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Respiration of mesopelagic fish: a comparison of respiratory electron transport system (ETS) measurements and allometrically calculated rates in the Southern Ocean and Benguela Current

Anna Belcher [1]*, Kathryn Cook[2], Daniel Bondyale-Juez[3], Gabriele Stowasser[1], Sophie Fielding[1], Ryan A. Saunders[1], Daniel J. Mayor[2], and Geraint A. Tarling [1]

[1]*Ecosystems, British Antarctic Survey, Cambridge CB3 0ET, UK*
[2]*Ocean Biogeochemistry and Ecosystems, National Oceanography Centre, Southampton SO14 3ZH, UK*
[3]*Las Palmas de Gran Canaria, EOMAR, Universidad de Las Palmas de Gran Canaria, Spain*

**Corresponding author: tel: 01223 221400; e-mail: annbel@bas.ac.uk.*

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Mesopelagic fish are an important component of marine ecosystems, and their contribution to marine biogeochemical cycles is becoming increasingly recognized. However, major uncertainties remain in the rates at which they remineralize organic matter. We present respiration rate estimates of mesopelagic fish from two oceanographically contrasting regions: the Scotia Sea and the Benguela Current. Respiration rates were estimated by measuring the enzyme activities of the electron transport system. Regression analysis of respiration with wet mass highlights regional and inter-specific differences. The mean respiration rates of all mesopelagic fish sampled were 593.6 and 354.9 µl O_2 individual^{-1} h^{-1} in the Scotia Sea and Benguela Current, respectively. Global allometric models performed poorly in colder regions compared with our observations, underestimating respiratory flux in the Scotia Sea by 67–88%. This may reflect that most data used to fit such models are derived from temperate and subtropical regions. We recommend caution when applying globally derived allometric models to regional data, particularly in cold (<5°C) temperature environments where empirical data are limited. More mesopelagic fish respiration rate measurements are required, particularly in polar regions, to increase the accuracy with which we can assess their importance in marine biogeochemical cycles.

Keywords: allometric, Benguela Current, ETS, mesopelagic fish, respiration, respiratory flux, Scotia Sea, Southern Ocean.

Introduction

The uptake of carbon dioxide (CO_2) by the ocean through the biological carbon pump plays an important role in the partitioning of CO_2 between the atmosphere and ocean (Kwon *et al.*, 2009). Understanding and quantifying the processes controlling the efficiency of this pump are therefore vital for predictions of future climate. Carbon may be transported by passive sinking (the gravitational pump), physical mixing, or active transport through vertical migration of metazoans (Boyd *et al.*, 2019). Previously, the gravitational pump was thought to be the dominant mechanism for transferring organic carbon to the deep sea. However, the importance of additional mechanisms, in particular the role of the

migrating mesopelagic community, has been recognized more recently (Bianchi *et al.*, 2013; Jónasdóttir *et al.*, 2015; Anderson *et al.*, 2019; Boyd *et al.*, 2019; Pakhomov *et al.*, 2019). Through diel (and seasonal) vertical migrations, organic carbon ingested in the epipelagic layer can be released in the mesopelagic layer through excretion, respiration, egestion and mortality (Longhurst *et al.*, 1990; Zhang and Dam, 1997; Steinberg *et al.*, 2000; Turner, 2002; Jónasdóttir *et al.*, 2015; Steinberg and Landry, 2017).

Acoustic estimates of mesopelagic fish biomass suggest that the global biomass of mesopelagic fish could be up to 15 gigatonnes (Gt) (Irigoien *et al.*, 2014), dramatically higher than trawl-based estimates of 1 Gt (Gjøsaeter and Kawaguchi, 1980). Model estimates are wide ranging (1.8–15.9 Gt) with the most likely model scenarios suggesting 2–9 Gt (Anderson *et al.*, 2019; Proud *et al.*, 2019). As a result of their substantial global biomass, combined with large diel vertical migrations (Klevjer *et al.*, 2016), mesopelagic fish can make a significant contribution to the biological carbon pump. Indeed, estimates of fish-mediated export (primarily estimates of respiratory flux, i.e. carbon respired at depth by the migrating community) can be up to 55% of the gravitational particulate organic carbon (POC) flux (Hidaka *et al.*, 2001; Davison *et al.*, 2013; Hudson *et al.*, 2014; Ariza *et al.*, 2015; Belcher *et al.*, 2019). The fish-driven respiratory flux in the mesopelagic layer is, therefore, an important component of ocean carbon budgets, yet measurements of mesopelagic respiration are difficult.

Obtaining live, heathy individuals from the mesopelagic through traditional net sampling techniques is not easy, resulting in a limited ability to incubate them under stress-free conditions and to obtain meaningful respiration rates. Previous studies (Hidaka *et al.*, 2001; Hudson *et al.*, 2014; Belcher *et al.*, 2019) attempting to quantify fish-driven respiratory fluxes have made use of allometric relationships to estimate respiration from more easily measurable parameters such as depth, temperature and biomass. Ikeda (2016) compiled global incubation measurements of the respiration of pelagic marine fishes, while Belcher *et al.* (2019) focused on myctophid fishes that included both incubation experiments and respiration rate estimates from measurement of the enzyme activity of the electron transport system (ETS). However, only 36% and 47% of the collated data from Ikeda (2016) and Belcher *et al.* (2019), respectively, were obtained from fish whose habitat depth is deeper than 50 m, meaning that the mesopelagic fish community was poorly represented in the relationships they derived. Clarke and Johnston (1999) compiled data on metabolic rates for a wide range of teleost fish and examined the effects of body mass and temperature, although the depth at which fish were sampled was not included.

The ETS method is currently the only method that can be used to estimate the respiration of fish sampled from the mesopelagic given our inability to catch and maintain them in a state where reliable respiration rates can be measured directly. For this method, nets are used to collect organisms from the mesopelagic layer and individuals are frozen immediately in liquid nitrogen. This allows measurement of the *in situ* enzyme activity as it is stable during the sampling process (Gómez *et al.*, 1996). Oxygen consumption occurs at the end of the ETS through the reduction of oxygen to water. The enzyme activity of the ETS can therefore be used to measure the potential respiration based on the respiratory capacity of the ETS. Despite not being sensitive to the short-term net sampling stress and avoiding the need for incubation, the ETS method is a measure of the maximum potential respiration and, thus, the ratio between respiration and ETS activity (*R*:ETS) must be known.

Considering the inherent difficulties of measurement, it is unsurprising that there is a lack of data on the respiration rates of mesopelagic fish (Ikeda, 2016). The empirically derived allometric relationships of Ikeda (2016) and Belcher *et al.* (2019) are global compilations of the available data, which are primarily from temperate and subtropical regions with little data from low temperature (<5°C) regions. As yet, there has not been sufficient data to validate these allometric methods in a robust way. In this study, we present estimates of respiration of mesopelagic fish derived from ETS activities. Our study focused on two oceanographically contrasting regions of the Atlantic, a low temperature, high productivity region of the Southern Ocean, the Scotia Sea, and a subtropical, high productivity region, the Benguela Current. We compare our ETS-derived respiration estimates with allometrically based estimates to assess their validity in these regions.

Methods

Data for this study were collected aboard the *RRS Discovery* during research cruises DY086 to the Scotia Sea in the Southern Ocean (12 November 2017–19 December 2017) and DY090 to the Benguela Current region offshore of Namibia (23 May 2018–28 June 2018), both in the Atlantic sector. During the Scotia Sea cruise, data were collected at station P3 (52.40°S, 40.06°W) to the northwest of South Georgia, and during the Benguela Current cruise, data were collected at station BN1 (18.00°S, 11.00°E). Vertical profiles of the water column at each site were made using a conductivity–temperature–depth unit (SBE 9 plus), which were used to determine *in situ* water temperatures and water mass properties.

Net deployments and sample processing

Mesopelagic fish samples were obtained using an opening and closing 25 m^2 rectangular mid-water trawl net (RMT25, minimum 4 mm mesh; Baker *et al.*, 1973; Piatkowski *et al.*, 1994). This system consists of two nets, which can be opened and closed on command to sample specific depth horizons. Nets were deployed at 0–125, 125–250, 250–500 and 500–750 m in the Benguela Current, and at 0–250 and 250–500 m in the Scotia Sea. These deployment depths were based on the observed oceanographic structure at each study site. Nets were towed obliquely at two knots, for 20–50 min at each depth layer, and were repeated during the day and night. Once aboard, all fish caught were sorted, identified to the lowest taxonomic level possible and the composite wet mass (WM) measured to the nearest 0.01 g using a motion-compensated balance. Representative subsamples from each of the numerically dominant fish species were taken for subsequent measurement of ETS activity in the laboratory (see below). These samples were immediately flash frozen in liquid nitrogen before storage at −80°C.

Respiration measurements—ETS activity

Frozen whole fish samples were reweighed for WM in the laboratory, and a subsample was taken from each individual (and weighed) for ETS analysis. The ETS activity was measured kinetically following the method of Owens and King (1975) with modifications from Gómez *et al.* (1996). Each subsample was homogenized in a phosphate buffer using an electric homogenizer for 45–60 s. Homogenates were then centrifuged for 10 min at

4000 rpm at 0°C. 100 µL of subsample of the homogenate was mixed with 300 µl of reaction buffer (0.1 M, pH 8.5) containing substrates NADH and NADPH (saturating concentrations of 1.7 and 0.25 mM, respectively) in a 1-cm path length cuvette. All procedures were carried out on ice. 100 µL tetrazolium chloride dye, INT (2-*p*-iodophenyl-3-*p*-nitrophenyl monotetrazolium chloride, 4 mM) was added to each cuvette, and the reaction was measured continuously for 8 min at 490 nm in a Cary 60 UV–Vis spectrophotometer (Packard and Christensen, 2004). The temperature of the reaction was controlled at 11.5–12°C. In addition, for each sample, a blank assay was performed without the ETS substrates to account for the contribution of the non-enzymatic reduction of INT (Maldonado *et al.*, 2012). Reagent blanks were also taken daily. In the kinetic assay, the INT replaces oxygen as the electron acceptor for the ETS, accepting two electrons where oxygen would accept four. The rate of formazan produced in the reduction of INT is therefore related to the oxygen consumption by a factor of two. The potential respiration rate (Φ, µmol O_2 h^{-1}) was calculated from the formazan production rate following Packard and Christensen (2004) using our measured INT extinction coefficient (at 490 nm) of 16.4 mM^{-1} cm^{-1}. Respiration (R) at the experimental temperature of 12°C was then estimated using a conservative R:ETS ratio of 0.5 based on measurements made on fish (Ikeda, 1989) and values in the literature for marine zooplankton (Hernández-León and Gómez, 1996, see Discussion section for detail on range in R:ETS ratios). Total respiration rates per individual (R_{IND_12}) at the experimental temperature of 12°C were calculated based on the wet weight of the subsample and total weight of the individual. In addition, we corrected respiration estimates to *in situ* temperature (ranging from 2 to 16°C) using the Arrhenius equation and an activation energy of 15 kcal mol^{-1} (Packard *et al.*, 1975; Ariza *et al.*, 2015; Hernández-León *et al.*, 2019), giving R_{IND_INSITU} for respiration rates per individual. These ETS-derived respiration estimates are a measure of routine respiration [i.e. between zero (resting) and maximum activity levels]. We define our ETS-derived respiration datasets as "Scotia Sea" and "Benguela", for our respective study sites. Throughout the manuscript, where respiration rates refer to rates at 12°C, we used the subscript "12", and where rates are for *in situ* temperatures, we use the subscript "INSITU".

Protein measurements

As an additional determinant of biomass, the protein concentrations of the homogenates used for ETS analysis were estimated according to the method of Lowry *et al.* (1951), with modifications as described by Rutter (1967). Calibration curves were made from standard solutions of bovine serum albumin. Total protein per individual was estimated using the ratio of total WM to the WM of the subsample used for ETS analysis.

Allometrically estimated respiration

Respiration rates were also estimated based on the empirical relationships defined for pelagic marine fishes by Ikeda (2016) and for myctophid fishes by Belcher *et al.* (2019). These are based on data compilations of field studies, with Ikeda (2016) utilizing incubation-derived measurements (in the absence of food) of routine respiration rates and Belcher *et al.* (2019) utilizing both incubation- and ETS-derived measurements. We apply the regression from Ikeda (2016) (their model 1, herein referred to as Ikeda2016$_{_INSITU}$) and the regression from Belcher *et al.* (2019) (herein referred to as Belcher2019$_{_INSITU}$) to the WM of each individual fish measured at our Benguela and Scotia Sea study sites and compare these predicted respiration rates with our ETS-based respiration measurements to assess the applicability of these two regressions to our study regions.

Ikeda2016$_{_INSITU}$ regression:

$$\mathrm{Ln}(R_{\mathrm{IND}_{\mathrm{INSITU}}}) = 19.491 + 0.885 \times \mathrm{Ln}(\mathrm{WM}) - 5.770 \times 1000/\mathrm{temp} - 0.261 \times \mathrm{Ln}(\mathrm{depth}), \quad (1)$$

where WM is the wet mass (mg) and temp is the temperature (K); depth is in metres.

Belcher2019$_{_INSITU}$ regression:

$$\mathrm{Ln}(R_{\mathrm{IND}_{\mathrm{INSITU}}}) = -1.315 + 0.734 \times \mathrm{Ln}(\mathrm{WM}) + 0.085 \times T, \quad (2)$$

where WM is the wet mass (mg) and T is the temperature (°C).

For comparison to our measurements, we adjust these respiration rate predictions to our experimental temperature of 12°C to give (R_{IND_12}), using the Arrhenius equation and an activation energy of 15 kcal mol^{-1} (Packard *et al.*, 1975; Ariza *et al.*, 2015).

Regression analysis

Regression analyses of ETS-derived respiration data collected in our study were carried out using a regression fitting model for multiple predictors and a response, where data were continuous and no additive terms were allowed. Regression analysis was carried out for R_{IND_12} to assess the effect of WM at our experimental temperature of 12°C; Equations were of the form:

$$\mathrm{Ln}(R_{\mathrm{IND}_{12}}) = a_0 + a_1 \times \mathrm{Ln}(\mathrm{WM}), \quad (3)$$

where a_0 and a_1 are regression coefficients and WM is the wet mass (mg). With only two large depth zones in the Scotia Sea (0–250 and 250–500 m), we lack sufficient depth resolution to include this variable appropriately in the model. WM and respiration data were transformed to the natural log prior to fitting the regression. Fitting was performed using the ordinary least squares method in Minitab (version 18.1).

To compare the regressions of our study with that of Ikeda (2016) and Belcher *et al.* (2019), we recalculated the linear regressions (following the aforementioned method) from the respective datasets (adjusted to our 12°C experiment temperature) with WM only (i.e. not including depth or temperature) as a predictor. We refer to these recalculated regressions as Ikeda2016R$_{_12}$ and Belcher2019R$_{_12}$. We then investigated statistical differences between the mass scaling coefficients (for R_{IND_12}) derived from our own datasets (Benguela and Scotia Sea) and the mass scaling coefficients derived from Ikeda2016R$_{_12}$ and Belcher2019R$_{_12}$ regressions. To do so, we calculated p-values for the interaction term of WM with dataset category (i.e. Ikeda2016R$_{_12}$, Belcher2019R$_{_12}$, Scotia Sea, Benguela) to see if the regression coefficients were significantly different. In addition, we performed regression analysis (as above) for all myctophid fish (termed "All Myctophid") by collating together the ETS measurements we made on myctophid fish species in the Scotia Sea and Benguela, with Belcher2019 (both incubation and ETS data). This was done for both R_{IND_12} and R_{IND_INSITU} data.

Results

ETS-derived respiration rates

Temperatures in the upper 500 m in the Scotia Sea ranged from 0.7 to 3.5°C, compared with the Benguela region, where temperatures in the upper 750 m ranged from 4.7 to 20.6°C. In the Scotia Sea, respiration rates of *Gymnoscopelus* spp., *Electrona antarctica* and *Krefftichthys anderssoni* were measured, with WM ranging from 0.2 to 16.1 g. Respiration rates ranged from 45.8 to 1837.8 µl O_2 individual^{-1} h^{-1} (0.139–7.447 µl O_2 mg prot.$^{-1}$ h^{-1}). The mean respiration rate of mesopelagic fish measured in the Scotia Sea was 593.6 µl O_2 individual^{-1} h^{-1}. In the Benguela region, respiration rates of *Bathylagus* spp., *Melamphidae* spp., *Gymnoscopelus* spp., *Cyclothone* spp., *Nemichthyidae* spp., and *Sternoptychidae* spp. were measured, with WM ranging from 0.1 to 20.9 g. The mean respiration rate of all mesopelagic fish measured in the Benguela was 354.9 µl O_2 individual^{-1} h^{-1} and ranged from 4.1 to 5245.8 µl O_2 individual^{-1} h^{-1} (see Supplementary Material for full dataset). The relationship between respiration and mass is strongest for mass units of protein rather than WM (Figure 1). As WM is more easily measured, and to allow comparison with previous studies, we present our data in terms of units of WM (see Supplementary Figures S1–S3 for data presented in units of protein).

Respiration rates at 12°C (predicted for each individual fish from Benguela and Scotia Sea datasets) by Ikeda2016$_{_12}$ range from 20.6 to 873.1 µl O_2 individual^{-1} h^{-1} for the Scotia Sea data, with Belcher2019$_{_12}$ predicting rates of between 44.9 and 1015.14 µl O_2 individual^{-1} h^{-1}. For the Benguela region, allometrically predicted respiration rates ranged between 5.8 and 680.4 µl O_2 individual^{-1} h^{-1} based on Ikeda2016$_{_12}$ and from 18.3 to 1170.3 µl O_2 individual^{-1} h^{-1} based on Belcher2019$_{_12}$.

Comparing respiration rates predicted from Ikeda2016$_{_12}$ and Belcher2019$_{_12}$ reveals that the allometric regressions are more representative of the Benguela data (Figure 2B) than the Scotia Sea data (Figure 2A). Both the Ikeda2016$_{_12}$ and Belcher2019$_{_12}$ equations underestimate respiration compared with ETS estimates of respiration rates made by the present study in the Scotia Sea. Our measured respiration rates are up to 32 and 13 times higher than predicted values from Ikeda2016$_{_12}$ and Belcher2019$_{_12}$, respectively (medians are eight and four times greater, respectively). To assess the applicability of the Ikeda2016$_{_12}$ and Belcher2019$_{_12}$ regressions, we examine the residuals of the predicted respiration rates against our ETS-based respiration rate measurements ($R_{\text{IND\_12}}$). Plotting these residuals against the WM (Figure 3) allows us to assess if the mass exponents of the Ikeda2016$_{_12}$ and Belcher2019$_{_12}$ regressions are appropriate for our datasets. The mean residuals between Scotia Sea respiration rates and predicted rates from Ikeda2016$_{_12}$ and Belcher2019$_{_12}$ were 1210.4 and 1047.7 µl O_2 individual^{-1} h^{-1}, respectively. The mean residuals calculated for Benguela respiration rates are 305.2 and 151.9 µl O_2 individual^{-1} h^{-1} for Ikeda2016$_{_12}$ and Belcher2019$_{_12}$, respectively. All residuals highlight the greatest underestimations for fish of larger body mass.

Regression analysis

Multiple linear regression of our ETS-derived fish respiration rates ($R_{\text{IND\_12}}$) reveals that WM is a significant predictor for both the Scotia Sea and Benguela fish data ($p < 0.05$ in all cases; Table 1). The model fit for $R_{\text{IND\_12}}$ was better for the Benguela data, R^2 of 46% compared with 36% for the Scotia Sea (Table 1). The mass exponent (a_1) of the Scotia Sea is low, at 0.33, compared with the mass exponent of 0.75 for the Benguela dataset.

The mass scaling coefficients (a_1) of the recalculated regressions Ikeda2016R$_{_12}$ (0.83) and Belcher2019R$_{_12}$ (0.76) were significantly different ($p < 0.001$) from the Scotia Sea mass scaling coefficient (0.33, $p < 0.001$) for $R_{\text{IND\_12}}$. In contrast, estimates of the mass scaling coefficients of Ikeda2016R$_{_12}$ and Belcher2019R$_{_12}$ did not differ significantly from the Benguela mass scaling coefficient (0.75, $p = 0.28$ and $p = 0.93$, respectively).

When combining all myctophid data [Myctophidae from this study and from Belcher *et al.* (2019)], we calculate a mass exponent of 0.89 and an R^2 of 82% for $R_{\text{IND\_12}}$, and a mass exponent of 0.52 and an R^2 of 70% for $R_{\text{IND\_INSITU}}$ (Figure 4).

Discussion

Methodological considerations

Our results highlight that while the regressions of Ikeda2016$_{_12}$ and Belcher2019$_{_12}$ are adequate at predicting the respiration

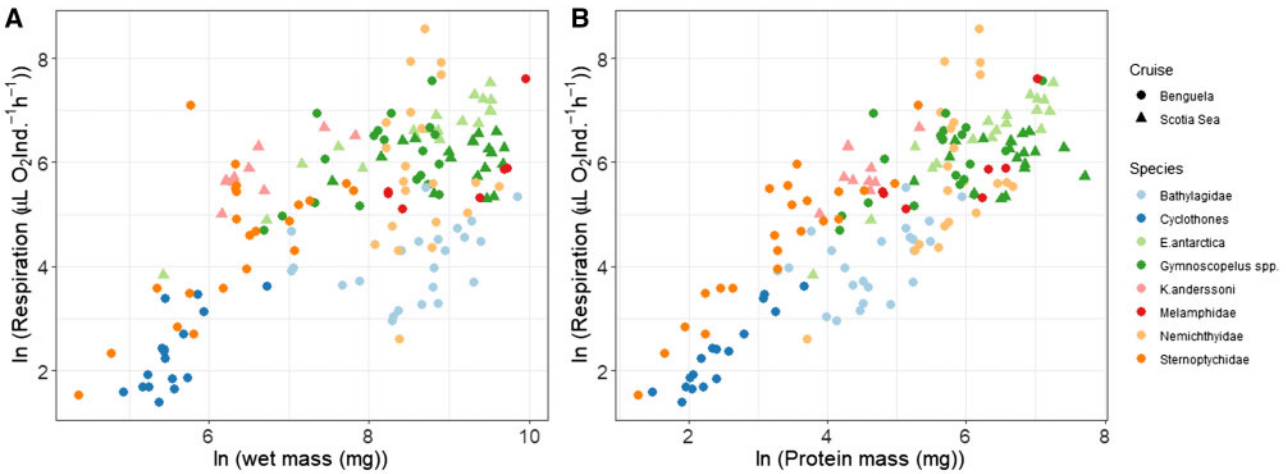

Figure 1. Respiration rates at *in situ* temperature ($R_{\text{IND\_INSITU}}$, µl O_2 individual^{-1} h^{-1}) of fish species measured at our study sites of the Benguela (circles) and the Scotia Sea (triangles) measured via ETS. Data are coloured by fish species. Respiration is plotted as a function of (A) the wet mass per individual (mg) or (B) protein mass per individual (mg). Note the natural logarithmic scale on both *x* and *y* axes.

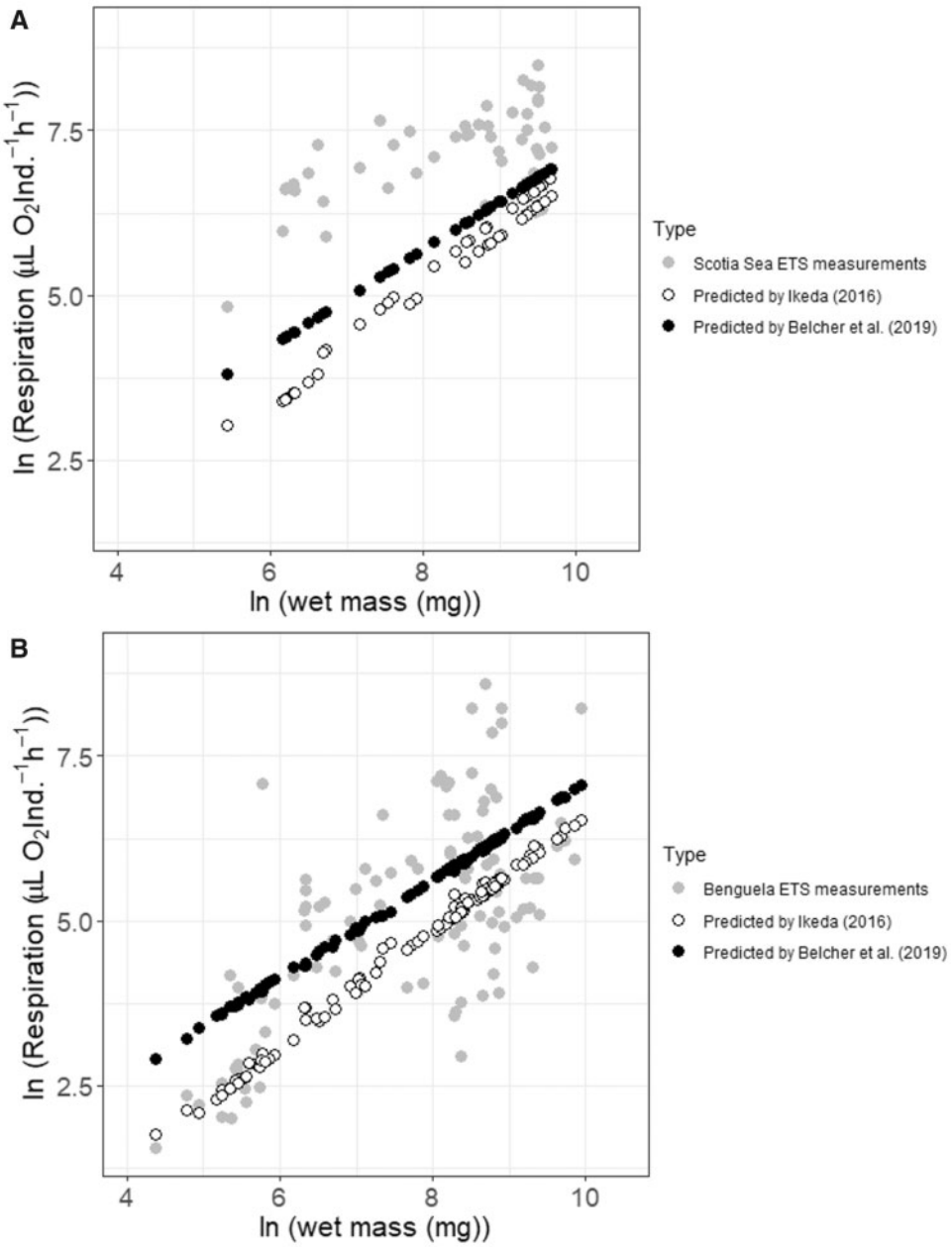

Figure 2. Comparison of respiration rates at 12°C (R_{IND_12}, µl O_2 individual^{-1} h^{-1}) calculated based on Ikeda2016$_{_12}$ (white circles) and Belcher2019$_{_12}$ (black circles) regressions and rates measured (via ETS) at our study sites (grey circles) of the (A) Scotia Sea and (B) Benguela Current. Respiration is plotted as a function of the wet mass per individual (mg). Note the natural logarithmic scale on both *x* and *y* axes.

rates of fishes from the Benguela study site (Figure 2B), both regression models substantially underestimate the respiration rates of fishes in the Scotia Sea when compared with ETS estimates of respiration (Figure 2A). As several different methods have been used in the various literature data compilations, we must first examine possible methodological differences that may contribute to the underestimation of respiration in the Scotia Sea by the regressions of Ikeda2016$_{_12}$ and Belcher2019$_{_12}$. Whereas Ikeda (2016) compile respiration rates derived from incubation experiments (by incubating net-caught fish), the Belcher2019$_{_INSITU}$ regression is based on both ETS- and incubation-derived data [Figure 1 in erratum to Belcher *et al.* (2019)]. A key difference between these two methods is the feeding state of the animals. The incubated fish were not fed, but the fish sampled for respiration via ETS are in their natural feeding state and thus gut fullness is variable. As there is an increase in energy expenditure during digestion, starved animals will have lower respiration rates than those that have just fed. Therefore, ETS-derived respiration rates could be higher than the respiration rates of starved animals, since ETS activity is less sensitive to the short-term effect of starvation (Ikeda and Skjoldal, 1980; Packard *et al.*, 1996), and thus our ETS-derived rates likely reflect the rates of fed animals. To adjust the

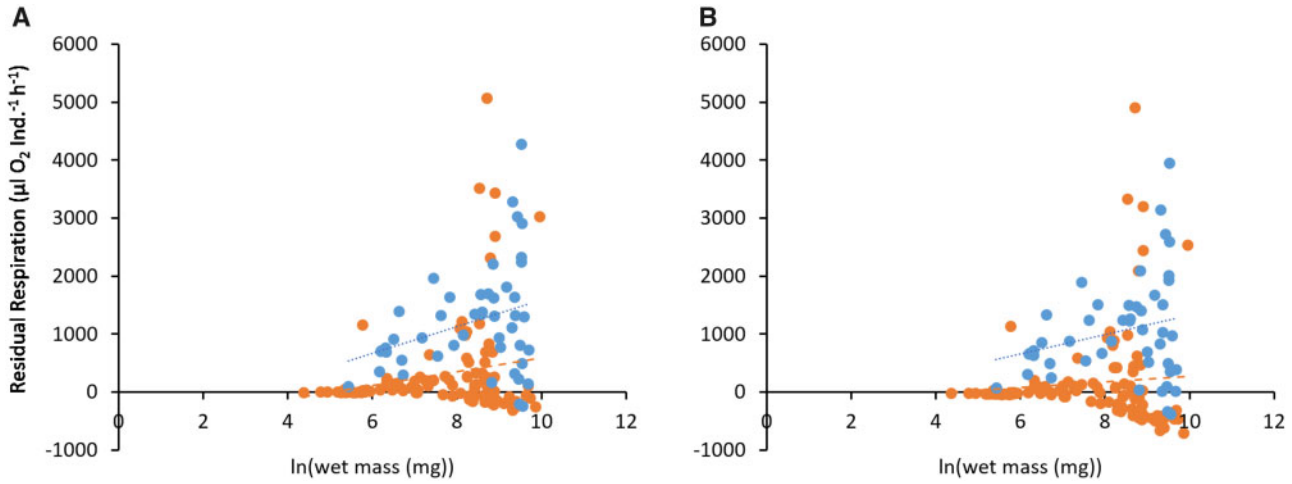

Figure 3. Calculated residuals of ETS-measured respiration (R_{IND_12}) in the Scotia Sea (blue circles) and Benguela (orange circles). Residuals are the difference between the measured data and the calculated respiration based on the regressions of (A) Ikeda2016$_{_12}$ and (B) Belcher2019$_{_12}$. Positive residuals indicate an underestimation by the regression. Linear regressions have been plotted through each data set (dashed lines).

Table 1. Regression coefficients and statistics derived from multiple regression of respiration rates R_{IND} (µl O_2 individual^{-1} h^{-1}) on WM (mg).

	a_0	a_1	a_2	a_3	n	R2 (%)	p-Values
Scotia Sea	4.359 (±0.556)*	0.334 (±0.066)*	–	–	47	36	Ln(WM) = 0.001
Benguela	−0.675 (±0.610)	0.752 (± 0.079)*	–	–	109	46	Ln(WM) = <0.001
Belcher2019$_{_INSITU}$	*−1.315 (±0.469)**	*0.734 (±0.052)**	*0.085 (±0.011)**	–	*74*	*77*	*T = <0.001*
							Ln(WM) = <0.001
Belcher2019R$_{_12}$ a	−0.469 (±0.185)*	0.760 (±0.030)*	–	–	74	90	Ln(WM) = <0.001
All Myctophid$_{_INSITU}$	−1.579 (±0.212)*	0.524 (±0.029)*	–	–	140	70	Ln(WM) = <0.001
All Myctophid$_{_12}$	−0.877 (±0.258)*	0.891 (±0.036)*	–	–	140	82	Ln(WM) = <0.001
Ikeda2016$_{_INSITU}$	*19.491 (±2.491)**	*0.885 (±0.021)**	*−5.770 (±0.752)**	*−0.261 (±0.032)**	*102*	*95*	*All <0.001*
Ikeda2016R$_{_12}$ a	*−1.133 (±0.202)**	*0.832 (±0.026)**	–	–	*102*	*91*	*Ln(WM) = <0.001*
Clarke and Johnston 1999[b]	*−5.43*	*0.80 (±0.13)*	–	–	*138*	–	–

The regression models of the Benguela and Scotia Sea are of the form: $Ln(R_{IND\_12}) = a_0 + a_1 \times Ln(WM)$, and based on respiration data at the experimental temperature of 12°C. We include literature data, *Ikeda2016$_{_INSITU}$*, *Belcher2019$_{_INSITU}$*, *and Clarke and Johnston (1999) for comparison (in italics)*, based on data at *in situ* temperatures. The Ikeda2016 regression is of the form: $Ln(R_{IND\_INSITU}) = a_0 + a_1 \times Ln(WM) + a_2 \times 1000/temp + a_3 \times Ln(depth)$, note that temperature here is represented in Kelvin. The Belcher2019 model is of the form: $Ln(R_{IND\_INSITU}) = a_0 + a_1 \times Ln(WM) + a_2 \times T$. In addition, we include the recalculated regressions, Ikeda2016R$_{_12}$ and Belcher2019R$_{_12}$, with only wet mass as a predictor. The "All Myctophid" category, combines data from fishes belonging to the order Myctophiformes from the Scotia Sea, Benguela, and Belcher2019 datasets.

[a]Regressions recalculated from routine respiration data from Ikeda 2016 (provided by T. Ikeda) and Belcher *et al.* (2019). Respiration data were adjusted to 12°C (see Methods section).

[b]Respiration in mmol O_2 individual^{-1} h^{-1}, mass in g.

*p-Value < 0.05.

predictions made with the Ikeda2016$_{_12}$ equation to represent fed animals, we can estimate the extra energy required for respiration due to the action of feeding. Applying a specific dynamic action (SDA; the energy expended on ingestion, digestion, absorption and assimilation of food) of 2.36 (Secor, 2009) brings the predictions of the Ikeda2016$_{_12}$ regression closer to our measurements, but predictions are still up to 14 times too low.

Sensitivity analyses

A number of assumptions were made in the estimation of respiration from ETS activity. We conduct here a sensitivity analysis to assess what changes to these assumptions would be required to make our ETS-derived data match the predictions of Ikeda2016$_{_12}$. We then consider whether such changes to our assumptions are feasible given the present knowledge on the biology and physiology of these organisms:

(i) R:ETS ratio: ETS is a measure of the potential respiration and, thus, it is possible that the conservative *R*:ETS ratio of 0.5 used to convert to respiration was not appropriate for the Scotia Sea data. The only studies measuring the respiration of mesopelagic fish via ETS (Ikeda, 1996; Ariza *et al.*, 2015; Hernández-León *et al.*, 2019) also use the conservative *R*:ETS ratio of 0.5, based on the typical range of values for zooplankton of 0.5–1 from Hernández-León and Gómez (1996) and fish (Ikeda, 1989). Hernández-León and Gómez (1996) investigated the *R*:ETS ratio in marine zooplankton, with most of their data falling between 0.4 and 0.6. However, there was variability around

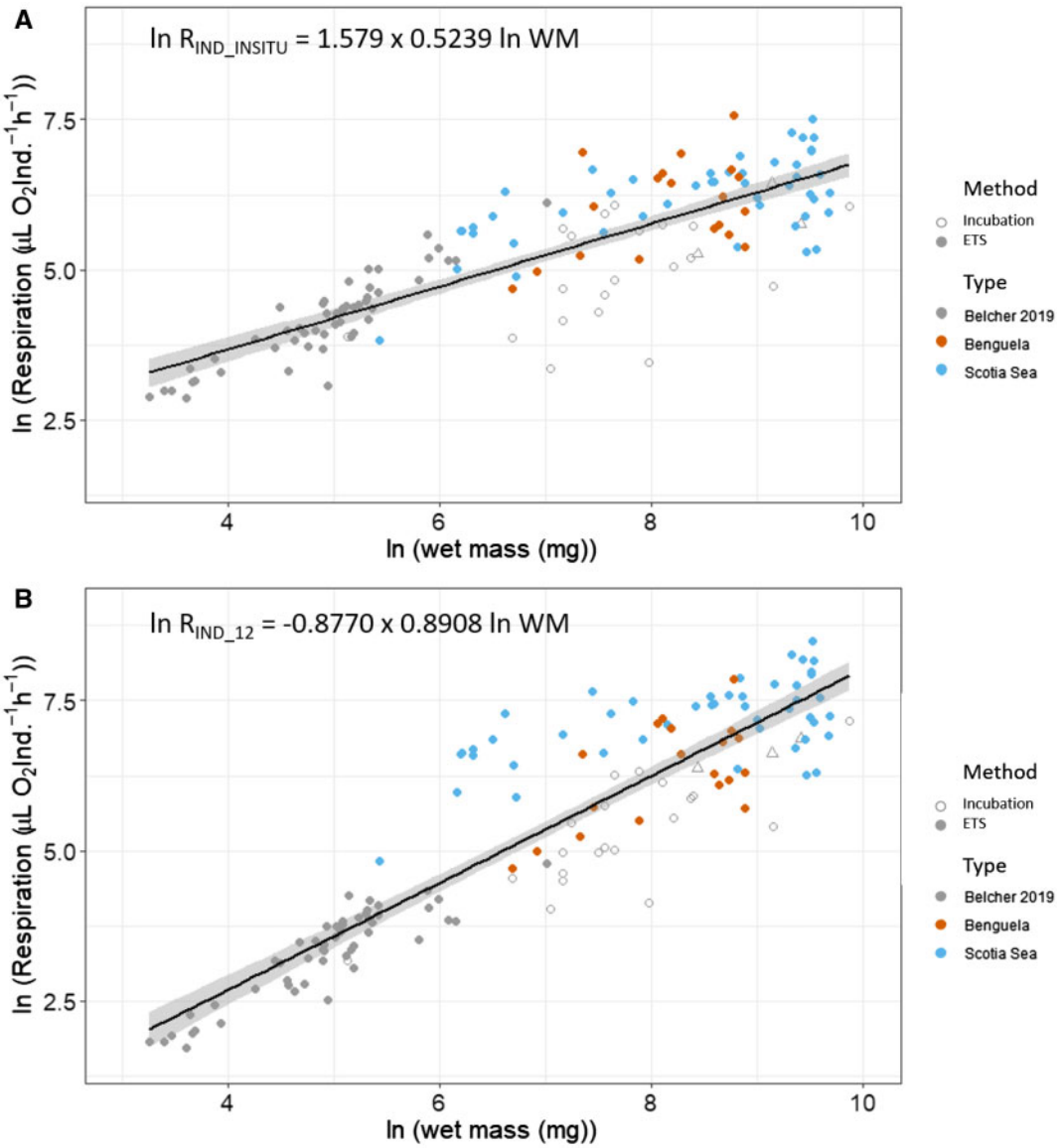

Figure 4. Compilation of respiration rates of only fishes belonging to the order Myctophiformes measured in this study (Benguela: orange circles, and Scotia Sea: blue circles) with the myctophid compilation of Belcher *et al.* (2019) (grey). (A) Respiration rates at *in situ* temperatures (R_{IND_INSITU}) and (B) respiration rates at 12°C (R_{IND_12}). The method of respiration rate measurement is defined by filled (ETS activity) or unfilled (incubation experiment) symbols. In addition, we highlight the literature data of Belcher *et al.* (2019) that represent incubation studies in Antarctic waters with grey open triangles. The regression fit of these data is shown by a black line with confidence intervals (0.95) in grey shading.

this, agreeing with the range of values measured by previous studies (0.16–2.34; Hernández-León and Gómez, 1996 and references within). Due to the difficulty in measuring the *R*:ETS ratio for mesopelagic fish, Ikeda (1989) incubated gobies and pomacentrids (sampled from a salt pond in Australia and not fed during the incubation) to estimate an *R*:ETS ratio of 0.62 and applied this to ETS activities of myctophid fishes sampled from surface waters. In addition, Schalk (1988) noted an *R*:ETS ratio of 0.16 measured in *Pomatoschistus* species in their own unpublished data. Based on the model of Packard *et al.* (1996), Osma *et al.* (2016) predicted the *in vivo* respiration rate of mysids from the ETS activity based on bisubstrate kinetics and measurements of kinetic constants and concentrations of the substrates NADH and NADPH. This model better takes into account the nutritional state of the organism compared with the use of a fixed value of the *R*:ETS ratio and is a promising alternative to incubating live animals to determine the *R*:ETS ratio. However, as yet, Osma *et al.* (2016) still recommend calibration of the predicted *in vivo* respiration rate with measured respiration rates.

We can calculate the *R*:ETS ratio required to bring the Scotia Sea estimates in line with the predictions of Ikeda2016$_{12}$ by assuming that the respiration predicted by

the Ikeda regression is correct and examining the ratio between this and our measured ETS activity. This results in a mean *R*:ETS ratio of 0.14 (median 0.09), which is at the very low end of observations in the literature (Hernández-León and Gómez, 1996). For the Benguela dataset, the mean calculated *R*:ETS ratio is 0.54 (median 0.30) highlighting the better agreement of Ikeda2016$_{12}$ with our ETS-derived respiration based on an *R*:ETS ratio of 0.5. If we must apply very different *R*:ETS ratios to the Benguela and Scotia Sea samples sets, it implies that environmental conditions must have a strong influence on this ratio, which no study has yet demonstrated.

(ii) Temperature correction: A major difference between the Benguela and Scotia Sea regions is the temperature range experienced by fish living in the mesopelagic (0.7–3.5°C in upper 500 m of Scotia Sea compared with 4.7–20.6°C in the upper 750 m of the Benguela). All ETS assays were carried out at a laboratory temperature of 11.5–12°C, and thus, the temperature correction is larger for the Scotia Sea data. Based on the available literature, we applied the Arrhenius equation and an activation energy (*E*a) of 15 kcal mol^{-1} to convert our measurements to *in situ* temperatures and to adjust the data of Ikeda (2016) and Belcher *et al.* (2019) to our experiment temperature of 12°C (R_{IND_12}). This has been used for micronekton (Ariza *et al.*, 2015; Hernández-León *et al.*, 2019) but is originally based on the mean of values measured on zooplankton (Packard *et al.*, 1975), which may not be ideal for mesopelagic fish. The *E*a may also change with environmental condition depending on the ability of enzymes to function at different temperature ranges (Simcic and Brancelj, 2004). However, to reduce our Scotia Sea respiration estimates to match the values predicted by the Ikeda2016$_{12}$ regression, we would require an *E*a of ∼40 kcal mol^{-1} (Q10 ∼ 12.5 for a temperature range of 2–18°C) which is not realistic (Supplementary Figure S3).

If we apply a more realistic *E*a of 20 kcal mol^{-1} [based on the range observed by Packard *et al.* (1975) of 11.7–21.9 kcal mol^{-1}, Q10 ∼ 3.5 for a temperature range of 2–18°C] and also apply an SDA factor of 2.36 to the Ikeda predictions, we find better agreement between the predictions of the Ikeda2016$_{12}$ regression and our ETS measurements in the Scotia Sea (calculated median *R*:ETS ratio of 0.46 assuming the predicted respiration from Ikeda2016$_{12}$ is correct). However, these adjustments would result in a median *R*:ETS ratio of 0.85 (mean 1.5) for the Benguela dataset, which is unlikely. In addition, although these methodological adjustments improve the general overall agreement, there is still disagreement on an individual fish basis (the range in calculated *R*:ETS for Scotia Sea is 0.05–2.35), highlighting the high degree of individual variability in metabolic rate for a given body mass and temperature.

Our sensitivity analysis suggests that, to bring the Ikeda2016$_{12}$ predictions in line with our observations, the necessary changes to the aforementioned parameters would be unrealistic given our present understanding. Although methodological factors may account for some of the differences between the regression-based predictions and our ETS-derived measurements, our data still suggest that there are regional differences in the scaling of mass with the respiration of mesopelagic fish.

Mesopelagic fish respiration: applicability of global equations to regional datasets

Cold temperature regions are not well represented in the existing mesopelagic fish regression models. Both Ikeda (2016) and Belcher *et al.* (2019) used global data to develop their regressions, with a dominance of data from lower latitudes [71% and 86% of respiration data at temperatures >5°C for Ikeda (2016) and Belcher *et al.* (2019), respectively]. Although our ETS-derived data sit in the cloud of myctophid respiration rate data compiled by Belcher *et al.* (2019) (Figure 4), comparison of the Belcher2019$_{12}$ regression and our All Myctophid regressions reveals that the addition of our myctophid data results in a lower mass scaling coefficient for data at *in situ* temperatures (Table 1, Figure 4B).

The poor fit of the Ikeda2016 and Belcher2019 allometric regressions to the Scotia Sea dataset could relate to the consumption of lipid-rich zooplankton that predominate in cold water regions (Lee *et al.*, 2006). Organisms respiring lipids have a low respiratory quotient [RQ; moles CO_2 produced/moles O_2 consumed relative to those respiring proteins or carbohydrates (RQs = 0.7, 0.8 and 1.0, respectively)]. Therefore, producing a mole of CO_2 when metabolizing fat will consume 1.4 moles of O_2, whereas producing 1 mole of CO_2 from carbohydrate will only require 1 mole of oxygen; fat metabolism is an oxygen-hungry process. The consumption of lipid-rich prey may also result in cold water mesopelagic fishes ingesting more carbon, e.g. relative to nitrogen, than their physiology requires. One mechanism through which organisms void excess dietary carbon is by "futile cycling", whereby excess carbon is disposed of via respiration that is decoupled from biochemical or mechanical "work" (Hessen and Anderson, 2008), resulting in elevated oxygen consumption rates. Further studies are required to examine these hypotheses. Nevertheless, environmentally driven and/or inter-species variation in diet may therefore contribute to the elevated respiration rates we measured in the Scotia Sea. The lack of data from cold water regions in the Ikeda (2016) and Belcher *et al.* (2019) datasets means that the potential for diet-driven increased rates of oxygen consumption in animals feeding on lipid-rich prey would not be well captured by their respective regression equations.

Mesopelagic fish respiration: inter- and intra-specific variabilities

The mass scaling coefficient has been shown to vary with phylogeny, although differences between taxa are not always statistically significant (Clarke and Johnston, 1999). Our data highlight a significantly lower mass scaling coefficient for the Scotia Sea data than for the global Ikeda (2016) dataset (Table 1), whereas no significant differences were found between mass scaling coefficients derived for the Benguela data and the Ikeda (2016) dataset. We can further examine the cause of the low-mass exponent of the Scotia Sea data by breaking the data down to the different species (Figure 5). Species-specific regression analysis revealed that the mass scaling coefficients for *E. antarctica* and *K. anderssoni* (R_{IND12}, 0.74 and 0.73 respectively) agree much better with the scaling coefficient of Ikeda (2016), and the low overall mass

scaling coefficient for the Scotia Sea data is driven by the lack of relationship between respiration and WM for *Gymnoscopelus* spp., and this likely explains the low R^2 of our relationship with mass for the Scotia Sea dataset (Table 1). This lack of scaling of respiration rate with WM for *Gymnoscopelus* spp. could simply suggest that the size range sampled was not large enough to see this. Although there was no significant difference between the mass scaling coefficient of Ikeda2016$_{_12}$ and the overall Benguela dataset, Figure 5B highlights that there are still inter-species differences in both mass scaling and mean respiration rate at the Benguela site. Our observations show that, within a given order, the metabolic rates can vary substantially for a given body mass and temperature. This is not surprising as the energetic requirements of different species vary with differences in lifestyle, e.g. activity, habitat, feeding mode, diet, swimming mode, as well as body composition (Clarke and Johnston, 1999). These differences also exist between individuals of the same species and will vary both spatially and temporally (Killen *et al.*, 2010).

There is evidence that, in the Scotia Sea, most myctophids vertically migrate to some degree (e.g. Collins *et al.*, 2012; Saunders *et al.*, 2014, 2015a, b), and this active lifestyle may contribute to their higher metabolic rates compared with other species (Figures 1 and 5). Visual predation on copepods and other relatively large prey (e.g. euphausiids and amphipods) in the surface likely requires greater levels of activity than an ambush feeding strategy employed by many non-migratory resident mesopelagic fish (Pavlov and Kasumyan, 2002). The vertical migrations of many myctophids may therefore reflect a metabolic strategy to allow feeding in warmer epipelagic layers where food is more available to visual predators, and assimilation of this food at depth where they can remain totally inactive (Barham, 1971; Pearcy *et al.*, 1979; Neighbours and Nafpaktitis, 1982). Conversely, *Cyclothone* spp. and *Bathylagus* spp. are predominantly found deeper in the water column and are not known to be diel vertical migrators (Sutton *et al.*, 2008; Bernal *et al.*, 2015). The ambush feeding strategy of non-migratory mesopelagic fish, combined with their low feeding intensity and energy requirements, likely comes at reduced metabolic demand. The lower level of activity associated with this more sedentary, deeper-dwelling lifestyle may therefore contribute to the low metabolic rate of these species. The presence/absence of a gas-filled swim bladder and energetic costs associated with this may also drive species differences in metabolic rate.

Bathylagidae have low-mass-specific respiration rates relative to other animals, but this difference is less pronounced when respiration is expressed as a protein-specific rate (Supplementary Figure S2). Thus, the low respiration rates of Bathylagidae could be explained by their high water content and low protein content (Tierney *et al.*, 2002; Schaafsma *et al.*, 2018). Species with low protein content will have lower mass-specific respiration rates than those with high protein content. Protein is more tightly related to aerobic respiration than WM, explained by the respiratory machinery being located in the mitochondria, and thus can be an informative measure when examining inter- and intra-species differences in respiration. Adoption of protein as a mass unit for allometric regressions may help to reduce uncertainty but comes at the cost of greater time and facilities needed to make these measurements.

Intra-specific variability, and the challenge of obtaining respiration measurements for mesopelagic fish, means that it is difficult to determine the species-specific drivers of metabolic rate. The differences we have observed, both between species and regionally, highlight the caution that needs to be taken when applying global derived relationships to regional datasets. This appears to be particularly true for cold temperature regions where data are sparser.

Respiration is a time-consuming parameter to measure, and many studies estimate respiration allometrically using the equations available in the literature (e.g. Takahashi *et al.*, 2009; Giering *et al.*, 2014; Belcher *et al.*, 2019; Pakhomov *et al.*, 2019). The accuracy of these allometrically derived respiration rates has knock-on effects for calculations of respiratory and active flux and can thus affect our conclusions as to the importance of a particular taxa in the biological carbon pump. Given the lack of suitability of either the Belcher2019$_{_12}$ or Ikeda2016$_{_12}$ regressions to the respiration rates of mesopelagic fish in the Scotia Sea, we recalculate the myctophidae (vertically migrating fraction) respiratory carbon fluxes of Belcher *et al.* (2019) using our Scotia Sea regression:

$$\mathrm{Ln}(R_{\mathrm{IND}_{12}}) = 4.359\ (\pm 0.556) + 0.334\ (\pm 0.066) \times \mathrm{Ln}(\mathrm{WM}). \quad (4)$$

We calculate the myctophid fish community respiration as outlined fully in Belcher *et al.* (2019). Briefly, respiration rates of myctophid fish (sampled during the Discovery 2010 expeditions to the Scotia Sea, Collins *et al.*, 2012; Tarling *et al.*, 2012) were estimated allometrically based on body mass. As our model (4) is for respiration at 12°C, we adjust respiration rates to the *in situ* respiration rate using the Arrhenius equation and an activation energy of 15 kcal mol^{-1} (Packard *et al.*, 1975; Ariza *et al.*, 2015). The *in situ* respiration rates of individual fish were summed for each net sample, and the daily respiratory flux by migrating myctophids estimated from the difference between day-time and night-time community respiration. Like Belcher *et al.* (2019), we do not take into account day-time net avoidance (Collins *et al.*, 2012; Fielding *et al.*, 2012) and, thus, estimates are of the maximum respiratory flux as we do not apply any corrections for lower catch efficiency during the day (i.e. total day-time community respiration is likely higher than estimated due to increased day-time biomass, which would result in a lower migratory respiratory flux).

We also recalculate the myctophidae (vertically migrating fraction) respiratory carbon fluxes of Belcher *et al.* (2019) based on model 1 (1 given above) of Ikeda (2016). At the present time, we are unable to perform these calculations for the Benguela region as we lack sufficient coverage of net samples over multiple seasons.

We find that estimates of respiratory carbon flux based on our Scotia Sea regression are 3.1–5.0 times greater than calculated by Belcher *et al.* (2019) and 4.0–8.3 times greater than predictions by the Ikeda2016$_{\mathrm{INSITU}}$ regression (Table 2). Based on the seasonal range of gravitational POC fluxes by Manno *et al.* (2014) in the Scotia Sea, Belcher *et al.* (2019) estimated that the myctophid respiratory carbon flux is equivalent to 9–47% and 1–2% of the gravitational POC flux at the North Scotia Sea (NSS) and Georgia Basin (GB) sites, respectively. The large range for the NSS estimate relates to the order of magnitude seasonal variability in POC flux at this site (Manno *et al.*, 2014). Using the Ikeda2016$_{\mathrm{INSITU}}$ regression gives 7–37% and 0.8–1.4% at NSS and GB, respectively. These estimates are much lower than estimates using our Scotia Sea regression (27–143% and 3.1–5.6% at

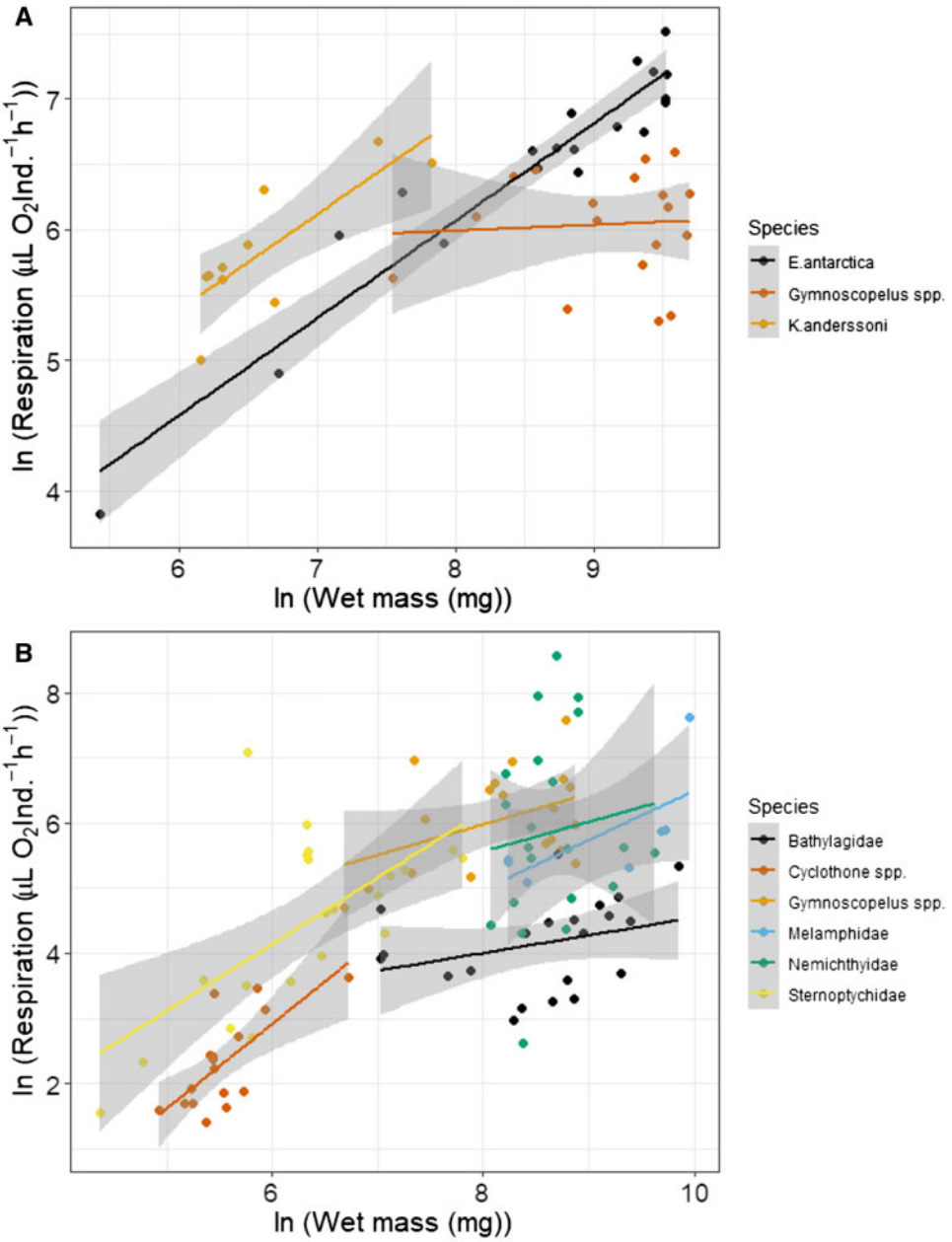

Figure 5. Species-specific respiration rates (R_{IND_INSITU}, µl O_2 individual^{-1} h^{-1}) for (A) Scotia Sea and (B) Benguela regions (respiration estimated from ETS measurements). Respiration is plotted as a function of the wet mass per individual (mg). Note the natural logarithmic scale on both *x* and *y* axes.

NSS and GB, respectively). Thus, even when POC fluxes are greatest, the respiration of myctophid fish alone (i.e. excluding other myctophid-driven carbon fluxes via excretion, mortality, and defaecation) could be equivalent to a minimum of 27% of the POC flux at NSS based on our new estimates. These calculations highlight (i) the importance of vertically migrating mesopelagic fish to carbon flux, at least in the Southern Ocean, and (ii) how the use of globally derived allometric regressions can result in substantial underestimates of respiratory and active fluxes in polar regions. As noted by Ikeda (2016), more data are needed from deep sea fishes to test and improve allometric models. We add to this that more data are required in regions of temperature <5°C to validate the much higher respiration rates observed at our Scotia Sea study site than predicted through allometric regression models.

Concluding remarks

It is time consuming and difficult to make respiration measurements in the field, particularly on fish collected from the mesopelagic. ETS provides a promising method for obtaining estimates of respiration from mesopelagic species, but there are still methodological considerations, especially regarding an appropriate *R*:ETS ratio to use.

Table 2. Comparison of respiratory carbon fluxes (mg C m^{-2} d^{-1}) calculated from the Discovery 2010 surveys in the Scotia Sea using allometric regressions; Scotia Sea, Belcher2019$_{_INSITU}$ and Ikeda2016$_{_INSITU}$.

	Respiratory flux mg C m^{-2} d^{-1} (% of Scotia Sea estimate)		
Site	**Scotia Sea (this study)**	**Belcher2019$_{_INSITU}$**	**Ikeda2016$_{_INSITU}$**
JR161 WSS, *n* (%)	0.25	0.05 (20.0)	0.03 (12.0)
JR161 NSS, *n* (%)	0.86	0.28 (32.6)	0.22 (25.6)
JR177 GB, *n* (%)	0.40	0.13 (32.5)	0.10 (25.0)
JR177 MSS, *n* (%)	0.91	0.27 (29.73)	0.20 (22.0)

To compare the results from the different regressions, we also express the Belcher2019 and Ikeda2016 estimates as a percentage of the Scotia Sea estimate, given in brackets (see Belcher *et al.* 2019 for details of calculation and Discovery 2010 surveys).

The data presented in this study demonstrate that globally derived allometric equations underestimate respiration rates of cold water mesopelagic fish. This potentially reflects the lipid-rich diets of these organisms. We have constructed a regression for myctophids (All Myctophid, Table 1) utilizing data across a range of regions and giving better representation of polar regions than previous equations. Nevertheless, even this myctophid-specific regression overestimates the respiration of the large *Gymnoscopelus* spp. Our data demonstrate that respiration rates can vary greatly between species, which may be related to differences in lifestyle, e.g. activity, habitat, feeding mode, diet, swimming mode, as well as body composition. We find high variability even between individuals of a given species with the same mass at the same environmental temperature. This variability means that allometric equations based solely on mass, or a combination of mass and temperature, will never be that precise in estimating respiration at an individual level. We stress that particular care should be taken when applying allometrical relationships to regional studies where the environmental conditions of that region are poorly represented in the data used to define the regression equation.

Underestimations of respiration rates of mesopelagic fish in cold water regions have implications on estimates of fish-driven respiratory flux and thus the biological carbon pump. Allometric estimates of fish-driven respiratory flux in the Scotia Sea were 67–88% lower than ETS-based estimates. Measurements of micronekton respiration are thus still needed, particularly for mesopelagic dwelling species and for under-sampled regions, because their contribution to carbon flux can be large. Only with these data we can build up our global databases sufficiently to be able to estimate total respiration confidently and parameterize respiratory flux or active flux. This is especially important to global biogeochemical models because the potential contribution of mesopelagic fish to carbon flux can be substantial.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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