# 1 Introduction

Mesopelagic fish (those living in the mesopelagic zone, between 150-1000 m) are an important component of the ocean biological carbon pump (Trueman et al. 2014, Anderson et al. 2018). Species often undertake diel vertical migrations, moving from depth to near-surface waters at night to feed on zooplankton under cover of darkness, before returning to the deep prior to daybreak. At the surface ocean, carbon readily exchanges with atmospheric carbon dioxide. Below 1000 m, carbon can take 100 years or more to reach the surface and re-enter atmosphere, a duration sufficient to qualify as long-term carbon sequestration (IPCC 2005, Passow & Carlson 2012). By predating on surface-dwelling zooplankton, mesopelagic fish ingest this surface carbon and export it to depth through respiration, excretion and mortality, where it is effectively sequestered. Non-migratory mesopelagic fish also contribute to the biological carbon pump by consuming migrating zooplankton when they enter the mesopelagic zone (Davison et al. 2013).

Myctophids (family Myctophidae) are the most abundant mesopelagic fish in the global oceans (Gjøsæter & Kawaguchi 1980, Catul et al. 2011). They dominate the ichthyofauna in the upper mesopelagic of the Scotia Sea; a highly productive area in the Atlantic sector of the Southern Ocean (Collins et al. 2008, Collins et al. 2012). Scotia Sea myctophids contribute an estimated 0.05 – 0.28 mg C m-2 d-1 to active carbon flux, equivalent to 9 – 47 % of gravitational particulate flux in the same area (Belcher et al. 2019). This figure was obtained by estimated individual myctophids mass-specific metabolic rate (MRW, μl O2 mg WM-1 h-1) according to the following equation:

(1)

Where T is temperature (˚C), and bW and bT are scaling exponents relating to body mass and temperature, respectively.

Equation 1 was parameterised using five studies of myctophid respiration rate, measure through either respirometry (Torres et al. 1979, Donnelly & Torres 1988) or electron transport system enzyme activity (ETS) (Torres & Somero 1988, Ikeda 1989, Ariza et al. 2015). These methods are somewhat problematic when applied to myctophids. They are delicate fish, and are often dead or damaged on landing (Catul et al. 2011). Consequently, fish which undergo respirometry are likely to be stressed, giving artificially high measures of resting metabolic rate. Torres et al. (1979) reported that “the most active fish were selected for measurements”, potentially biasing oxygen consumption values towards higher metabolic rates. ETS circumvents the issue of landing dead or damaged fish by measuring the respiration potential of a sample of fish tissue. This is converted to metabolic rate using a ratio of ETS to whole organism oxygen consumption (Ikeda 1989, Cammen et al. 1990, Ariza et al. 2015). No direct calibrations between ETS and whole organism oxygen consumption are available for myctophids, which may lead to inaccurate estimates.

Respirometry aims to measure standard metabolic rate (SMR); the minimum metabolic expenditure of a resting, post-absorptive organism at a known temperature. In reality, respirometry often yields resting or routine metabolic rate (RMR), which is similar to SMR but with some “routine” activity by the organism (Treberg et al. 2016). In the context of the biological carbon pump, both of these measures are less useful than field metabolic rate (FMR). FMR is the time-averaged energy expenditure of an organism free ranging in its natural habitat (Treberg et al. 2016, Trueman et al. 2016, Chung et al. 2019a, Chung et al. 2019b). As with SMR and RMR, FMR includes energy expended on basal costs, but also incorporates the thermic effect of food (also called specific dynamic action), as well as energy expended for growth, reproduction, excretion and movement (Treberg et al. 2016, Chung et al. 2019a, Chung et al. 2019b).

This study uses otoliths to calculate a mass-specific proxy for FMR (Moto). Otoliths are paired calcium carbonate structures found in the inner ears of teleost fishes. They grow sequentially and are metabolically inert. New otolith carbonate material is deposited from the endolymph, which surrounds the otolith (Campana 1999, Solomon et al. 2006). This otolith carbon is derived from carbon in the fish’s blood, which originates from two sources. Metabolic or dietary carbon is produced from the oxidation of food during cellular respiration, while dissolved inorganic carbon (DIC) is ingested from the ambient water through the gills and gut (Solomon et al. 2006, Chung et al. 2019a).

Stable isotopes are atoms of the same element, which have different atomic weights, but are not radioactive. In ecology, stable isotopes are used as naturally occurring tracers, as the proportion of heavier and lighter isotopes varies in the environment (Fry 2006). The Moto proxy takes advantage of the naturally occurring isotopic distinction between metabolic carbon and DIC. DIC has a greater proportion of carbon-13 to carbon-12 than metabolic carbon, so the δ13C of DIC is around 15‰ more positive than δ13C of metabolic carbon (Tagliabue & Bopp 2008, Magozzi et al. 2017). Fish with relatively higher metabolic rates have higher respiration rates, and so produce more metabolic carbon. As fish regulate the levels of carbonate in the blood, this increase in metabolic carbon is compensated for a decrease in blood DIC. This increases the proportion of metabolic carbon in the blood, meaning the overall blood carbon has a more negative δ13C (Kalish 1991, Trueman et al. 2016, Chung et al. 2019a, Chung et al. 2019b). This is supported by a negative correlation between otolith δ13C and caudal aspect ratio (a morphometric proxy for activity levels) among fish families (Sherwood & Rose 2003). The overall otolith δ13C value is a weighted average of δ13C values from the metabolic carbon and DIC (Trueman et al. 2016, Chung et al. 2019a, Chung et al. 2019b). If δ13C values of the otolith, metabolic carbon and DIC are known, the proportion of metabolic carbon (Moto) can be calculated. While this is a novel method, a recent study confirmed that Moto and mass-specific oxygen consumption were positively correlated in Atlantic cod (*Gadus morhua*), providing empirical support for the use of Moto as a metabolic proxy (Chung et al. 2019b).

This study applies the Moto proxy to the study of FMR in Scotia sea myctophids. Our primary aim is to use Moto to compare relative FMR between six species of myctophids common in the Scotia Sea: *Electrona antarctica*, *E. carlsbergi*, *Gymnoscopelus braueri*, *G. nicholsi*, *Protomyctophum bolini*, and *Krefftichthys anderssoni* (Piatkowski et al. 1994, Collins et al. 2008, Collins et al. 2012). We investigate the scaling relationships of Moto with body mass and temperature. According to the metabolic theory of ecology, log metabolic rates scale linearly with log body mass with an exponent of 0.75 (equivalent to -0.25 for mass-specific metabolic rates) (Brown et al. 2004). Metabolic theory further predicts that log mass-normalised metabolic rate will increase with temperature (Gillooly et al. 2001). We examine these relationships among and within species. Finally, we compare estimates of mass-specific metabolic rate generated using equation 1 to Moto values.

# 2 Methods

## 2.1 Samples

Otoliths and muscle samples were obtained from fish collected during four cruises of the RRS *James Clark Ross* in the Scotia Sea during the austral summer (JR38, December 1998 - January 1999; JR177, December 2007 - February 2008; JR15004, January - February 2016; JR16003 December 2016 - January 2017). Six species of myctophids were studied: *Electrona antarctica* (n = 19), *E. carlsbergi* (n = 17), *Gymnoscopelus braueri* (n = 20), *G. nicholsi* (n = 13), *Krefftichthys anderssoni* (n = 20) and *Protomyctophum bolini* (n = 20). Due to a labelling error, eight *E. carlsbergi* individuals did not have body mass recorded, therefore we omitted data from these individuals during body mass analyses.

## 2.2 Stable Isotope Analysis

Prior to stable isotope analysis, each otolith was manually cleaned in water, blotted and allowed to air dry. Large otoliths (>1mm diameter) were mounted onto a backing plate (Struers EpoFix resin) and milled to a depth of 100-200μm using an ESI New Wave Micromill to obtain a sample for analysis. In this way, only otolith material deposited most closely to the time of fish capture was analysed. Small otoliths (<1mm diameter - all *K. anderssoni* and some *P. bolini*) were crushed whole to obtain a sample, incorporating otolith material throughout the fish’s life. Supplementary analyses were run to test if this used a significant difference in resulting Moto. Muscle tissue was freeze dried using a Heto PowerDry LL3000 freeze dryer for 24-48 hours and crushed using a mortar and pestle.

Stable isotope analysis was carried out at the Stable Isotope Ratio Mass Spectrometry Laboratory (SIRMS Laboratory, Southampton, UK). Carbon and oxygen stable isotopes of otolith samples were analysed using a Kiel IV Carbonate device coupled with a MAT253 isotope ratio mass spectrometer. Replicates of the international standards NBS 19 and NBS 18, as well as the in-house standard GS1 (Carrara marble), were run for quality control and calibration. Carbon isotopes of muscle samples was analysed using a Vario Isotope select elemental analyser, coupled with an Isoprime 100 isotope ratio mass spectrometer. Replicates of the international standards USGS 40 and USGS 41, as well as the in-house standards acetanilide, glutamic acid and fish muscle were run for quality control and calibration. All stable isotope values are reported in permil using delta notation (δ13C and δ18O), relative to Vienna Pee Dee Belemnite (VPDB).

## 2.3 Parameter Calculations

All parameters were calculated in R version 3.4.4 (R Development Core Team 2010) using the Bayesian framework JAGS (Plummer 2016), with 100000 iterations, 50000 burn-in, 3 chains and thinning parameter of 50. Models were checked for mixing and convergence using trace plots, Geweke’s diagnostic and the Gelman-Rubin diagnostic. The proportion of metabolic carbon in the blood, Moto, was calculated using the following equation (Chung et al. 2019a, Chung et al. 2019b):

(2)

Where δ13Coto is the δ13C of the otolith sample, δ13CDIC-SW is the value for δ13C of dissolved inorganic carbon (DIC) in the ambient seawater ingested by the fish, δ13Cdiet is the δ13C of the fish’s diet and εtotal is the total isotopic fractionation (i.e. fractionation from DIC and diet to blood, blood to endolymph and endolymph to otolith). Estimates of Moto were calculated using MixSIAR version 3.1, with uninformative priors (Stock & Semmens 2016). δ13CDIC-SW was set using an isoscape (Tagliabue & Bopp 2008) based on catch location, and adjusted for the Suess effect. δ13Cdiet was set using muscle δ13C from the corresponding fish, minus the trophic enrichment factor for carbon (DeNiro & Epstein 1978). εtotal was set to zero and assumed to be invariant across species (Solomon et al. 2006).

δ18O of otolith aragonite can be used to estimate the ambient temperature experienced by each fish (Thorrold et al. 1997, Høie et al. 2004). Experienced temperature values (T, °C) were reconstructed using the following equation:

(3)

Where δ18Ooto is the δ18O of the otolith, δ18OSW is the δ18O of the ambient seawater, and a and b are parameters, set according to Høie et al. (2004). δ18OSW was set using catch location and depth (Schmidt et al. 1999). We used an informative prior for temperature (normal distribution, μ = 2, σ = 3) based on measurements from JR16003, JR15004 and JR177.

Estimates of mass-specific oxygen consumption (resting metabolic rate) were calculated using equation 1 with uninformative priors. Parameters were set according to Belcher et al. (2019), so that a = -1.315 (± 0.468), bW = -0.2665 (± 0.0516), and bT = 0.848 (± 0.0108).

## 2.4 Statistical Analyses

All statistical analyses were carried out in R version 3.4.4 (R Development Core Team 2010). Hamiltonian Monte Carlo (HMC) models were fitted in RStan version 2.17.3 (Stan Development Team 2018) using the rethinking package version 1.59 (McElreath 2016). A single chain of 10000 iterations, 5000 warmup and a thinning parameter of 1 was run for each model. Models were checked for convergence and mixing using traceplots, number of effective samples and the Gelman-Rubin diagnostic.

To examine the effect of body mass and temperature on estimates of field metabolic rate, we modelled Moto as a linear function of log body mass (W, g) and temperature (T, ˚C). Body mass and temperature were z-scored before input to the model. Species was included in the model as a random factor (a\_VarSpecies), both to address pseudoreplication, and to assess differences in Moto among species while accounting for body mass and temperature variation.

(4)

The same model, less a\_VarSpecies was run within species to test for intraspecific effects of body mass and temperature on Moto.

(5)

Moto was modelled as a linear function of estimated mass-specific resting metabolic rate (RMR) using the following model.

(6)

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