artificially lowered pCO_2-levels. In some experiments (e.g. respiration rate of *U. vulgaris* at 11˚C and 10% air saturation) the number of replicates was low, but also in cases where many replicates were available, as well as with the GLM no significant effects were found. The approximate detection level of our approach was a 1.2-fold difference in respiration and a 1.5-fold difference in ammonia excretion. We did not acclimatize the animals to the respective test conditions, as we aimed to mimic DVM changes in temperature, pO_2 and pCO_2 experienced by the animals when migrating from the OMZ to the surface layer and back. These migrations are conducted within 1 to 5 hours (Fischer and Visbeck 1993, Heywood 1996) and the animals should therefore be able to cope with fast changes in environmental conditions. Previously, copepods were found to be relatively robust if it comes to acute changes in pCO_2 (Isari et al., 2015; Thor and Dupont 2015, Thor and Oliva 2015). Food quality and quantity, life history (Thor and Dupont 2015, Thor and Oliva 2015) and other environmental constraints (e.g. temperature, pO_2) seem to be more important than pCO_2 in determining metabolic rates. Thor and Dupont (2015) for example found that respiration rates of *Pseudocalanus*

acuspes copepods reared in the laboratory for two generations at elevated CO_2-levels (0.09 kPa) had 1.14-fold higher respiration rates than copepods reared at 0.04 kPa pCO_2. Respiration rates of copepods reared at 0.15 kPa were significantly reduced by a factor of 0.84 compared to those of copepods reared at 0.04 kPa pCO_2. However, if animals reared at 0.09 kPa were tested at 0.04 kPa or vice versa, no significant changes in respiration rates were found. The same holds true for tests of animals raised at 0.04 kPa and 0.15 kPa. Isari et al (2015) found no statistically significant differences in respiration rates in *Acartia grani* and *Oithona davisae* upon acute exposure to 0.12 kPa pCO_2, compared to 0.04 kPa. Results are however difficult to assess as the number of replicates was low (n=2), and mortality in the batch incubations seemingly was not zero. Thor and Oliva (2015) found both increases and decreases in respiration rates after exposure to elevated pCO_2 for 10 days in a Skagerrak population of *P. acuspes*, but not in a Svalbard population. Food was a better predictor of respiration rate than pH in both populations. Until now, only one study assessed metabolic responses of Euphausiids to pCO_2-level changes (Saba et al., 2012). A statistically significant 3-fold increase in DOC excretion in response to an elevated pCO_2 (0.07 vs. 0.04 kPa; 24 hour incubations) was found for *E. superba*, whereas ammonium, phosphate and urea excretion were not significantly impacted. The increase in DOC excretion might be related to an observed increased feeding activity at elevated pCO_2, which currently remains unexplained (Saba et al., 2012).

The fact that *Pleuromamma abdominalis*, *E. gibboides* and *E. mucronata* are naturally exposed to daily varying pCO_2-levels when migrating into and out of the OMZ might also explain why no consistent changes in respiration or excretion rates under CO_2-plus vs. CO_2-minus conditions were found in these species. Migrating organisms that regularly are exposed to pCO_2-changes may be less sensitive than those that live at stable conditions. Rosa and Seibel (2008) found that in the cephalopod (*Dosidicus* gigas)

routine metabolism was not impacted by exposure to a pCO_2 of 0.1 kPa at low temperatures characteristic for the ETSP OMZ. Maas et al. (2012a) found no significant differences in respiration rates when pteropods migrating into the OMZ were exposed to OMZ CO_2-levels (0.1 kPa) compared to surface CO_2-levels (0.04 kPa) at 20˚C. Acute CO_2 effects on metabolic activity in copepods and other DVM organisms therefore seem to be small and might also be masked by the response to concomitant changes in O_2-levels the animals experience when migrating into and out of the OMZ.

4.2 Effects of temperature and oxygen on metabolic activity

That temperature strongly affects metabolic activity is well established and our results are consistent with the general observation that zooplankton metabolic activity doubles with a 10˚C increase in temperature within the thermal window of the species (Ikeda, 2014). The Q_{10} of respiration and ammonium excretion was relatively close to 2 in *Euphausia gibboides* and *Pleuromamma abdominalis*. The Q_{10} was below 2 in *Undinula vulgaris*, but this might be related to the fact that this surface dwelling species is seldom exposed to 11˚C and the Q_{10} therefore might not be representative for the normal thermal range of this species. An increase in temperature furthermore impaired hypoxia tolerance as indicated by a higher lethal pO_2, respiration p_{crit} and ammonium excretion p_{crit} in all species tested. That elevated temperatures impair hypoxia tolerance has been found for numerous other marine species and quantitative estimates of the p_{crit} can help to understand distribution patterns of marine organisms (e.g. Deutsch et al., 2015). Consistent with previous studies (e.g. Childress and Seibel 1998), respiratory p_{crit} values of *E. mucronata* from the ETSP were found to be lower than in species from weak- (*E. gibboides*, *P. abdominalis* and *U. vulgaris*) or non-OMZ regions (Childress and Seibel 1998). No large differences in respiratory p_{crit} could be observed when comparing the tropical Atlantic copepod and krill species. In general, our findings support the

hypothesis that the critical oxygen partial pressure evolved to largely match the minimum oxygen level to which a species is regularly exposed (Seibel, 2011; Richards, 2011).

It seems reasonable to also transfer this concept to the impact of oxygen levels on ammonium excretion rates. A reduction of ammonium excretion under severely hypoxic or anoxic conditions was observed in *E. mucronata* and is consistent with similar observations from the squat lobster *Pleuroncodes monodon* (Kiko et al., 2015) and from several calanoid copepods (Cass and Daily, 2014). It follows that both respiration and ammonium excretion are drastically reduced when crustacean zooplankton organisms are exposed to severe hypoxia or anoxia. The characterization of metabolic rates across the entire tolerated oxygen level spectrum (including, if possible, anoxia) is key to properly estimate effects of hypoxia and anoxia on species distribution and activity, as well as related biogeochemical fluxes. Furthermore, the determination of respiration and excretion rates over a wider temperature range and at higher resolution could be helpful to better understand differences in performance between migratory and non-migratory species. The inclusion of further factors (e.g. time of day and feeding status) could also lead to a more precise predictive model of zooplankton and nekton respiration and excretion rates.

4.4 Implications for the calculation of biogeochemical fluxes of Oxygen, Carbon and Nitrogen

Several studies have assessed the active DVM-mediated fluxes and the passive particle-mediated fluxes of carbon and nitrogen in regions that mostly do not feature severe hypoxia (pO_2 < 1 kPa) or anoxia at midwater depths (Longhurst et al., 1990; Zhang and Dam, 1997; Hidaka et al., 2001; Steinberg et al., 2002; Davison et al., 2013). Excretion rates used in these studies to calculate the active flux were obtained at mildly hypoxic (pO_2 > 1 kPa) to normoxic conditions (e.g. Donnelly and Torres, 1988; Dagg et

al., 1980; Steinberg et al., 2002). For OMZs demonstrating only mild hypoxia, such as the ETNA OMZ, this approach seems reasonable. The respiration and ammonium excretion p_{crit} of *Pleuromamma abdominalis* were found to be 0.6 kPa and 1.8 kPa at 11˚C, respectively. The respiratory p_{crit} of *Euphausia gibboides* was found to be 2.4 kPa at 13˚C and no significant reduction of ammonium excretion was observed at a mean pO_2 of 5.1 kPa for *E. gibboides* at this temperature. *P. abdominalis* and *E. gibboides* normally do not encounter extremely low oxygen levels in their natural habitat. Oxygen concentrations in the ETNA seldom fall below 40 µmol kg^{-1} (~ 3.4 kPa at 12˚C, Karstensen et al., 2008). It follows that zooplankton respiration and excretion rates for biogeochemical flux calculations in the ETNA can be calculated from published empirical models of zooplankton respiration and excretion (Ikeda 2014), if oxygen levels above ~ 2.4 kPa are encountered. However, food conditions should also be taken into account. Excretion rates of *E. gibboides* at normoxia were lower than predicted via the empirical model by Ikeda (2014), possibly due to low food availability as indicated by low midgut coloration scores.

Estimates of DVM-mediated fluxes to midwater depth with drastically reduced oxygen levels in the Pacific (e.g. Longhurst et al., 1990; Escribano et al., 2009; Bianchi et al., 2014) are likely too high. *E. mucronata* migrates to the core of the ETSP OMZ that features oxygen concentrations below 4 µmol O_2 kg^{-1} (~0.34 kPa) (Antezana 2009). Later work by Thamdrup et al. (2012) showed that the core of the ETSP OMZ is often anoxic. Antezana (2002) tested the dependence of respiration on oxygen concentration at 13˚C, but only tested oxygen levels > 1.8 kPa (21 µmol O_2 kg^{-1}) and therefore did not characterize the response of *E. mucronata* to the ETSP OMZ core conditions. A reduction in respiration activity at oxygen levels of 1.8 to 3.5 kPa (21 to 43 µmol O_2 kg^{-1}) in comparison to measurements at normoxia was not observed (Antezana 2002). We here show that reduction in respiration and ammonium excretion sets in at about 1 kPa

(~12 µmol O_2 kg^{-1}) in *E. mucronata*. Specimens migrating to the OMZ core therefore are exposed to oxygen concentrations below their p_{crit} for respiration and ammonium excretion. We obtained similar results for the squat lobster *Pleuroncodes monodon* that also seems to conduct regular migrations to the OMZ core (Kiko et al., 2015) and Cass and Daly (2014) report a strong reduction of nitrogen excretion in several copepod species at oxygen levels of 4 to 17 µmol O_2 kg^{-1} (0.3 to 1.3 kPa). It follows that calculations of DVM-mediated respiratory carbon dioxide and ammonium release into the anoxic core of the Pacific OMZ need to be adjusted. We here observed a 5.3-fold reduction of ammonium excretion in *E. mucronata* and found a 4-fold reduction in *P. monodon* (Kiko et al., 2015). Generalizations that DVM mediated ammonium export is 20% of the passive export flux (Bianchi et al., 2014) seem invalid and it seems unlikely that DVM mediated ammonium supply to anoxic OMZs can support anammox to a large extent. Parameterizations that account for changes in respiration and ammonium excretion with decreasing oxygen levels should be applied in biogeochemical modeling studies to calculate DVM-mediated impacts on biogeochemical cycles in a consistent model framework.

OMZs are thought to expand due to climate change, as warming reduces the solubility of oxygen in seawater and enhances stratification (Stramma et al., 2008). During the last decades, it has been observed that the vertical extent and the area covered by tropical oceanic OMZs expanded in the Eastern North Pacific (Bograd et al., 2008) as well as in the Indian Ocean, the ETSP and the ETNA (Stramma et al., 2008). Modeling studies predict a further decrease in the global oxygen inventory (Bopp et al., 2013; Cocco et al., 2013). However, the detailed extension of OMZs seems to be difficult to model and Cocco et al. (2013) come to the conclusion that “projections of the evolution of low O_2 regions will vary among models and be affected by large uncertainties”. Median deviations of the water volumes with oxygen levels below 5 and 50 µmol oxygen kg^{-1}

predicted for the year 1990 by the 17 models investigated (Cocco et al., 2013; Bopp et al., 2013) from the volumes observed in 1990 (Bianchi et al., 2012) are 6.7 (range: 1.0 to 21.1) and 1.9 (range: 1.1 to 4.8), respectively. To some extent problems to predict the observed OMZ extensions might be related to an unrealistic representation of oxygen dependent processes, including the regionally varying dependency of DVM-mediated export processes on the physiological capacities of the migrating organisms to cope with low oxygen levels. We expect that a decrease in oxygen levels below approximately 2.4 kPa (~ 30 µmol O_2 kg^{-1}, at 11 to 13˚C) in the ETNA will cause avoidance of these regions and will therefore result in a reduction of DVM mediated export. Expansion of the anoxic core of the ETSP should also result in a reduction of DVM-mediated fluxes, not via exclusion of anoxia-tolerant migrators, but via repression of their metabolic activity. Both effects would weaken the biological pump and would therefore affect the oceanic CO_2 uptake capacity, possibly enhancing global warming.

Conclusions

We here show that variations in the environmental pCO_2 do not result in a perceptible change in the respiration or ammonium excretion rates of tropical calanoid copepods or euphausiids. Decreases in temperature and pO_2 result in the expected reduction of respiration rates in these species. We show for the first time that also the ammonium excretion rates are reduced at low oxygen levels in calanoid copepods and euphausiids, which speaks in favor of an overall suppression of metabolism in these species at low oxygen levels. It is therefore necessary to consider the effects of temperature and pO_2 on metabolic activity of zooplankton and nekton when calculating or modelling diel vertical migration mediated fluxes in OMZ regions or on a global scale.

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Tables:

Table 1: Volumetric composition of used premixed gases.

		50% air sat.	33% air sat.	10% air sat
ETNA CO_2-plus	Nitrogen	89.58	93.02	97.85
	Oxygen	10.36	6.91	2.07
	Carbon Dioxide	0.06	0.07	0.08
ETSP CO_2-plus	Nitrogen	89.53	92.96	97.77
	Oxygen	10.36	6.91	2.07
	Carbon Dioxide	0.11	0.13	0.16
CO_2-minus	Nitrogen	89.64	93.09	97.93
	Oxygen	10.36	6.91	2.07
	Carbon Dioxide	0.00	0.00	0.00

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Table 2: Recommended volumetric composition for premixed gases for the ETSP OMZ.

		50% air sat.	33% air sat.	10% air sat
ETSP CO_2-plus	Nitrogen	89.56	93.00	97.81
	Oxygen	10.36	6.91	2.07
	Carbon Dioxide	0.08	0.09	0.11

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Table 3: Lethal pO_2, parameter estimates (a, b) of fitted power functions, p_{crit} and Q_{10} values for respiration and excretion rate data. [#] = approximate p_{crit} deduced from Fig. 2 in Teal and Carey (1967). NA = not applicable.

Species	Target Temp. (˚C)	mean lethal pO_2(kPa), (SD, n)	minimum lethal pO_2 (kPa)	a_{resp}	b_{resp}	$p_{crit\ resp}$ (kPa)	$a_{NH4\text{-}excr}$	$b_{NH4\text{-}excr}$	$p_{crit\ NH4\text{-}excr}$ (kPa)	$Q10_{resp}$	$Q10_{NH4\text{-}excr}$
U. vulgaris	11	2.7 (0.3, 6)	2.3	15.48	0.20	2.4	2.29	0.13	2.9	1.4	1.6
U. vulgaris	23	6.6 (0.7, 6)	5.5	27.55	0.17	6.2	NA	NA	NA		
P. abdominalis	11	NA	NA	9.62	0.33	0.6	1.51	0.30	1.8	2.0	2.3
P. abdominalis	23	6.3 (1.0, 2)	5.3	31.31	0.25	6.5	6.37	0.13	6.5		
E. gibboides	13	2.5 (1.4, 20)	1.1	19.70	0.22	2.4	NA	NA	NA	2.0	2.3
E. gibboides	23	7.5 (1.9, 20)	4.5	27.12	0.33	6.2	NA	NA	NA		
E. mucronata	13	NA	NA	10.00	0.39	0.6	1.27	0.36	0.73	NA	NA
E. mucronata	20	NA	NA	NA	NA	2 to 4[#]	NA	NA	NA	NA	NA

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Figures

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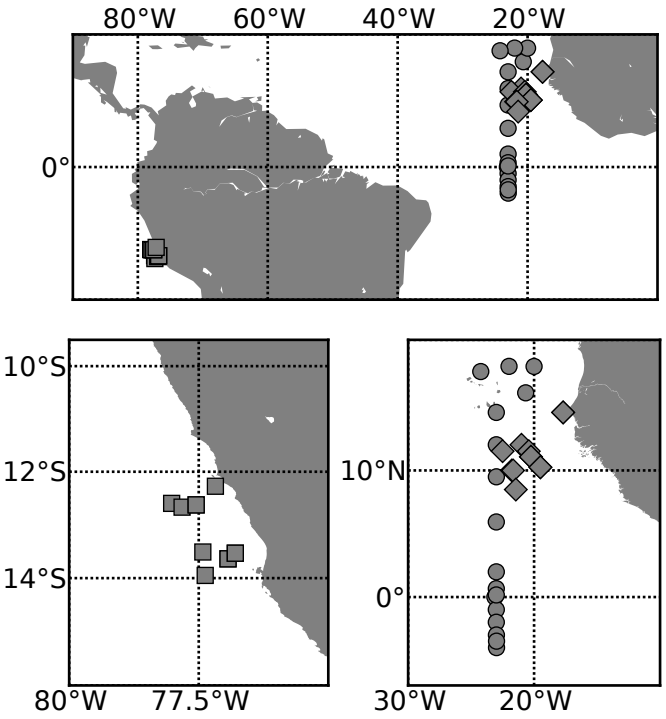

Fig.1: Sampling locations of specimens for respiration and excretion measurements. 965 Circles: *Pleuromamma abdominalis* and *Undinula vulgaris* during cruise MSM22 (Oct/Nov 2012); Squares: *Euphausia mucronata* during cruise M93 (Feb 2013); Diamonds: *Euphausia gibboides* during cruise M97 (May 2013).

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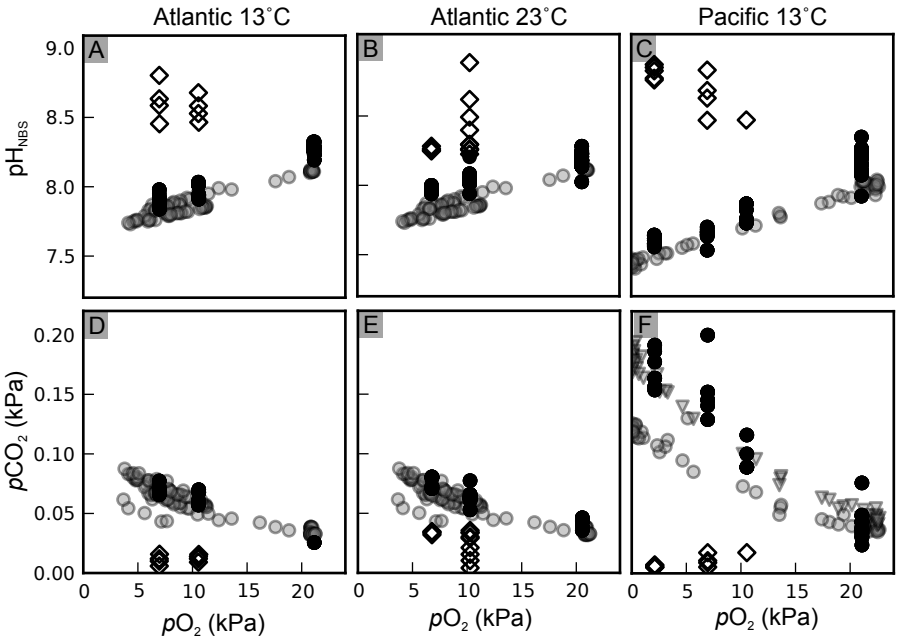

Fig.2: pH_{NBS} and pCO_2 in incubation water (black symbols), compared to environmental levels (grey symbols) for the ETNA OMZ and the ETSP OMZ. Black circles: CO_2-plus treatment. Open diamonds: CO_2-minus treatment. Grey circles: environmental pH or pCO_2 data, pCO_2 calculated from A_T and C_T. Grey triangles in F: pCO_2 calculated from pH and A_T

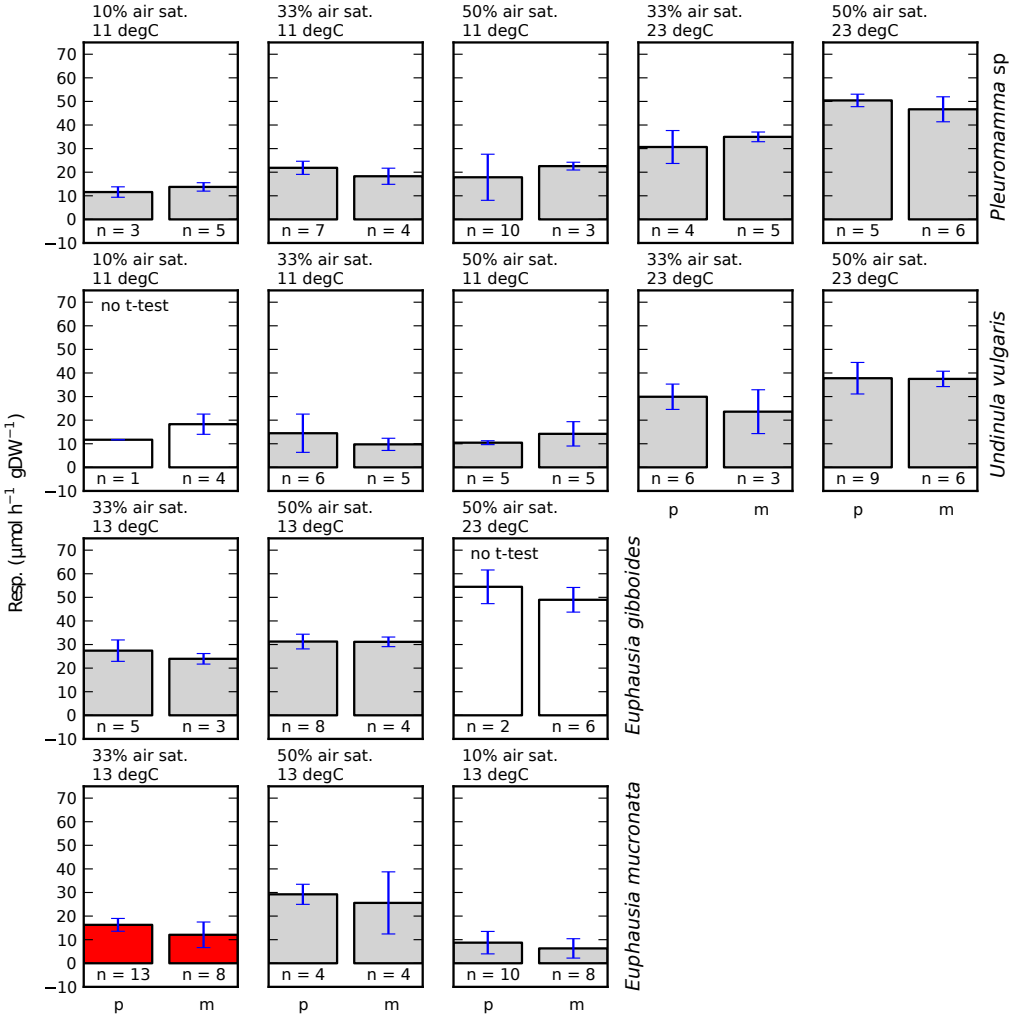

Fig. 3: Weight-specific respiration rates of calanoid copepods and euphausiids at different temperatures, air saturation and CO_2-levels. p = CO_2-plus, m = CO_2-minus. Red barplot color indicates a statistically significant difference (t-test, $p < 0.05$) between CO_2-plus and CO_2-minus treatments. Each row contains data for only one species, with low temperatures on the left side of the row and high temperatures on the right side and air saturation increasing from left to right.

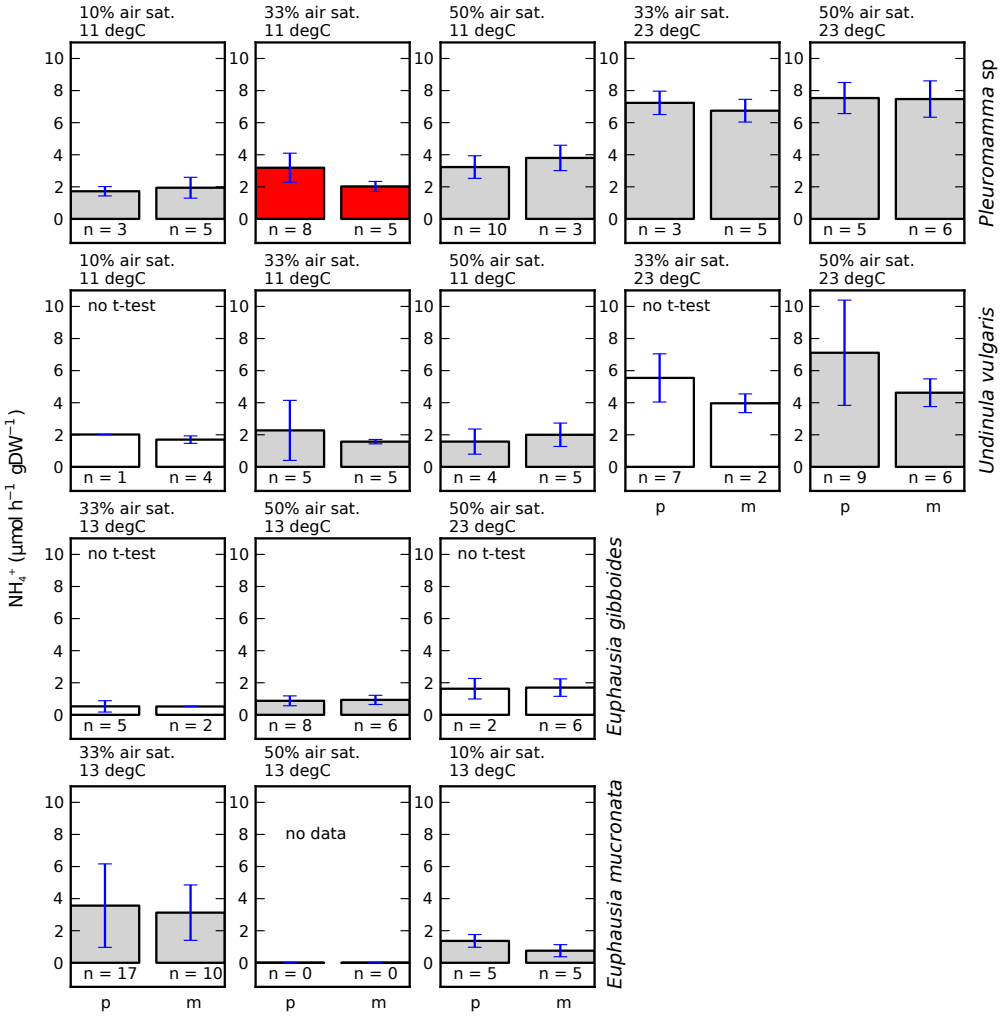

Fig. 4: Weight-specific ammonium excretion rates of calanoid copepods and euphausiids at different temperatures, air saturation and CO_2-levels. p = CO_2-plus, m = CO_2-minus. Red barplot color indicates a statistically significant difference (t-test, $p < 0.05$) between CO_2-plus and CO_2-minus treatments. Each row contains data for only one species, with low temperatures on the left side of the row and high temperatures on the right side and air saturation increasing from left to right.

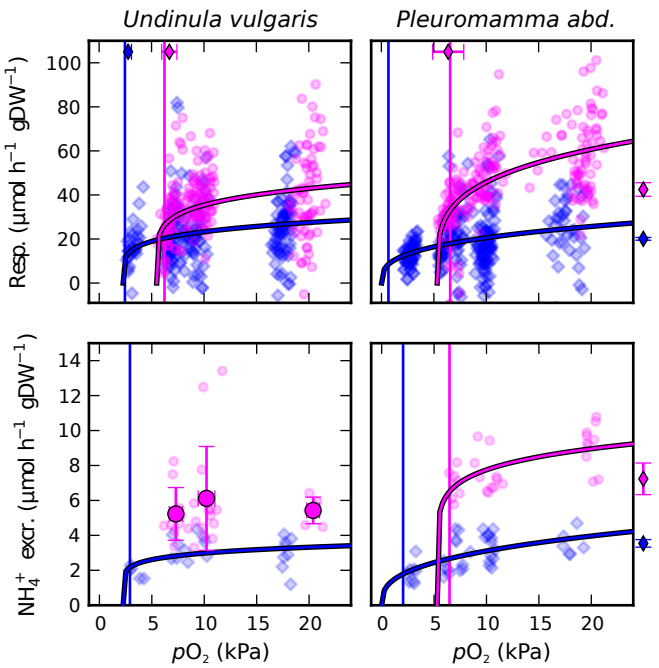

Fig. 5: Weight-specific respiration and ammonium excretion rates of *Undinula vulgaris* and *Pleuromamma abdominalis* at different oxygen partial pressures and temperatures (CO_2-plus and CO_2-minus treatments combined). Magenta symbols = 23˚C, Blue symbols = 11˚C. Transparent dots represent individual measurements. Solid curves indicate the power function fits to the data. Regression coefficients are given in Table 3. Solid vertical lines indicate the respective p_{crit}. Horizontal error bars in the upper panels indicate the mean (± SD) lethal pO_2 at the respective temperature. A power function could not be fitted to the ammonium excretion data for *U. vulgaris* at 23˚C, therefore the mean (± sd) excretion rates for the three pre-set oxygen levels (100% air saturation, 50% and 33%) are plotted with vertical errorbars. Respiration and excretion rates were standardized to a mean dry mass of 0.1 g. Error bars right beside the plots indicate the respiration or ammonium excretion rate (± SE) calculated according to Ikeda (2014) for calanoid copepods (0.1 gDW) at the respective temperature.

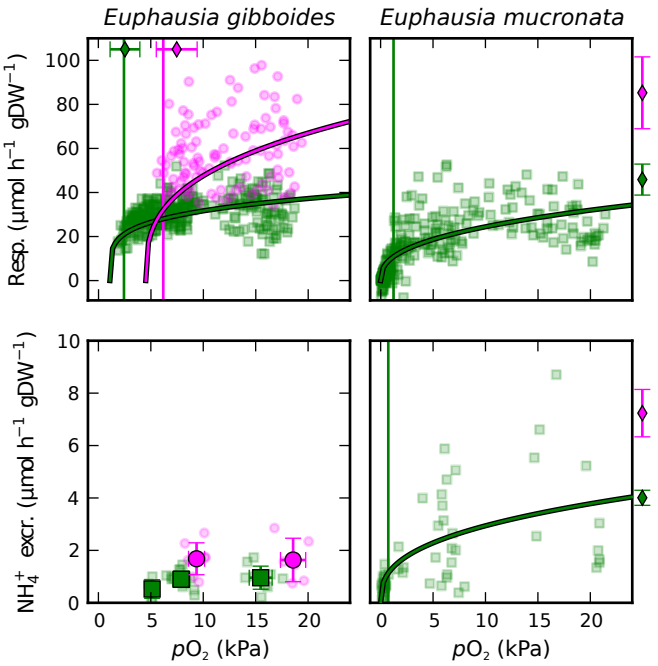

Fig. 6: Weight-specific respiration and ammonium excretion rates of *Euphausia gibboides* and *Euphausia mucronata* at different oxygen partial pressures and temperatures (CO_2-plus and CO_2-minus treatments combined). Magenta symbols = 23˚C, Green symbols = 13˚C. Transparent dots represent single measurements. Solid curves indicate the power function fits to the data. Regression coefficients are given in Table 3. Solid vertical lines indicate the respective p_{crit} for respiration and ammonium excretion. Horizontal error bars indicate the mean (± SD) lethal pO_2 at the given temperature for *E. gibboides*. A power function could not be fitted to the ammonium excretion data for *E. gibboides*, therefore the mean (± sd) excretion rates for the three pre-set oxygen levels (100% air saturation, 50% and 33%) are plotted with vertical errorbars. Respiration and excretion rates were standardized to a mean dry mass of 0.1 g. Error bars right beside the plots indicate the respiration or ammonium excretion rate (± SE) calculated according to Ikeda (2014) for euphausiids (0.1 gDW) at the respective temperature.