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ORIGINAL ARTICLE

Allometry and the calculation of zooplankton metabolism in the subarctic Northeast Pacific Ocean

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Using measurements of respiration and dissolved organic carbon (DOC) excretion from the subarctic Northeast Pacific Ocean (August 2018), we explore the efficacy of pre-existing allometric relationships to predict metabolic rates of diel vertically migrating zooplankton, and to test taxon-specific influences on these calculations at our study site. Non-taxon-specific allometric equations were associated with our best predictive model, and they underestimated measured respiratory values by ~10%. The best prediction of DOC release from estimates of biomass used taxon-specific coefficients and overestimated DOC production by 12%. There is a distinct allometric relationship for DOC excretion that varies between taxa, and slightly higher DOC production in more carnivorous groups. This study provides uncertainty estimates for zooplankton active flux analyses in the region, and identifies important research directions for allometry in biogeochemical studies.

KEYWORDS: respiration; DOC; excretion; POC; metabolism; diel vertical migration

INTRODUCTION

Global biogeochemical cycles are strongly influenced by the uptake of inorganic carbon and nutrients by phytoplankton, and the subsequent export of this biologically fixed organic matter out of surface waters to depth (the biological pump; Longhurst and Harrison, 1989). The contributing processes to the biological pump include the sinking of particles, advective mixing of dissolved or suspended carbon and "active" transport of carbon via zooplankton vertical migration (Longhurst *et al.*, 1990; Siegel *et al.*, 2016). Particulate organic carbon (POC) that is consumed in surface waters and transported to the

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mesopelagic zone by migrating zooplankton is metabolized and released at depth via respiratory release of carbon dioxide (CO_2), excretion of dissolved organic carbon (DOC) compounds, egestion of POC as fecal pellets, production of feeding structures or molts (other forms of POC) and mortality at depth. The magnitude of active transport of carbon by diel vertical migration of zooplankton varies considerably, and at times of high migrating biomass can exceed carbon exported by sinking particles (reviewed in Steinberg and Landry, 2017). A recent model estimates that on average, across regions and seasons, zooplankton active transport accounts for 16% of total global carbon export (Archibald *et al.*, 2019). Active transport mechanisms are arguably the least sampled of the export pathways, and are considered a community priority for future research on the biological pump (Burd *et al.*, 2016).

A common approach to estimate zooplankton active flux is to calculate the differences between (total or size-fractionated) nighttime and daytime net samples from the euphotic zone to define the biomass, or abundance by taxon, of the vertically migrating community, then to apply experimentally-derived allometric (i.e. body size—usually dry or carbon weight) and temperature corrections to predict respiratory CO_2 produced at depth (Al–Mutairi and Landry, 2001; Steinberg *et al.*, 2008; Alcaraz *et al.*, 2010; Stukel *et al.*, 2013; Ikeda, 2014b). There is error associated with net avoidance by organisms during the daytime (Ianson *et al.*, 2004), and thus recent studies have made similar calculations based on diel changes in midwater zooplankton abundances (e.g. Kiko *et al.*, 2020). Although taxon-specific allometric equations exist for respiration, historically most datasets used for active flux calculations do not distinguish among taxa; thus, a general equation is usually selected to represent each size class or the whole zooplankton community. Recent endeavors have begun to use these taxon-specific equations (Kiko and Hauss, 2019; Kiko *et al.*, 2020; Kwong *et al.*, 2020), although the impact of using taxon versus non-taxon-specific approaches for these calculations has rarely been quantified.

In the compromise between specificity and feasibility, researchers have gravitated toward a set of equations generated by Ikeda that compile > 50 years of zooplankton metabolic rate measurements (Ikeda, 2014b) to provide estimates of body size (allometric) and temperature dependence of the metabolic rate of various zooplankton groups. These are based on a long history of estimating zooplankton respiration by oxygen consumption, typically using closed chamber respiration experiments where the change in oxygen is directly measured over time (Hernández-León and Ikeda, 2005; Ikeda, 2014b). Alternatively, and less common, the respiratory CO_2 produced by zooplankton (the change in dissolved inorganic carbon; (DIC)) is directly measured (Steinberg *et al.*, 2000). Simultaneous measurements of these two factors provide a respiratory quotient (RQ) that allows conversion between the substrate (O_2) and byproduct (CO_2) of aerobic metabolism. The RQ can vary among taxa and has only been thoroughly studied in a few groups, most predominantly crustaceans (Mayzaud *et al.*, 2005).

In contrast to respiration rate, measurements of DOC excretion rates or fecal pellet POC production by vertical migrators are rare (Steinberg *et al.*, 2000; Schnetzer and Steinberg, 2002), and the contributions of feeding structures and molts have not yet been considered for migrators. Additionally, although there are no direct measures of mesozooplankton mortality, there are models developed for fish by Peterson and Wroblewski (1984) that have been applied to mesozooplankton (Zhang and Dam, 1997), as well as an allometric approach based on copepod life history traits by Hirst and Kiørboe (2002). This mortality term, which mainly accounts for midwater predation of zooplankton, has only recently been included in estimates of active flux in an allometric fashion (Kiko *et al.*, 2020; Kwong *et al.*, 2020), despite that modeling efforts suggest mortality at depth accounts for ~50% of zooplankton contributions to midwater carbon flux (Kelly *et al.*, 2019). As attempts are made to better parameterize and synthesize the results of existing field data on active flux into global biogeochemical models, it is important to assess the current methods of estimating active flux, and to provide a characterization of the uncertainties for the different contributing pathways. Although the equations generated by available meta-analyses help describe broad patterns in the physiology of zooplankton, they do not necessarily have strong predictive power for a specific community. It is therefore valuable to ground truth predictive equations with experimentally-derived measures of physiology to best estimate regional active flux.

There is a paucity of direct measurements of DOC production by migrating zooplankton, with much of the ecosystem scaling estimates relying on the DOC excretion: respiratory CO_2 ratio of 0.31. This ratio was derived from a study of active flux in the Sargasso Sea (Steinberg *et al.* 2000). These experiments were conducted on pooled sets of organisms, primarily using a range of crustaceans (copepods, euphausiids, amphipods and shrimps) and one group of polychaetes. Analyses of DOC production by other zooplankton taxa in different regions are relatively rare (e.g. Condon *et al.*, 2010; Saba *et al.*, 2012; Thibodeau *et al.*, 2020). Due to the scarcity of zooplankton DOC excretion measurements, a concerted attempt to establish scaling relationships between organismal mass and DOC production across a variety of taxa has not

been attempted. Similarly, estimates of the production of fecal pellet POC at depth are limited and based on comparisons between gut passage time and speed of migration to predict what portion of fecal pellets are egested at depth (Atkinson *et al.*, 1996; Schnetzer and Steinberg, 2002; Gleiber *et al.*, 2016). The assumptions are that both gut passage time and fecal pellet size/carbon content are related to zooplankton size, but these allometric relationships have never been thoroughly examined across a broad taxonomic range.

Calculations of active flux rely on a suite of assumptions, primarily since up to this point, individual physiological measurements were onerous and often did not produce sufficient signal to resolve analytically, making the scaling of allometric relationships to environmental samples difficult to validate. Additionally, high taxonomic resolution with individual biomass measurements of the community is laborious, making application of taxon-specific allometric equations impractical. Recently, advances in respiration measurements using non-invasive optical spot (Optode) sensing have substantially improved the ability to make a large number of respiration measurements of even very small organisms, or those with slow metabolic rates (Burke *et al.*, in review; Lilley and Lombard, 2015; Brown *et al.*, 2018; Maas *et al.*, 2018b). Pairing these measurements of respiration directly with DOC production from the same individual allows for assessment of how these factors vary with allometry and relate to each other. Similarly, new advances in imaging technology allow for more rapid classification of the entire zooplankton community by size and taxon (Alcaraz *et al.*, 2010; Gorsky *et al.*, 2010; Lombard *et al.*, 2019; Maas *et al.*, in review; Ohman *et al.* 2019).

These technological advances allow us to more clearly define allometric relationships between zooplankton and their active flux excretory products. The objective of this paper is thus to compare field measurements with various allometric relationships to both determine the best approach for calculating the components of active flux, and to establish the sources and levels of uncertainty for these calculations for our region of interest. To achieve this goal, we conducted a set of physiological experiments as part of the EXport Processes in the Ocean from Remote Sensing (EXPORTS) program in August 2018 near Ocean Station Papa in the subarctic Northeast Pacific Ocean (Siegel *et al.*, 2016). In this study, paired measures of respiration (O_2 consumption), and DOC excretion production were collected from individual organisms across a range of vertically migrating taxa. We used these data to test various body mass and taxon-based equations for the prediction of zooplankton physiological rates relevant to active carbon flux calculations at our study site. This dataset allows for a determination of how sensitive estimates of community active flux are to variability in allometric relationships. The intention is to demonstrate which equations have the most predictive power for estimation of active flux, and to provide uncertainty estimates when scaling zooplankton biomass and distribution datasets.

METHODS

Zooplankton capture

Sampling during the EXPORTS field campaign occurred in proximity of Station P (50°N 145°W, also known as Ocean Station Papa or P26) aboard the R/V *Roger Revelle*, in late summer 2018 (August 10–September 13). Twelve sets of experiments were performed over the duration of the cruise. Zooplankton were collected for live experiments within the epipelagic zone between the chl-a maximum (~60 m; Seigel *et al.*, in review) and surface using a vertically-towed (0–100 m), slowly-retrieved (10 m/min), 1-m diameter ring net equipped with a non-filtering cod end. Net mesh size varied (200, 500 or 1600 μm) based on community composition and abundance of gelatinous organisms. Upon retrieval, the contents of the net's cod end were gently transferred into 10 L of unfiltered mixed layer seawater until further processing. Four liters of water were collected at 330 m using the CTD-Rosette, filtered with 0.2-μm Supor® 200 membranes (Pall Corporation) mounted on a 142-mm acrylic in-line standing filter holder, and stored in a dark stand-up incubator at mesopelagic temperature (ranging between 4 and 9°C) for at least 1.5-h prior to each experiment. Water from this depth was chosen to more closely align experimental conditions with the oxygen, carbonate chemistry and nutrient characteristics experienced by migrators at their daytime mesopelagic residence depth. After the filtration process, the starting oxygen concentration was, therefore, substantially lower than saturation, with an average 183 ± 33 μmol (SE), and was consistent with conditions between 50 and 200 m (Fig. 1). The acoustic backscatter from the shipboard 150 KHz acoustic doppler current profiler (ADCP) was processed according to standard CODAS methods and binned into 2-min intervals to achieve a general profile of migratory patterns. This frequency is optimized for fluid-like organisms (e.g. copepods, euphausiids and chaetognaths) 6–15 mm and, although it will return scattering for smaller copepods (2–6 mm), it likely under-samples the smaller sized taxa (Lavery *et al.*, 2007). Data were collected with vertical bin spacing was 8 m; no averaging was done in the vertical (Firing *et al.*, 2012). Anomalies in the acoustic backscatter were calculated by subtracting the signal at

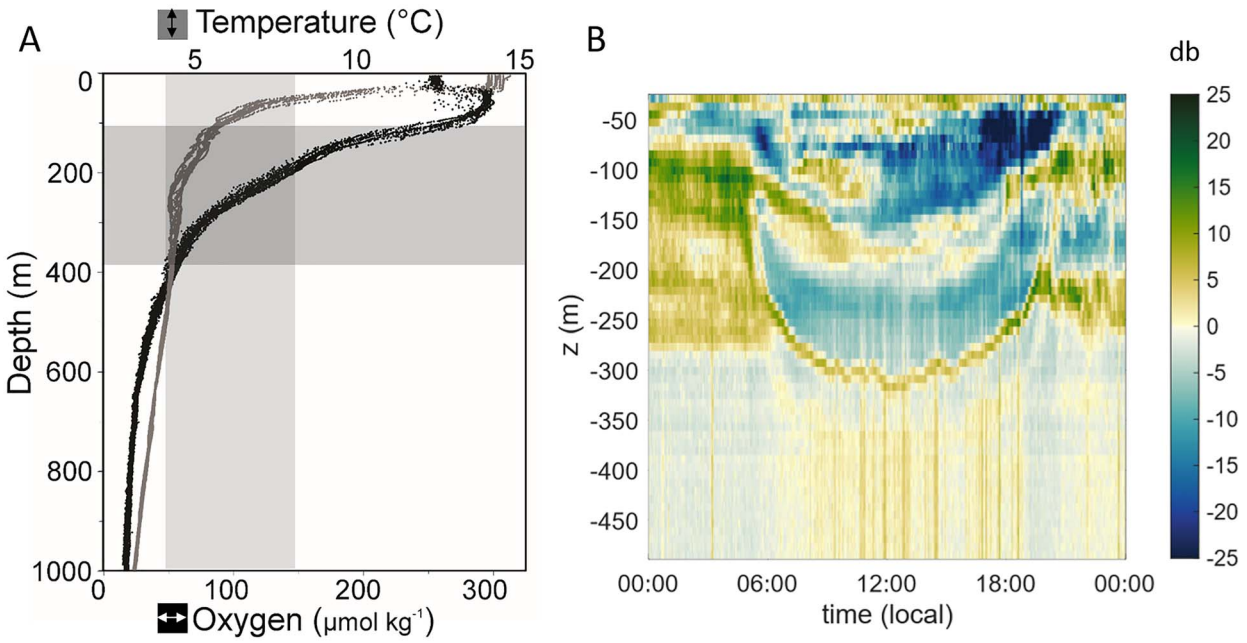

Fig. 1. The environmental conditions and acoustic backscatter of the upper water column. (**A**) The oxygen (black) and temperature (gray) conditions measured during MOCNESS deployments are consistent with those experienced by organisms in the experiments from ~50 to 375 m (shaded gray box represents the experimental conditions). (**B**) The acoustic backscatter from the 150 KHz ADCP suggests that many of the migrators are present in this depth range. The colors represent the anomaly of the acoustic backscatter in decibel (db).

each depth bin from the time-averaged signal, such that positive values indicate higher than average scattering for that isobar.

Respiration experiments

Dominant migratory species for live individual respiration experiments were gently selected using a small ladle. The following species were sampled: *Neocalanus cristatus* and *Metridia pacifica* (Order Calanoida), *Themisto pacifica* and *Vibilia propinqua* (Order Amphipoda), *Thysanoessa inspinata*, *Tessarabrachion oculatum*, *Stylocheiron maximum* and *Euphausia pacifica* (Order Euphasiacea), *Parasagitta elegans* and *Sagitta elegans* (Phylum Chaetognatha) and *Pneumoderma sp.* (Order Pteropoda).

Animals were placed into 15- and 50-cc metal Luer lock tip glass syringes (micro-mate) customized with optically-isolated oxygen sensor spots (code SC7-537-198, PyroScience GmbH). Syringes were filled with the previously filtered seawater with a 1-mg glass bead added to prevent organisms from swimming into the syringe tip. Oxygen concentrations were recorded using three 2016 generation multi-channel FireStingO_2 optical Optode oxygen meters (PyroScience GmbH) equipped with TSUB21 temperature sensors. O_2 meters were operated through the proprietary Pyro Oxygen Logger software (v3.314) and at the start of each 8-day sampling cycle were manually calibrated over the 0–100% range of dissolved O_2 (2-Point in Water or Humid Air mode; 0% = sodium sulfite O_2-depleted MilliQ water; 100% = *in situ* marine water; gently aerated to ensure saturation; 33.9 psu). After the process of filtering and temperature acclimation, the starting oxygen in our experimental chambers was on average (±SD) 204 ± 20 µmol (range 170–250 µmol). Both chambers and oxygen meters were maintained for 12 h in dark incubators set at mesopelagic temperature. In continuous experiments, measurements were taken at a 1-min interval for calculation of metabolic rates. To increase sample size (with limited instrumentation), end-point trials were also conducted. In these trials, measurements were taken manually every 2 h. Chambers containing only filtered sea-water and glass beads served as controls.

At the end of the incubations, animals were checked to ensure they were active (with the occasional moribund animal removed from our analyses). Water from the 50 mL chambers was filtered through an inline high density polyethylene cartridge containing a pre-combusted (450°C) 25-mm GF-75 glass fiber filters (Advantec) into pre-combusted (450°C) 40-mL EPA vials (I-CHEM, Thermo Scientific). The resulting DOC samples were acidified with 60 µL 4 N HCl to a pH < 3 and stored at ~14°C until analysis. DOC was only sampled from the

50 mL chambers. Animals from all experiments were then removed, rinsed twice with deionized water and stored at −80°C in individual cryovials. Water and filters were examined for fecal pellets which were enumerated. Upon return to land, specimens were wet- and dry-weighed (after 3 days at 55°C) with a microbalance (Mettler-Toledo) and imaged on a stereomicroscope (Leica Microsystems). Individuals were sent to the Barcode of Life Data System for analysis of the COI gene for species verification (website + accession number).

For both end-point and continuous measurements the oxygen concentration (μmol O_2 L^{-1}) in each chamber was plotted over time and the slope (reduction in oxygen per hour) was calculated. The slope of the control was subtracted from that with the organism and was then multiplied by the volume of the chamber to calculate the respiration rate (μmol O_2 ind^{-1} h^{-1}). Due to occasional temperature fluctuations in the incubator, all rate analyses were conducted with data from periods when temperature remained relatively constant (<0.5°C change h^{-1}) and there were no large deviations in the slope of the O_2 concentration in the control chamber. For consistency, the first 2 h of temperature acclimation were excluded from analyses of each experiment. Consequently, generally calculations were made over a 10-h period, although occasionally rates were calculated over a shorter duration (minimum 4 h).

It was not the objective of this analysis to determine the O_2 concentration at which the metabolic rate of the zooplankton was influenced by O_2 concentration (pCrit), so any time there appeared to be a change in slope of the respiration rate, the reading was truncated. Owing to the diffusion of gas into the experimental water during the filtering process and the natural variability in the rate of respiration of different sized organisms, the starting and ending O_2 concentrations ranged substantially across experiments (Fig. 2). Similarly, the temperature of the experiments varied over the course of the cruise, ranging from 4.06 to 8.66°C. Experimental temperature and oxygen values are consistent with those *in situ* between ~50 and 400 m (Fig. 1A), the depth range where acoustics datasets suggest a large number of migrators are located during the day (Fig. 1B). This variability in experimental conditions allows for the analysis of Q_{10} by fitting a slope to the rate data based on the experimental temperature (Fig. 2A) and then calculating the average respiration rate at the minimum and maximum temperature. These were implemented in the standard temperature coefficient equation of:

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

where R_2 and T_2 are the respiration rate and temperature, respectively at the maximum value, and R_1 and T_1 are at the minimum measured temperature. A linear regression was performed to determine whether there was a significant effect of starting or ending O_2 concentration and respiration rate. Finally, to synthesize the dataset and explore internal allometric relationships (the measured respiration and DOC production rates), the measured values were scaled to the average temperature of the experiments (5.7°C) using a standard Q_{10} of 2.

DOC analysis

DOC concentrations were measured using the high-temperature combustion method on a modified TOC-V or TOC-L analyzer (Shimadzu) at the University of California, Santa Barbara as described in Carlson *et al.* (2010). Concentrations were estimated using glucose standard solutions with nano-pure (low carbon) water. Reference waters were collected from 5 to 3000 m during the cruise from and were calibrated against consensus reference material provided by D. Hansell (University of Miami; https://hansell-lab.rsmas.miami.edu/consensus-reference-material/index.html). Each set of reference was analyzed every 6–8 samples for each analytical run to assess machine performance. The resulting precision of the TOC analyzer for surface samples was CV of < 2% or ~1 umol L^{-1} C for this data set. The final concentration of DOC produced by experimental animals during the incubations was calculated as the difference between the measured experimental value and the DOC measured from the control chamber at T-final of the same set of incubations.

Analysis of allometric active flux calculations

To compare the observed respiration and DOC excretion rates with published values and to those predicted from allometric scaling, oxygen consumption was calculated per individual (μmol O_2 ind^{-1} h^{-1}). Two analyses were run, one where all organisms were treated similarly, with generic (copepod-based) allometric relationships, and the second using taxon-specific conversion factors (Table I). Two types of currently existing allometric relationships for zooplankton respiration were assessed. The first used phylogenetically distinct equations generated by Ikeda (Ikeda *et al.*, 2001; Ikeda and Takahashi, 2012; Ikeda, 2013a, b, 2014a) that describe the allometric and temperature sensitivity of each taxonomic group (herein referred to as the "taxonomic rate") with species-specific constants

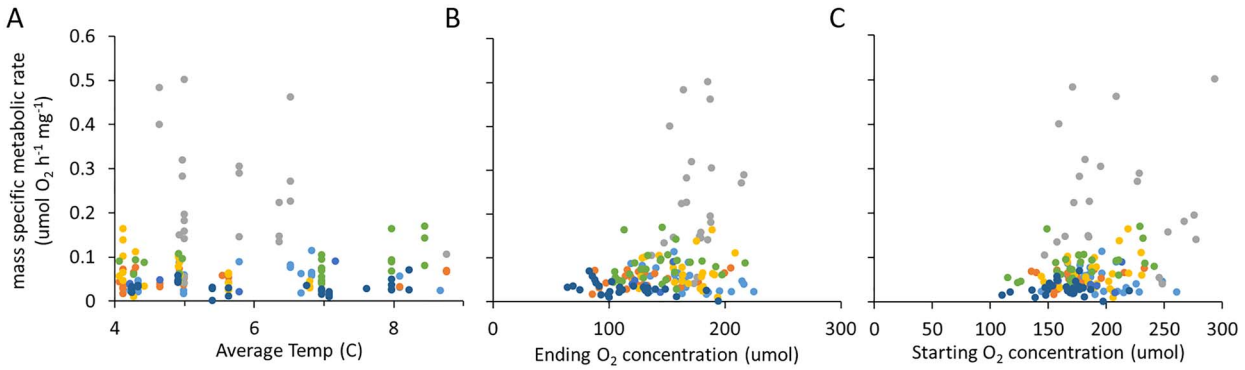

Fig. 2. Temperature and oxygen conditions experienced in respiration experiments. (**A**) Experimental average temperature, (**B**) ending oxygen concentration and (**C**) starting oxygen concentration were not strongly correlated with the metabolic rate of any group of organisms ($R^2 < 0.3$).

Table I: Taxon-specific RQ, allometric, and temperature scaling coefficients

Model	Taxonomic group	RQ	a_0	a_1	a_2	a_3	b	Source
ES	Amphipod	1.35	0.407	0.743	0.037			Ikeda (2013a)
ES	Chaetognath	1.35	−0.173	0.805	0.068			Ikeda and Takahashi (2012)
EC, ES	Copepod	0.87	−0.399	0.801	0.069			Ikeda *et al.* (2001)
ES	Euphausiid	1.35	0.392	0.753	0.046			Ikeda (2013b)
ES	Mollusca	0.94	−0.56	0.82	0.046			Ikeda (2014a)
AS	Amphipod	1.35	18.775	0.766	−5.256	−0.113	0.416	Ikeda (2014b)
AS	Chaetognath	1.35	18.775	0.766	−5.256	−0.113	−0.448	Ikeda (2014b)
AC, AS	Copepod	0.87	18.775	0.766	−5.256	−0.113	0	Ikeda (2014b)
AS	Euphausiid	1.35	18.775	0.766	−5.256	−0.113	0.697	Ikeda (2014b)
AS	Mollusca	0.94	18.775	0.766	−5.256	−0.113	0	Ikeda (2014b)

Notes: RQ from Mayzaud *et al.* (2005). Coefficients are from taxon-specific models (Ikeda *et al.*, 2001; Ikeda and Takahashi, 2012; Ikeda, 2013a, b, 2014a), or from the aggregate model of Ikeda (2014b). Analyses using a generic value applied to all taxa (AC, AC) were conducted using the copepod-specific values.

for a_0, a_1 and a_2 (Table I):

$$\text{Taxonomic Rate} = \text{EXP}\left(a_0 + (a_1 * \ln(\text{DM})) + (a_2 * (\text{TempC}))\right)$$

The second relies on the combined model from Ikeda (2014b) which assumes that the effects of body mass and temperature of all taxa are the same as that of copepods (herein referred to as the "aggregate rate"):

$$\text{Aggregate Rate} = \text{EXP}\left(a_0 + (a_1 * \ln(\text{DM})) + (a_2 * (1000/\text{TempK})) + (a_3 * \ln(\text{d})) + \text{b}\right)$$

For each of these equations the predicted respiration rate is related to the dry mass (DM), temperature (Temp) in either degrees Kelvin (TempK, aggregate) or Celsius (TempC, taxonomic) of the experiment, and depth (d, meters) of the average depth of the organism. The Ikeda (2014b) equation is adjusted for taxonomic variation by adding a species-specific dummy variable to account for phylogenetic differences (b; Table I). Additionally, the Ikeda (2014b) model tested for habitat depth, which has been suggested to independently (of temperature, for example) play a role in setting metabolic rate (Seibel and Drazen, 2007; Brey, 2010). Diel vertical migrator activity level during migrations, and their upper water column nighttime distribution would cause migrator metabolism to be more closely aligned with surface-living species (vs. that of full-time mesopelagic residents). Thus, we chose a representative habitat depth of 25 m, also a migrator nighttime biomass peak–as verified by acoustics and depth-discrete net collections. In an additional calculation we excluded the depth factor from the analysis (by choosing a depth of 1 m) to test the usefulness of this component of the equation for our data.

The DOC excretion rate was used to test the prediction that it is equivalent to 0.31 of respiratory carbon production (Steinberg *et al.*, 2000). The predicted DOC excretion rate of each individual was calculated using (i) the measured respiration rate converted to respired CO_2 by applying either species-specific or taxon-specific RQ (Table I), (ii) based entirely on animal DM, using the aggregate or taxonomically-derived respiration

Table II: Calculated Q_{10} temperature coefficient

	Max final O_2	Min final O_2	Max temp	Min temp	Q_{10}	Equation
Chaetognath	224.7	119.1	8.66	4.21	3.5	$0.0044x + 0.0076$
Euphausiid	205.1	85.6	8.76	4.06	1.7	$0.0027x + 0.0359$
Metridia (copepod)	216.2	137.0	8.76	4.63	0.0	$-0.0898x + 0.8016$
Neocalanus (copepod)	209.6	120.8	8.22	4.05	0.0	$-0.0242x + 0.2025$
Themisto (amphipod)	217.7	103.9	8.45	4.06	2.2	$0.0063x + 0.0405$
Vibilia (amphipod)	194.6	64.3	8.22	4.24	1.1	$0.0003x + 0.0288$

Notes: The minimum and maximum final oxygen concentration (μmol L^{-1}) and minimum and maximum average temperature is reported for each experiment. Using a plot of the respiration rates (μmol O_2 mg^{-1} h^{-1}) versus experimental temperature (Fig. 2A) a regression was plotted (equation) that was then used to determine a Q_{10} for the experiments. The R_2 of each of these regressions was < 0.3 emphasizing the large biological variability in the dataset.

rates as calculated above with either the taxon- or copepod-specific coefficients or (iii) using the allometric relationship between DM and DOC excretion rates generated by this dataset. We then calculated the regression fit (R^2), the mean square error (MSE), and the average % error ((measured-predicted)/measured) for the various predictive approaches to determine which best fit our experimental data.

RESULTS

Effect of temperature and oxygen on metabolic rate

There was no statistical correlation between starting or ending O_2 concentration and respiration rate, suggesting that organisms were not physiologically influenced by the dynamic range of measured O_2 saturation states (down to 33 μmol L^{-1}). Data from any trials where the O_2 concentration in the chamber fell below 60 μmol L^{-1} (20% of surface O_2 saturation, and O_2 concentration at 350 m at Station P) were, however, excluded from the dataset to ensure that there was no unintended effect of low O_2 on zooplankton metabolism. Q_{10} was calculated for each taxonomic groups with a sufficient number of individuals tested at multiple temperatures (Table II), and broadly fell close to the range expected from marine ectotherms (2–3; Hochachka and Somero, 2002), with the exception of *Metridia* and *Neocalanus* copepods which showed no substantial temperature dependence of metabolism ($Q_{10} = 0$).

Respiratory and DOC model assessment

Measured allometric scaling relationships for respiration (Fig. 3A) were highly significant (analysis of variance, ANOVA: $F_{1,148} = 225.03$, $P < 0.001$) with an $R^2 = 0.61$ (Table III). Taxon-specific respiratory scaling relationships were typically statistically significant (with the exception of the less abundant *Pneumoderma* pteropod and the *Neocalanus* and *Metridia* copepods). Although the DOC excretion rate was statistically related to DM (Fig. 3B; ANOVA: $F_{1,62} = 27.15$, $P < 0.001$), the R^2 was lower (0.54), the taxon-specific relationships were mostly not statistically significant (with the exception of Euphausiids), and the scaling factors were different from respiration rate (Table III). Wet mass (WM) and DM were strongly correlated ($R^2 = 0.70$), although there were clear differences between taxa in the relationship, particularly with an offset in the intercept for the gelatinous *Pneumoderma* sp. pteropods and a shallower slope for *V. propinqua* amphipods (Fig. 3C). The relationship between respiration and excretion rate for the whole dataset was weak but significant, ($R^2 = 0.37$, $P < 0.001$), but improved substantially ($R^2 = 0.72$, $P < 0.001$) when chaetognaths were excluded (Fig. 3D). When taxon-specific RQs were applied to respiration O_2 values there was an average DOC excreted: respiratory CO_2 ratio of 0.37 for crustacean zooplankton ($R^2 = 0.74$, $P < 0.001$; Fig. 4A). A slightly higher and substantially more variable proportion of carbon was released as DOC in chaetognaths (0.40; $R^2 = 0.15$, $P = 0.197$). When the copepod-specific RQ was applied, the overall pattern did not change; however, the fraction partitioned as DOC excreta increased to 0.62 and 0.57 of respiration for chaetognatha and crustacea, respectively (Fig. 4B).

The taxonomic rate equation for respiration rate based on copepod values provided the best prediction of overall respiration, using overall R^2 and mean squared error as selection metrics (Fig. 5A; Table IV). The smaller copepods (*M. pacifica*) had consistently higher respiration rates than predicted, while the carnivorous chaetognaths consistently had lower than predicted respiration rate (Fig. 5A). Although the taxonomic copepod rate was the best for the full dataset, it was not always the best choice when considering each taxon independently (Supplementary Table I). Estimates of DOC release based on mass alone had less predictive power than estimates based on measured respiration rate (Fig. 5B; Table IV). The best model for DOC excretion resulted from using the measured respiration rates and the taxon-specific RQ. If predicting exclusively from mass the results

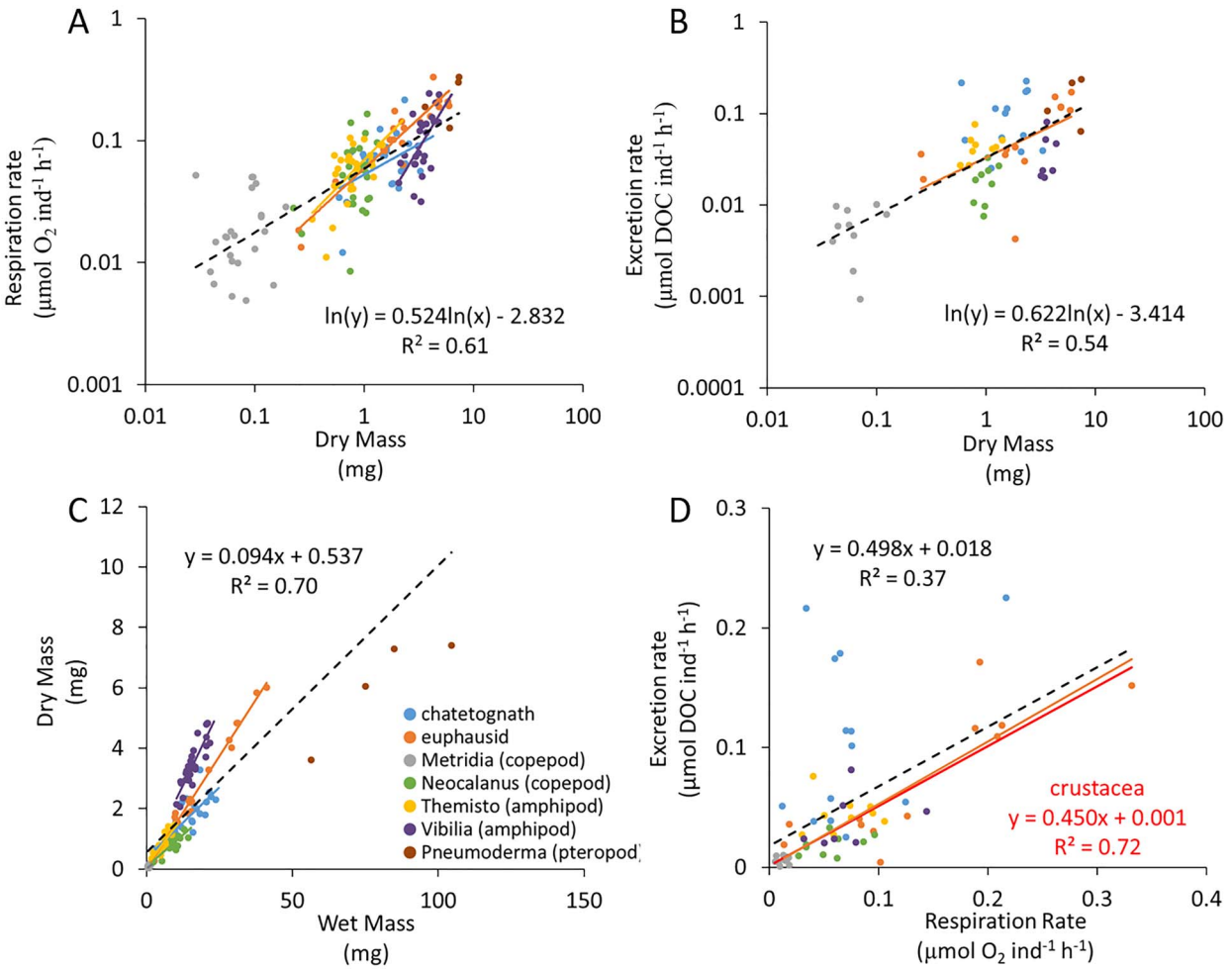

Fig. 3. Scaling relationships. DM vs. (**A**) respiration rate, (**B**) DOC excretion rate, and (**C**) WM. (**D**) Measured respiration rate (O_2) vs. excretion rate. Regressions (equation and line) for the full dataset are in black (dashed lines), while those for individual taxonomic groupings are marked by the colors in the legend (solid lines) only when they are significant ($P < 0.05$). Taxon-specific relationships are reported in Table III. All data have been converted to a single average experimental temperature (5.7°C) using a $Q_{10} = 2$.

were less conclusive. Use of the taxonomic rate model was the best predictor of DOC excretion (based on % error) as it underestimated the DOC by ~12%. The allometric equation for DOC excretion generated by this study had the lowest MSE, but the lowest R^2 and overestimated DOC production by 55%.

DISCUSSION

The objective of this analysis was to determine how detailed the application of taxon-specific and allometric-derived metabolism must be to accurately predict the metabolism of diel vertical migratory species at Station P. The results provide a reasonable compromise between specificity and feasibility as we seek to use our species- and site-specific metabolic rate measurements to estimate community physiological rates. Our study explicitly sought to study the physiology of organisms above the oxygen saturation state at which their physiology is influenced by oxygen availability (their pCrit). At our site acoustic datasets suggest that migrators are found in a number of bands from 50 to 300 m (as well as deeper), where they are able to reach 0.001 PAR (Fig. 1; Omand *et al.*, in review), and would still experience oxygen concentrations above 100 µmol. Based on our respiration experiments, there is no reason to think that they would reach their pCrit within the range of oxygen saturation states they encounter. Thus, the measured rates and the associated predictive allometric equations presented here are appropriate for scaling to active flux in the region. In addition, the findings provide a quantitative estimate of how much the chosen models deviate from direct measurements within the system,

Table III: Measured taxon-specific allometric relationships

	Taxonomic group	*n*	Value (standard error)	R^2	*P*
DM = *f*(WM) (linear)	*Themisto* (amphipod)	27	DM = 0.132(0.030)WM + 0.101(0.174)	0.76	<0.001
	Vibilia (amphipod)	26	DM = 0.197(0.047)WM + 0.315(0.749)	0.76	<0.001
	Chaetognath	23	DM = 0.950(0.029)WM + 0.343(0.424)	0.67	<0.001
	Metridia (copepod)	23	DM = 0.650(0.054)WM + 0.260(0.051)	0.23	0.022
	Neocalanus (copepod)	25	DM = 0.085(0.027)WM + 0.068(0.257)	0.65	<0.001
	Euphausiid	20	DM = 0.150(0.008)WM + 0.005(0.176)	0.99	<0.001
	Pneumoderma (pteropod)	4	DM = 0.080(0.108)WM − 0.366(8.899)	0.84	0.086
	All	150	DM = 0.094(0.010)WM + 0.537(0.184)	0.70	<0.001
O_2resp = *f*(DM) (power)	*Themisto* (amphipod)	27	$\ln(O_2) = 0.917(0.485)\ln(DM) - 2.668(0.212)$	0.38	<0.001
	Vibilia (amphipod)	26	$\ln(O_2) = 1.474(0.857)\ln(DM) - 4.149(1.052)$	0.34	0.002
	Chaetognath	23	$\ln(O_2) = 0.498(0.452)\ln(DM) - 2.944(0.273)$	0.18	0.032
	Metridia (copepod)	23	$\ln(O_2) = 0.306(0.709)\ln(DM) - 3.303(1.862)$	0.04	0.379
	Neocalanus (copepod)	25	$\ln(O_2) = 0.638(0.673)\ln(DM) - 2.891(0.300)$	0.14	0.062
	Euphausiid	20	$\ln(O_2) = 0.823(0.156)\ln(DM) - 2.833\ (0.175)$	0.87	<0.001
	Pneumoderma (pteropod)	4	$\ln(O_2) = 0.656(3.507)\ln(DM) - 2.671(6.284)$	0.24	0.505
	All	150	$\ln(O_2) = 0.524(0.068)\ln(DM) - 2.832(0.089)$	0.61	<0.001
DOCex = *f*(DM) (power)	*Themisto* (amphipod)	9	$\ln(DOC) = 0.401(0.894)\ln(DM) - 3.097(0.282)$	0.138	0.324
	Vibilia (amphipod)	7	$\ln(DOC) = 1.504(5.172)\ln(DM) - 5.322(6.651)$	0.100	0.489
	Chaetognath	13	$\ln(DOC) = -0.006(0.945)\ln(DM) - 2.462(0.626)$	0	0.989
	Metridia (copepod)	10	$\ln(DOC) = 0.130(1.712)\ln(DM) - 4.954(4.822)$	0.004	0.866
	Neocalanus (copepod)	9	$\ln(DOC) = 1.375\ (2.450)\ln(DM) - 4.047(0.384)$	0.202	0.225
	Euphausiid	12	$\ln(DOC) = 0.574(0.557)\ln(DM) - 3.407(0.697)$	0.350	0.044
	Pneumoderma (pteropod)	4	$\ln(DOC) = 0.294(5.548)\ln(DM) - 2.506(9.944)$	0.025	0.841
	All	64	$\ln(DOC) = 0.622(0.146)\ln(DM) - 3.415(0.207)$	0.54	<0.001
DOCex = *f*(O_2res) (linear)	*Themisto* (amphipod)	9	$DOC = -0.013(0.453)O_2 + 0.046(0.037)$	0	0.954
	Vibilia (amphipod)	7	$DOC = 0.227(0.697)O_2 + 0.022(0.056)$	0.123	0.441
	Chaetognath	13	$DOC = 0.539(0.863)O_2 + 0.067(0.076)$	0.147	0.197
	Metridia (copepod)	10	$DOC = 0.042(0.546)O_2 + 0.006(0.007)$	0.004	0.864
	Neocalanus (copepod)	9	$DOC = 0.163(0.289)O_2 + 0.010(0.018)$	0.202	0.225
	Euphausiid	12	$DOC = 0.522(0.232)O_2 + 0.001(0.038)$	0.72	<0.001
	Pneumoderma (pteropod)	4	$DOC = -0.078(2.668)O_2 + 0.175(0.669)$	0.007	0.912
	All	64	$DOC = 0.498(0.165)O_2 + 0.018(0.018)$	0.370	<0.001

Notes: The sample size (*n*) are reported for respiratory O_2 (μmol ind^{-1} h^{-1}; O_2resp) and excretory DOC (μmol ind^{-1} h^{-1}; DOCex) comparisons. The correlation between DM (mg) and WM (mg) are also shown. The coefficients are denoted as value (95% confidence interval). The R^2 of the correlation and *P*-value for each relationship are also shown. All data for these regressions have been converted to a single average experimental temperature (5.7°C) using a $Q_{10} = 2$.

and which taxonomic groups' metabolic rates are best and most poorly predicted by the models. By applying these models to community composition data we could create an uncertainty envelope for regional active flux estimates, one of the improvements recommended for estimates of zooplankton respiration in future studies (Burd *et al.*, 2010).

Respiration

Allometric relationships provided a reasonable, accurate prediction of the experimentally-measured respiration rates in our region. The best model for the full dataset, the taxonomic estimate using copepod-specific scaling coefficients, underestimates respiration on average by ∼10%. Although application of a single non-taxonomically resolved equation was best for the full dataset, it was not always the best equation when considering each taxon independently. This suggests that estimates may be improved by applying variable taxon-specific equations as long as the same choice of equation is not required for each taxonomic group. It is important to note that many of the organisms used in the Ikeda (2014b) meta-analyses (which established the coefficients for these equations) were held for some period in captivity for gut clearance and acclimation. The individuals in our experiments were transferred within 1 h from capture into the experimental chambers to minimize captivity effects and to most closely approximate the respiration and excretion rates of these diel vertical migrators at their daytime resident depths. Thus, differences in metabolic rates due to specific dynamic action (metabolic cost of feeding and digestion; Svetlichny and Hubareva, 2005; Secor, 2009) may contribute to this deviation from the predicted rates. Our approach, which captures the metabolic rate at the start of the decline in metabolism

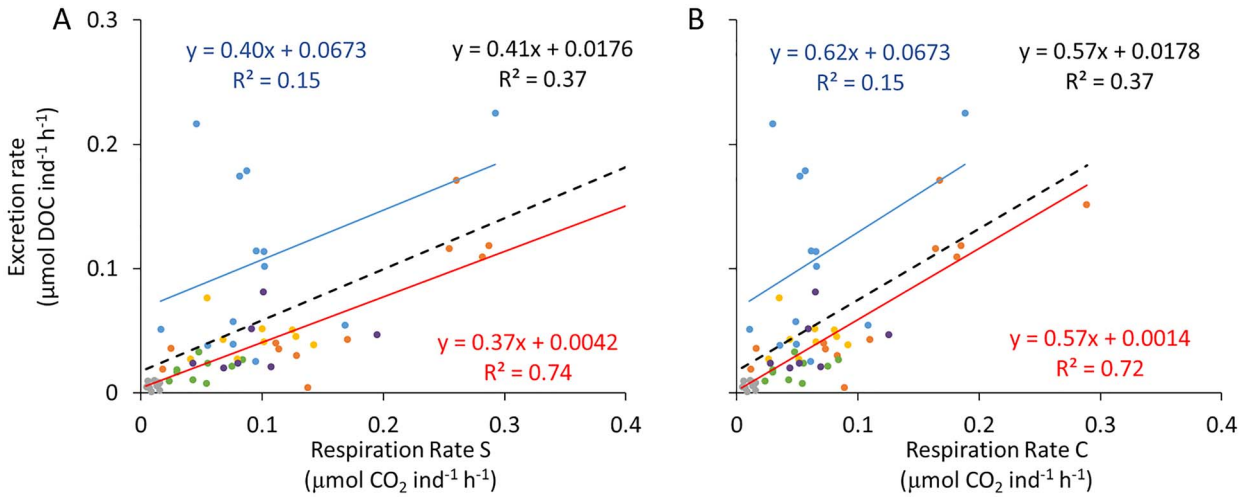

Fig. 4. Analysis of respiratory CO_2 to DOC excretion. The respiratory CO_2 production was estimated using either (**A**) species-specific RQ (S; from Table I), or (**B**) using a standard copepod RQ of 0.87, and then compared with the measured DOC excretion rate. Chaetognaths (blue) and crustacea (red) had distinctly different excretion patterns, causing the aggregate regression (dashed black) to inaccurately represent either group.

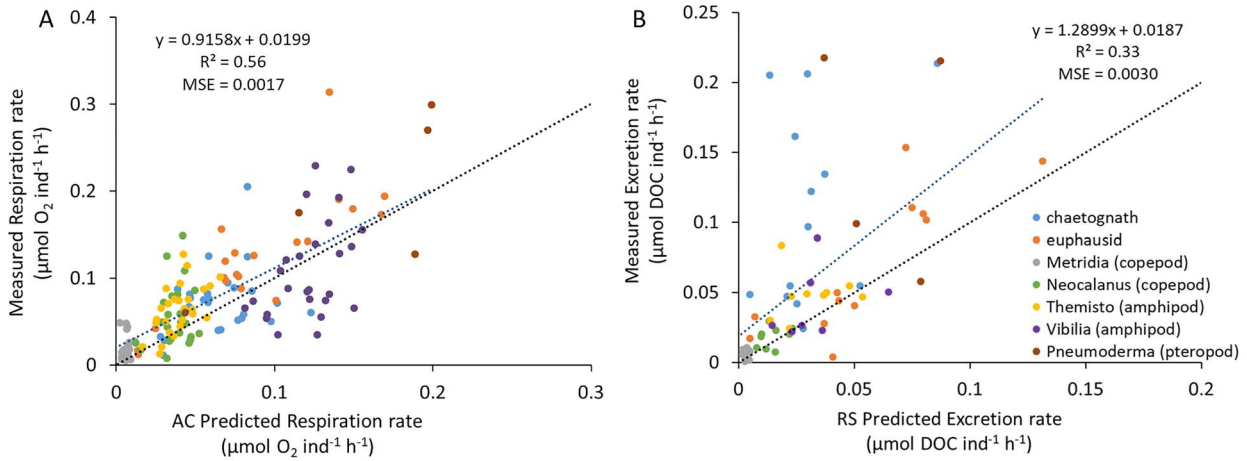

Fig. 5. Analysis of allometric relationships. The measured versus predicted (**A**) respiration rates, and (**B**) excretion rates based on the method that resulted in the regression (dotted blue line) with the highest R^2 and lowest MSE. Points that fall above the 1:1 (dotted black line) suggest higher metabolism than predicted from allometric estimates.

that is evident in filtered water in captivity (Maas *et al.*, 2018a), is likely more representative of the field conditions for migratory organisms.

The presence of waste products (POC and DOC) in the chambers could increase respiration rates of bacteria in the chambers, as both sources of waste stimulate microbial activity (Köster *et al.*, 2014; Maas *et al.*, 2020), but we posit this was a minor source of error in our study. First, incubation times were limited to 12 h to reduce conflation of microbial and zooplankton respiration. Second, potential microbial activity using direct measurements of microbial respiration on fecal pellets (Shek and Liu, 2010; Köster and Paffenhöfer, 2013) is low. Applying the maximum rates reported in these studies (329 pmol O_2 pellet^{-1} h^{-1}) to the number of pellets we observed in a chamber, and correcting for our experimental temperatures using a $Q_{10} = 2$, we calculate microbes would on average increase oxygen consumption by only 0.5% h^{-1} when fecal pellets were present. Similar direct measurements of microbial metabolism from zooplankton DOC production in our experiments are unavailable, although Steinberg *et al.* (2000) found no significant bacterial growth in incubations with and without migrators in analogous experiments conducted at higher temperature. Even if microbial growth on DOC were twice as fast as growth on POC this would still result in < 2% of the observed difference between measured O_2 consumption and predicted values.

Table IV: Efficacy of different prediction methods for respiratory O_2 consumption and DOC excretion

Prediction calculation	Equation	R^2	MSE	Avg. error
O_2 AC	$y = 1.044x + 0.018$ ($y = 1.502x + 0.018$)	0.56	0.0032	−4.07% (−41.71)
O_2 AS	$y = 0.584x + 0.033$ ($y = 0.840x + 0.033$)	0.49	0.0026	−16.14% (−27.66)
O_2 TC*	$y = 0.916x + 0.020$	0.56	0.0018	−8.75%
O_2 TS	$y = 0.510x + 0.029$	0.48	0.0038	33.24%
DOC RC	$y = 1.690x + 0.017$	0.33	0.0035	−28.10%
DOC RS*	$y = 1.182x + 0.018$	0.32	0.0028	−4.92%
DOC AC	$y = 2.289x + 0.014$	0.28	0.0041	−44.58%
DOC AS	$y = 1.466x + 0.017$	0.24*	0.0034	−23.70%
DOC TC	$y = 1.754x + 0.018$	0.24	0.0039	−36.45%
DOC TS*	$y = 1.092x + 0.022$	0.20	0.0032	−11.51%*
DOC M*	$y = 1.135x + 0.008$	0.22	0.0026*	54.62%

Notes: The linear regression relating the measured respiration/excretion value (y) to the predicted (x), as well as the R^2, mean squared error (MSE) and average % error of the relationships. Relationships were derived based on aggregate (A) or taxonomic (T) models and using either copepod (C) or taxon-specific (S) coefficients (See Table I). We tested two depth factors for the Ikeda (2014b) meta-analysis of respiration (AC and AS) using either a depth factor of 1 m (results in plain text) or 25 m (results in parentheses). For DOC excretion an additional analysis using the measured respiration rate (R) and either C or S respiratory coefficients is shown, as well as a test of the measured allometric rate from this study (M). The best model is demarcated by an *. A negative value indicates that measured value in the experiments was higher than would have been predicted by the model. Predicative ability of the models for each specific taxonomic group is reported in Supplementary Tables I and II.

Additionally, although the Ikeda (2014b) model aims to test the effect of depth on metabolism, the datasets from which they draw are skewed toward euphotic zone species. Furthermore, by conflating depth of capture or average depth of distribution with the evolutionary pressures that modify physiology, which are posited to be related to mode of life or visual predation pressure (Childress, 1995; Thuesen *et al.*, 1998; Seibel and Drazen, 2007), this depth approach lumps migratory species with endemic mesopelagic organisms. All of the organisms in this study were chosen for their migratory behavior, and spend more than half of their time in the mesopelagic zone. Our results suggest that the addition of depth as a variable reduces the predictive power of the aggregate equation, causing it to further underestimate the metabolic rates of vertical migrators in this region.

Excretion of DOC

DOC is a significant proportion of the carbon excreted by migrators in our study. Our DOC excreted: respiratory CO_2 ratio average of 0.37 for crustacea (copepods, euphausiids and amphipods) closely aligns with that measured (0.31) by Steinberg *et al.* (2000) for a predominantly crustacean dataset of vertically migrating zooplankton in the Sargasso Sea. A recent study of pteropod metabolism near the Antarctic Peninsula similarly reported DOC excretion: respiratory CO_2 ratios of 0.32 and 0.30 for two species of thecosome pteropods (assuming a RQ of 0.94; Thibodeau *et al.*, 2020). Together these data support our approach of applying the DOC excretion: respiratory CO_2 ratio of 0.31 generated by Sargasso Sea experiments across a broad set of oceanic ecosystems (Al-Mutairi and Landry, 2001; Steinberg *et al.*, 2008; Hernández-León *et al.*, 2019; Kiko *et al.*, 2020; Kwong *et al.*, 2020). It is important to note that our analyses, as well as those of Thibodeau *et al.* (2020), were derived from rates of O_2 consumption that were converted to CO_2 with a taxon-specific RQ. In our dataset the use of a non-taxon-specific RQ of 0.87 resulted in a much higher DOC excreted: respiratory CO_2 ratio of 0.57. Since no DIC measurements were made during our experiments, these conversion factors could not be thoroughly tested in our study and remain a source of uncertainty.

Additional sources of uncertainty in our measurements are the leaching of DOC from fecal pellets and the consumption of DOC by microbes during the incubations. Although some studies document negligible DOC leaching from fecal pellets (Steinberg *et al.*, 2000; Saba *et al.*, 2011), others suggest as much as 20% of fecal pellet C becomes dissolved within the first hour (Møller *et al.*, 2003). Using our calculated carbon per gut load this results in an estimated leaching of 1 ng of C h^{-1} as DOC, equivalent to 0.1–2.9% of the taxa-specific average DOC excretion rate, indicating this was a minor source of error. There are no good estimates of the rates of DOC uptake by microbes at this site, but the same experimental protocols that were designed to reduce microbial respiration, i.e. the use of filtered seawater and a 12-h incubation period, would similarly result in low levels of microbial DOC uptake. Despite these inherent uncertainties, our findings increase confidence in the application of a 0.31 ratio of DOC excreted: respiratory CO_2 using a taxon-specific RQ, as application of this value resulted in only a

~5% underestimation using the respiration-based taxon-specific model. This approach does, however, require future field or modeling analyses to have experimental measurements of the metabolic rates of organisms to attain a similar level of accuracy. Models based exclusively on biomass resulted at best in a 12% underestimation of the observed DOC excretion rate (the ES model). This lower predictive power for DOC excretion rate based on biomass alone may be due to the differences in temperature and scaling coefficients between respiration and DOC metabolic waste pathways. Although the experimentally-derived allometric relationship between DM and DOC excretion rate resulted in the lowest MSE, it overestimated observed DOC excretion rate by 55%.

Our analyses indicate that taxon-specific coefficients improve predictive power in models of DOC production. This taxonomic variability was suggested previously, with the ratio between DOC excretion and respiratory CO_2 production ranging from 5 to 72% based on taxon and temperature changes in experiments at multiple temperatures with pooled organisms studied in the Sargasso Sea (Steinberg *et al.*, 2000). Taxonomic patterns observed for our study region include the consistently lower production of DOC by the *Neocalanus*, which were actively preparing to diapause, and the higher production by carnivorous taxa like *Themisto* sp. amphipods and chaetognaths. These findings suggest that trophic level or feeding state may contribute to DOC excretion allometric patterns. To determine the factors driving the observed variation, and to provide data in support of DOC excretion prediction based entirely upon biomass, taxonomically diverse DOC excretion studies are a high research priority. Predictive estimates of zooplankton DOC release are important, as they constitute a substantial portion of the active flux pathway. Furthermore, recent work demonstrates that this component of active flux can provide important dissolved organic compounds, including vitamins, to midwater prokaryotic organisms, influencing the biodiversity, abundance and metabolism of the mesopelagic biota (Clifford *et al.*, 2017; Valdés *et al.*, 2017; Calleja *et al.*, 2018; Maas *et al.*, 2020). As a consequence, refining our estimates of DOC excretion will be important for future investigations of metabolic processes in the ocean's twilight zone.

Scaling to field data

This analysis suggests that taxon-specific allometric equations do not outperform copepod-based estimates of community respiration for the dominant migratory species near Station P. This means that even coarse taxonomic identification of the community is not required to make good estimates of respiratory active flux. It does suggest that size-based analyses of the community may become increasingly useful as optically-derived datasets (e.g. Zooscan: Kiko and Hauss, 2019), with their associated organismal size output, allow for wider application of allometric equations. We note that there is substantially less metabolic data from non-crustacean (i.e. gelatinous) taxa, potentially leading to incorrect allometric, RQ and temperature coefficients for these groups. Additionally, most of the metabolic experiments presented here were conducted with robust crustacean taxa and thus our conclusions may not be applicable to physiology of the whole community, which would include gelatinous species such as the abundant migratory salps at our site (Steinberg *et al.*, in prep). In regions or seasons where non-crustacean organisms dominate the migratory community, taxonomic differences may be more important. Similar validation of alternative methods, including the electron transport system (ETS) enzyme assays, could provide alternative methods to verify active transport (Hernández-León *et al.*, 2019). Continued efforts to identify a unified and robust approach to calculating active flux will be useful as additional datasets are applied to inform biogeochemical modeling of carbon cycling.

CONCLUSION

Our study uses field-based measurements of respiration (O_2 consumption) and DOC excretion, to explore the efficacy of current allometric methods for estimating zooplankton metabolism in the northeast subarctic Pacific. Our results suggest that current methods for estimating respiratory active flux are effective using non-taxon-specific allometric equations, resulting in a 10% underestimation of respiratory flux. The best equation is not, however, the same among taxon groupings. This suggests that taxonomy is still worth considering and incorporating into future analyses when possible. Using the standard 0.31 DOC excretion: respiratory CO_2 ratio based on biomass data results in a 12% underestimation of DOC excretion, and appears to be improved by inclusion of allometric and taxon-specific information. It is important to acknowledge, however, that most of our experiments were run with crustacea, and further validation with other phyla is warranted. Our DOC excretion dataset, which is one of the largest and most taxonomically diverse in the literature, provides an allometric relationship that is closely in line with prior DOC production measurements but emphasizes the need for greater taxonomic verification. This validation approach will become increasingly valuable as new imaging systems become more widely employed and community biomass-based estimates are

replaced or augmented by individual biovolume estimates with greater taxonomic resolution.

SUPPLEMENTARY DATA

Supplementary data can be found at *Journal of Plankton Research* online.

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DATA AVAILABILITY

All metabolic data is available in Supplementary Table III and Seabass under the DOI: 10.5067/SeaBASS/EXPORTS/DATA001 stored in the EXPORTS archive: https://seabass.gsfc.nasa.gov/archive/BIOS/Maas/EXPORTS/EXPORTSNP/archive/EXPORTS-EXPORTSNP_zooplankton_active_flux_experiments_process_R2.sb

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