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Review

Otolith $\delta^{13}C$ values as a metabolic proxy: approaches and mechanical underpinnings

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Abstract. Knowledge of metabolic costs associated with maintenance, foraging, activity and growth under natural conditions is important for understanding fish behaviours and the bioenergetic consequences of a changing environment. Fish performance in the wild and within a complex environment can be investigated by analysing individual-level field metabolic rate and, at present, the natural stable carbon isotope tracer in otoliths offers the possibility to reconstruct field metabolic rate. The isotopic composition of carbon in fish otoliths is linked to oxygen consumption through metabolic oxidation of dietary carbon. The proportion of metabolically derived carbon can be estimated with knowledge of $\delta^{13}C$ values of diet and dissolved inorganic carbon in the water. Over the past 10 years, new techniques to study fish ecology have been developed, and these can be used to strengthen the application of otolith $\delta^{13}C$ values as a metabolic proxy. Here, we illustrate the great potential of the otolith $\delta^{13}C$ metabolic proxy in combination with other valuable and well-established approaches. The novel approach of the otolith $\delta^{13}C$ metabolic proxy allows us to track the effects of ontogenetic and environmental drivers on individual fish physiology, and removes a major obstacle to understanding and predicting the performance of free-ranging wild fish.

Additional keywords: bioenergetics, field metabolic rate, isotopic mixing models, oxygen consumption.

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Introduction

Metabolic rate is a proxy of the energy used by individual animals and provides a physiological perspective to interpret behavioural ecology. Laboratory-based measurement of fish standard and maximum metabolic rate (SMR and MMR respectively) is a common approach to investigating fish physiology in response to environmental changes (Killen *et al.* 2007, 2010; Chabot *et al.* 2016; Metcalfe *et al.* 2016), but has limited value for explaining fish behaviours in the field. The relevant trait to measure is the field metabolic rate (FMR). Unfortunately, FMR is challenging to measure with conventional methods for estimating metabolic rates in free-living fish (Treberg *et al.* 2016). A method of *in situ* oxygen consumption measurement termed field-based respirometry has been tried to investigate the metabolic rate and swimming activity of wild fish in natural environment (Bailey *et al.* 2002; Farrell *et al.* 2003). However, the methods do not allow for monitoring realistic energy demands of, for example, prey–predator interactions or recording a time-integrated total metabolic rate in a wild individual. The stable carbon isotope composition of otolith aragonite (expressed as $\delta^{13}C_{oto}$ values) may provide an answer to this problem. $\delta^{13}C_{oto}$ values have been studied for decades and show potential as a metabolic proxy because the carbon used to precipitate otolith aragonite is drawn from both metabolic (dietary) and ambient water sources. Therefore, the isotopic composition of carbon in otolith aragonite is a weighted average between the isotope compositions of metabolic carbon released from respiration and the dissolved inorganic carbon from ambient water (Kalish 1991*a*, 1991*b*; Iacumin *et al.* 1992; Gauldie *et al.* 1994; Gauldie 1996; Thorrold *et al.* 1997; Wurster and Patterson 2003; Wurster *et al.* 2005; Solomon *et al.* 2006; Dufour *et al.* 2007; Tohse and Mugiya 2008). However, despite a clear theoretical basis backed up by consistent observational data, relating variations in $\delta^{13}C_{oto}$ values directly to alternative measurements of metabolic rate have proven challenging, partly due to the difficulty of estimating FMR in aquatic organisms. Over the past 10 years, new knowledge and enhanced analytical techniques have been developed, and the potential for using $\delta^{13}C_{oto}$ values as a metabolic proxy should be updated. This short paper briefly overviews the use of $\delta^{13}C_{oto}$ values in relation to metabolism and illustrates a way forward to improve the methodology, and thereby provide fish ecologists and

physiologists with a strong tool to explore some of the current challenges in fish and fisheries ecology. First, we outline the mechanism underpinning carbon incorporation into otolith aragonite and describe analytical approaches to quantify the otolith metabolic proxy. Second, we summarise efforts to describe the relationship between the otolith metabolic proxy and oxygen consumption. Finally, we show the great potential of using otolith metabolic proxy in combination with other otolith-based analyses to answer physiological questions.

$\delta^{13}C_{oto}$ metabolic proxy expressed as a two-component mixing model

Previously, $\delta^{13}C_{oto}$ values have often been used as natural tracers for differences in water or diet source between or within individuals (Nonogaki *et al.* 2006; Ashford and Jones 2007; Schloesser *et al.* 2009; Elsdon *et al.* 2010; von Biela *et al.* 2015; Fraile *et al.* 2016) to answer various fishery and ecological questions, such as stock identification (Gao and Beamish 1999; Gao *et al.* 2001; Bastow *et al.* 2002; Correia *et al.* 2011; Shen and Gao 2012) and fish movements and migration (Augley *et al.* 2007; Kimirei *et al.* 2013; Currey *et al.* 2014; Javor and Dorval 2014; Gerard *et al.* 2015). Other applications have associated $\delta^{13}C_{oto}$ values with metabolic rate to reveal variation in fish physiological performance and the factors affecting it (Wurster and Patterson 2003; Wurster *et al.* 2005; Shephard *et al.* 2007; Hanson *et al.* 2013). However, we need to carefully evaluate the drivers behind variation in $\delta^{13}C$ values of dissolved inorganic carbon (DIC) in the water ($\delta^{13}C_{DIC}$) and $\delta^{13}C$ values of the diet ($\delta^{13}C_{diet}$) in order to use $\delta^{13}C_{oto}$ as an accurate estimate of fish FMR.

The $\delta^{13}C_{oto}$ value is the weighted average of the isotopic composition of carbon in two main carbon sources, DIC and diet, which are typically very distinct (~1 and −16‰ respectively; Sherwood and Rose 2005; Tagliabue and Bopp 2008). Therefore, the $\delta^{13}C_{oto}$ value can be described as the outcome of a two-component mixing model (Schwarcz *et al.* 1998; Solomon *et al.* 2006):

$$\delta^{13}C_{oto} = M_{oto} \times \delta^{13}C_{diet} + (1 - M_{oto}) \times \delta^{13}C_{DIC} + \varepsilon_{total} \quad (1)$$

where M_{oto} is the proportion of metabolically derived carbon (from the diet) in otolith carbonate and ε_{total} is the total net isotopic fractionation during carbon exchange between DIC and blood, as well as between the blood and endolymph in which the otolith is formed. DIC uptake is primarily across the gut and gills (Solomon *et al.* 2006). In contrast, metabolic carbon is released into the blood through the respiration and oxidisation processes, and the rate of oxidation of dietary carbon by definition reflects metabolic rate. Therefore, the weighted average of the isotopic values between DIC and diet is controlled by fish metabolism, and M_{oto} is viewed and named as a metabolic proxy in the following discussion. Below we review sources of uncertainty in evaluating Eqn 1 and therefore M_{oto} values.

$\delta^{13}C_{DIC}$ values

$\delta^{13}C_{DIC}$ values vary between water masses, geographic locations and time, influenced by the release of carbon from the lithosphere, carbon flux exchange within the atmosphere and respiration and photosynthesis from the biosphere. Fresh waters have a wide range of $\delta^{13}C_{DIC}$ values among rivers and lakes (for a review, see Bade *et al.* 2004). For example, $\delta^{13}C_{DIC}$ values range from −16 to −8‰ in the Ottawa River basin (Canada; Telmer and Veizer 1999) and from 2.6 to −31‰ among 104 lakes on four different continents (for details, see Bade *et al.* 2004). The variation of freshwater $\delta^{13}C_{DIC}$ values is strongly dependent on geological chemistry, water metabolism and biogeochemical process. By contrast, $\delta^{13}C_{DIC}$ values are relatively constant in marine systems, with values that generally vary between 0 and 3‰ on the horizontal spatial scale, and ~1‰ in the vertical gradient (Kroopnick 1985; Tagliabue and Bopp 2008; Schmittner *et al.* 2013; Becker *et al.* 2016). In addition to spatial variations, temporal differences in the $\delta^{13}C_{DIC}$ values, such as seasonal or annual changes, have been noticed. In areas with strong phytoplankton booms, rates of removal of CO_2 from DIC may exceed diffusion rates of atmospheric CO_2 into surface waters, and preferential uptake of ^{12}C into algal cells can cause a temporary increase in $\delta^{13}C_{DIC}$ values. Over the past 100 years, oceanic $\delta^{13}C_{DIC}$ values have declined continuously because anthropogenic carbon decreases the oceanic $\delta^{13}C_{DIC}$ values by CO_2 exchanges between the atmosphere and the ocean. This has been termed the Suess effect and, interestingly, the Suess effect has been recorded in otoliths from Atlantic bluefin tuna (Fraile *et al.* 2016). According to a recent biogeochemical model, the oceanic $\delta^{13}C_{DIC}$ value decreased 0.07‰ per decade from 1860 to 2000, whereas in the recent period from 1970 to 2000 it decreased at a rate of −0.18‰ per decade (Tagliabue and Bopp 2008). The decreasing rate speeds up with time. If we want to use $\delta^{13}C_{oto}$ metabolic proxy to compare fish metabolism between decades with an assumed $\delta^{13}C_{DIC}$ value but without calibrating the Suess effect, it will overestimate the metabolic rate of fish caught in a recent year. Therefore, we suggest using a model calibration to predict $\delta^{13}C_{DIC}$ values or reconstructing $\delta^{13}C_{DIC}$ values with given oceanographic parameters for the specific year.

It is possible to acquire $\delta^{13}C_{DIC}$ values from the direct measurement of water samples, but it is not always feasible, particularly where studies are based on historical otolith collections or from remote oceanic locations. Nevertheless, there are several ways to acquire $\delta^{13}C_{DIC}$ values through modelling predictions. $\delta^{13}C_{DIC}$ values can be predicted with a given value of apparent oxygen utilisation (AOU) in the world's ocean (Kroopnick 1985), and Filipsson *et al.* (2017) presented a regional relationship between $\delta^{13}C_{DIC}$ values and AOU with salinity revision in the Baltic–Skagerrak region at water depths below the halocline:

$$\delta^{13}C_{DIC} = 0.032 \times S - 0.01 \times AOU - 0.12 \quad (2)$$

where S is salinity and AOU is measured in micromoles per kilogram. In addition, a regional multiple linear regression model predicting $\delta^{13}C_{DIC}$ values from salinity, temperature and DIC concentrations was used by Becker *et al.* (2016) to model $\delta^{13}C_{DIC}$ values at a depth of more than 1500 m in the North Atlantic Ocean:

$$\delta^{13}C_{DIC} = -16.9 + 0.80 \times S - 0.080 \times \Theta - 0.0045 \times \mathrm{DIC} \quad (3)$$

where Θ is potential temperature (°C) and *DIC* is measured in micromoles per kilogram. $\delta^{13}C_{DIC}$ values can also be extracted from interpolated spatial models (McMahon *et al.* 2013) or biogeochemical models (Tagliabue and Bopp 2008; Schmittner *et al.* 2013). Biogeochemical models take into account both the spatial and temporal factors and yield a global pattern that is necessary for studies on large-scale fish migration and movement.

$\delta^{13}C_{diet}$ values

Distinguishing and measuring the isotopic values of metabolically derived carbon from DIC in blood and endolymph is difficult. Hence, using the $\delta^{13}C$ values in various tissues, such as muscle, liver and heart, is an alternative approach to estimating the $\delta^{13}C$ values of metabolically derived carbon, $\delta^{13}C_{diet}$. Tissue $\delta^{13}C$ values represent a weekly to monthly average of diet signals, depending on the tissue turnover rates among species, the types of tissue and diet preferences (Ankjærø *et al.* 2012). Isotopic enrichment from diets to tissues is also influenced by various biological and environmental factors, such as growth rate, metabolism and temperature, with typical isotopic offsets between diet and muscle tissue ranging from −1.75 to 3.7‰ (Sweeting *et al.* 2007). Post (2002) reported an average value of 0.4‰ in carbon isotope enrichment, but Sweeting *et al.* (2007) suggested out that 1.5‰ is a more appropriate value. These reported values are species averages, but in reality tissue–diet isotopic spacing is a dynamic variable rather than a fixed trait, varying within and among individuals and species depending on physiological status, life history traits and feeding histories. Despite the variation found between species and studies, muscle $\delta^{13}C$ values provide a reasonable approximation of $\delta^{13}C_{diet}$ in the M_{oto} estimation, because a 1‰ variation of $\delta^{13}C_{diet}$ values only contributes a maximum of ~0.005 to the uncertainty in the M_{oto} term (see details in the following sections). A drawback of using soft tissue is that individual trophic history cannot be reconstructed from these tissues because their $\delta^{13}C$ values are continuously changing due to variable diet and their metabolic turnover.

$\delta^{13}C$ values recorded in otolith organic matters have been recently used to indicate diet signals and trophic information in wild fish (Sirot *et al.* 2017). Compared with muscle tissue, otolith organic materials have the advantage that their $\delta^{13}C$ values appear close to those of the diet (i.e. show little trophic enrichment; Grønkjær *et al.* 2013). Moreover, otoliths grow continuously and record ontogenetic information and, in theory, if we can extract the organic materials from otolith aragonite formed at different periods or life stages of an individual, it would be possible to reconstruct that individual's trophic history. This would allow estimates of FMR through the life of a single individual. However, the proportion of organic material in otoliths is extremely small (<10%), and analysis of individual trophic history is at present only feasible with fish species possessing large otoliths.

The ε_{total} term

Physiology controls carbon isotope incorporation into otoliths and it directly affects the isotopic fractionation factor, ε_{total}. There are three different settings of ε_{total} that have been used in previous studies. Schwarcz *et al.* (1998) used a value of 2, which was based on the findings of carbon isotope enrichment from ambient fluids (HCO_3^-) to biogenic aragonite carbonates at 5°C (Grossman and Ku 1986). Høie *et al.* (2003) and Wurster and Patterson (2003) adopted a value of 2.7, which was derived from the inorganic precipitation of aragonite carbonate where the enrichment factor was temperature independent (Romanek *et al.* 1992). Solomon *et al.* (2006) used rainbow trout (*Oncorhynchus mykiss*) and conducted a controlled laboratory experiment with ^{13}C-enriched diets and a ^{13}C bicarbonate spike in water, finding that ε_{total} was slightly negative (−1.8), but not significantly different from zero. The determination of ε_{total} is still unresolved and remains a source of uncertainty in M_{oto} measurements (Dufour *et al.* 2007). Further research is needed to investigate the specific ε_{total} values among species and minimise the bias of M_{oto} estimations.

M_{oto} estimations

Two notable studies have conducted controlled laboratory experiments to estimate the proportion of metabolic carbon in fish otoliths. Solomon *et al.* (2006) reared juvenile rainbow trout (*O. mykiss*) in water with different $\delta^{13}C_{DIC}$ values and fed them food with different $\delta^{13}C_{diet}$ values, and reported a M_{oto} value of 0.17. Tohse and Mugiya (2008) used the isotope labelling technique on goldfish (*Carassius auratus*) to estimate the proportion of metabolically derived carbon, which they found to account for 25% of overall otolith carbon composition (M_{oto} value of 0.25). The percentage of metabolically derived carbon was higher (28%; M_{oto} value of 0.28) during the day and lower (13–20%; M_{oto} value of 0.13–0.20) during the night. In most other previous studies, M_{oto} values estimated from the two-component mixing model fell in the range 0–0.5 (Table 1). High values over 0.5 suggested by Wurster and Patterson (2003) and Hanson *et al.* (2013) reflect consideration of a range of possible $\delta^{13}C_{DIC}$ and $\delta^{13}C_{diet}$ values and associated the uncertainty in M_{oto} estimations.

Uncertainty in the $\delta^{13}C_{DIC}$ and $\delta^{13}C_{diet}$ values determine the precision of M_{oto} estimations. As an example, we performed a sensitivity test considering the effect on estimates of M_{oto} rising from the sources of variation in Eqn 1.

We calculated M_{oto} values corresponding to simulated values of $\delta^{13}C_{oto}$ ranging between 0 and −6‰. We allowed $\delta^{13}C_{DIC}$ values to vary by 1‰, capturing the likely uncertainty in most marine applications (Kroopnick 1985; Tagliabue and Bopp 2008; Schmittner *et al.* 2013; Becker *et al.* 2016). We varied $\delta^{13}C_{diet}$ values in a range from −16 to −22‰, reflecting typical isotope values of dietary items for benthic to pelagic fish species in temperate latitudes. The ε_{total} term was assumed to be 0 based on the observations by Solomon *et al.* (2006). Varying the $\delta^{13}C_{DIC}$ term across a range of 1‰ resulted in M_{oto} values ranging between ~0.05 and 0.35, depending on the $\delta^{13}C_{diet}$ and $\delta^{13}C_{oto}$ values used in the calculation (Fig. 1*a*). The s.d. of the M_{oto} term varied between 0.01 and 0.02, and systematically changed with $\delta^{13}C_{oto}$ and $\delta^{13}C_{diet}$ values. This suggests variation in the precision of M_{oto} within the fish functional groups. Fish with more positive $\delta^{13}C_{diet}$ values, such as benthic fishes, usually also have higher $\delta^{13}C_{oto}$ values (Sherwood and Rose 2003). Higher $\delta^{13}C_{oto}$ values mean that the difference between $\delta^{13}C_{oto}$ and $\delta^{13}C_{DIC}$ values is smaller, and therefore the uncertainty associated with the M_{oto} term will increase. This is seen in our sensitivity tests,

Table 1. Estimations of the proportion of metabolically derived carbon (M_{oto}) in the literature
Information before 2006 is extracted from table 2 in Solomon *et al.* (2006)

Source	Species	Type	System	M_{oto} value
Radtke (1984)	*Mugil cephalus*	Experimental	Marine	>0
Kalish (1991*a*)	Various species	Observational	Marine	>0
Kalish (1991*b*)	*Arripis trutta*	Experimental	Marine	0.317–0.349
Thorrold *et al.* (1997)	*Micropogonias undulatus*	Experimental	Marine	>0
Schwarcz *et al.* (1998)	*Gadus morhua*	Observational	Marine	0.07–0.43
Weidman and Millner (2000)	*Gadus morhua*	Observational	Marine	0.2
Guiguer *et al.* (2003)	*Salvelinus alpinus*	Experimental	Freshwater	0.067
	Oncorhynchus mykiss			0.014
Høie *et al.* (2003)	*Gadus morhua*	Experimental	Marine	0.28, 0.32
Wurster and Patterson (2003)	*Aplodinotus grunniens*	Observational	Freshwater	<0.95[A]
Wurster *et al.* (2005)	*Oncorhynchus tshawytscha*	Observational	Freshwater	0.24–0.44
Solomon *et al.* (2006)	*Oncorhynchus mykiss*	Experimental	Freshwater	0.17
Dufour *et al.* (2007)	*Coregonus lavaretus*	Observational	Freshwater	<0.56[B]
Weidel et al. (2007)	*Lepomis macrochirus*	Observational	Freshwater	0.35, 0.45
Tohse and Mugiya (2008)	*Carassius auratus*	Experimental	Freshwater	0.25
Elsdon *et al.* (2010)	*Fundulus heteroclitus*	Experimental	Coastal area	0.297–0.369
Nelson *et al.* (2011)	*Sciaenops ocellatus*	Experimental	Marine	0.08, 0.15
Hanson *et al.* (2013)	*Salmo salar*	Observational	Anadromous species	0.04–0.81[C]
Trueman *et al.* (2013)	*Hoplostethus atlanticus*	Observational	Deep sea	0.06–0.19
Chung (2015)	*Alepocephalus bairdii*	Observational	Deep sea	0.125–0.349
	Antimora rostrata			
	Coryphaenoides rupestris			
	Spectrunculus grandis			
Gerdeaux and Dufour (2015)	*Coregonus lavaretus*	Observational	Freshwater	0.1–0.35
	Salvelinus alpinus			
	Esox lucius			
	Perca fluviatilis			
	Rutilus rutilus			
	Tinca tinca			
Trueman *et al.* (2016)	30 deep-sea fish species	Observational	Deep sea	<~0.3
Martino *et al.* (2019)	*Chrysophrys auratus*	Experimental	Marine	0.21–0.28

[A]Fossil otoliths; the variation in M_{oto} values was evaluated by possible changes in $\delta^{13}C$ values of dissolved inorganic carbon (DIC) in the water ($\delta^{13}C_{DIC}$) and $\delta^{13}C$ values of the diet ($\delta^{13}C_{diet}$).
[B]The variation in M_{oto} values was evaluated by different fractionation factors.
[C]The variation in M_{oto} values was evaluated by possible changes in $\delta^{13}C_{DIC}$ and $\delta^{13}C_{diet}$ values; the M_{oto} value of the marine life stage in *S. salar* is 0.033–0.048.

because benthic fish have a systematically higher uncertainty (s.d.) in the M_{oto} estimations than pelagic fish (with more negative $\delta^{13}C_{diet}$ and $\delta^{13}C_{oto}$ values; Fig. 1*b*). Therefore, uncertainty in $\delta^{13}C_{DIC}$ values contributes more to estimated M_{oto} values of benthic or low metabolic rate fishes than to pelagic or higher metabolic rate fishes.

Similarly, we conducted sensitivity tests on the effect of $\delta^{13}C_{diet}$ uncertainty, which was set as a 1‰ variation between −18 and −19‰. $\delta^{13}C_{DIC}$ values were set to range between 0 and 3‰, which basically covers $\delta^{13}C_{DIC}$ values in the surface ocean around the world (Tagliabue and Bopp 2008). $\delta^{13}C_{oto}$ values ranged from 0 to −6‰. As expected, higher M_{oto} values were accompanied by a higher uncertainty (Fig. 1*c*, *d*). However, compared with DIC, a smaller s.d. was observed from the diet sensitivity test even with the same setting of 1‰ variation. The range of $\delta^{13}C_{diet}$ values is comparable to those commonly seen in temperate and subtropical marine environments, but in coastal or freshwater ecosystems the uncertainty will be amplified according to a wider range of $\delta^{13}C$ baseline changes. Moreover, higher uncertainty is expected in the migratory species with habitat changes ($\delta^{13}C_{DIC}$ variation), especially if these habitat changes infer diet shifts ($\delta^{13}C_{diet}$ variation).

To deal with the variation in both $\delta^{13}C_{DIC}$ and $\delta^{13}C_{diet}$ values, as well as uncertainty in ε_{total}, a Bayesian framework for isotopic mixing models offers an attractive statistical solution. This method provides the likelihood of a given M_{oto} term determined using Bayesian methods and considering the uncertainty of the two sources in terms of $\delta^{13}C_{DIC}$ and $\delta^{13}C_{diet}$ variations. It also facilitates comparing metabolic performance (M_{oto} term) between fish populations, and is easy to conduct within the well-established R software package MixSIAR (see https://github.com/brianstock/MixSIAR, accessed 21 March 2019; Stock *et al.* 2018).

Relationship between M_{oto} and oxygen consumption

Although a $\delta^{13}C_{oto}$ metabolic proxy corresponding to fish mass-specific metabolism has been described (Dufour *et al.* 2007; Trueman *et al.* 2013, 2016; Chung 2015), there are limited

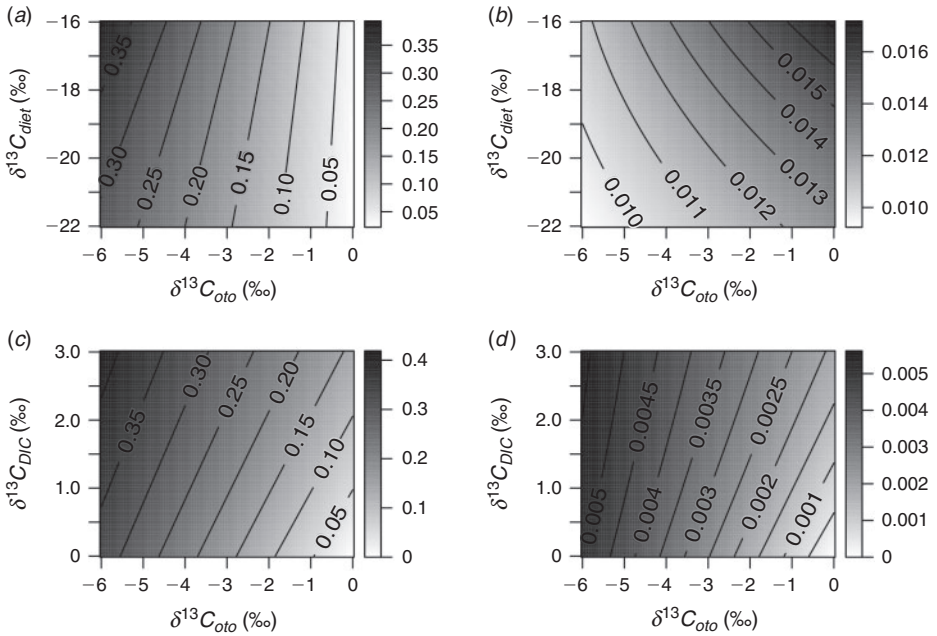

Fig. 1. Sensitivity tests on the proportion of metabolically derived carbon (M_{oto}) estimations by 1‰ variations in (*a*, *b*) $\delta^{13}C$ values of dissolved inorganic carbon (DIC) in the water ($\delta^{13}C_{DIC}$) and (*c*, *d*) $\delta^{13}C$ values of the diet ($\delta^{13}C_{diet}$). The mean (*a*, *c*) and s.d. (*b*, *d*) of the M_{oto} term were estimated using 1000 Monte Carlo simulations. The 1‰ variation of $\delta^{13}C_{DIC}$ is set from 0 to ~1‰ and, each run, a $\delta^{13}C_{DIC}$ value is randomly chosen from a uniform distribution U[0,1]. One thousand $\delta^{13}C_{DIC}$ vectors were produced, and each $\delta^{13}C_{DIC}$ vector was used to estimate M_{oto} with a given value of $\delta^{13}C_{diet}$ and $\delta^{13}C_{oto}$ ($\delta^{13}C$ values of otoliths). As a result, 1000 values of M_{oto} were generated and calculated as a mean and s.d. (*a*, *b*) Across a range from 0 to −6‰ for $\delta^{13}C_{oto}$ and from −16 to −22‰ for $\delta^{13}C_{diet}$, we produced a contour plot with a resolution of 100 × 100 grids for M_{oto} mean (*a*) and s.d. (*b*). Similarly, $\delta^{13}C_{diet}$ was set from −18 to −19‰ for uniform distribution U[−18,−19]. We followed the same procedure of simulation to estimate M_{oto} values and make contour plots showing the mean (*c*) and s.d. (*d*) with a resolution of 100 × 100 grids across a range from 0 to −6‰ for $\delta^{13}C_{oto}$ and from 0 to 3‰ for $\delta^{13}C_{DIC}$.

studies describing the scaling of M_{oto} values with mass-specific oxygen consumption. Here, we introduce a standard bioenergetics model to evaluate the likely relationship between M_{oto} values and oxygen consumption. The model allocates energy intake into three compartments: metabolism, growth and waste (Treberg *et al.* 2016; Deslauriers *et al.* 2017):

$$Consumption = Metabolism + Growth + Waste \quad (4)$$

$$Metabolism = SMR + Activity + SDA \quad (5)$$

where *SDA* is specific dynamic action. SMR can be predicted by measuring experienced temperature and body mass of the fish according to the metabolic theory of ecology (MTE; Brown *et al.* 2004):

$$SMR = B_0 \times BM^{\alpha} \times e^{-\frac{0.65}{(8.62\times10^{-5})\times T}} \quad (6)$$

where the B_0 is the normalised constant, *BM* is the body mass and *T* is temperature in kelvin; α is the allometric scaling exponent of body mass, which follows the three-quarters power law in MTE (as −0.25 for mass-specific metabolism; Brown *et al.* 2004) but was found to be 0.79 for teleost fishes (Clarke and Johnston 1999; Clarke 2006).

For wild-caught fishes, experienced temperature can be estimated from otolith $\delta^{18}O$ values (e.g. Shirai *et al.* 2018 and references therein). Second, otolith increment analysis provides a chronological record of body mass. A lifelong history of body mass can be reconstructed from von Bertalanffy growth curves with given age inferred by the otolith increment numbers. Otherwise, it is possible to back-calculate fish body mass from fish length, obtained from otolith back-calculations (Campana 1990). Using these methods, several previous studies present expected relationships between $\delta^{13}C_{oto}$ or M_{oto} values and temperature (Kalish 1991*a*; Høie *et al.* 2004*a*; Gao *et al.* 2010) and body mass (Trueman *et al.* 2013; Chung 2015).

The M_{oto} value is regarded as a proxy of FMR, corresponding to the sum of SMR, activity and SDA. To examine the relationship, we obtained M_{oto} values as well as fish length data and the otolith $\delta^{18}O$ values of Atlantic cod (*Gadus morhua*) extracted from Jamieson (2001) and Jamieson *et al.* (2004). Fish lengths and otolith $\delta^{18}O$ values were used to reconstruct body mass and experienced temperature of fish, which are critical for metabolic rate estimations (Table 2). The three metabolic compartments (i.e. SMR, activity and SDA) are estimated theoretically with the body mass and temperature by Fish Bioenergetics (ver. 4.0, see http://fishbioenergetics.org, accessed 21 March 2019), a package in R programming software (Deslauriers *et al.* 2017). The metabolic rate of the sum of the three metabolic compartments

Table 2. Individual information used to construct Fig. 2

Fish length, $\delta^{13}C_{oto}$ (δ^{13}C values of otoliths), $\delta^{18}O_{oto}$ (δ^{18}O values of otoliths) and the proportion of metabolically derived carbon (M_{oto}) values are extracted from Jamieson (2001). Fish weight is derived from the length–weight relationship from FishBase (R. Froese and D. Pauly, see www.fishbase.org): Weight = 0.0071 × Length$^{3.08}$. Temperature is reconstructed following the $\delta^{18}O_{oto}$–temperature equation given by Høie *et al.* (2004*b*) and seawater δ^{18}O is set as −2‰. Oxygen consumption is estimated with body mass and temperature according to Deslauriers *et al.* (2017)

Length (cm)	Weight (g)	$\delta^{13}C_{oto}$ (‰)	$\delta^{18}O_{oto}$ (‰)	M_{oto}	Temperature (°C)	Oxygen consumption (mg O_2 kg^{-1} h^{-1})
4.9	0.9	−4.7	−0.44	0.35	11.7	499
5.6	1.4	−4.7	−0.44	0.35	11.7	463
6	1.8	−3.5	−0.44	0.3	11.7	446
5.9	1.7	−4.9	−0.44	0.36	11.7	450
6.6	2.4	−3.5	−0.44	0.3	11.7	423
6.3	2.1	−3.8	−0.44	0.31	11.7	434
7.3	3.2	−4	−0.44	0.32	11.7	400
7.3	3.2	−4.1	−0.44	0.33	11.7	400
8.2	4.6	−3.2	−0.44	0.29	11.7	375
8.4	5.0	−3.8	−0.44	0.31	11.7	370
8.8	5.8	−3.6	−0.44	0.31	11.7	360
8.7	5.6	−4	−0.44	0.32	11.7	362
9.1	6.4	−3.6	−0.44	0.3	11.7	353
9.1	6.4	−4.1	−0.44	0.32	11.7	353
9.8	8.0	−3.2	−0.44	0.28	11.7	339
14.3	25.7	−2.3	0.43	0.26	7.35	217
14.5	26.8	−3.5	0.43	0.31	7.35	215
14.6	27.4	−2.5	0.43	0.27	7.35	214
14.7	28.0	−2.9	0.43	0.29	7.35	213
14.6	27.4	−2.8	0.43	0.29	7.35	214
15	29.8	−2.2	0.43	0.26	7.35	210
14.7	28.0	−2.6	0.43	0.28	7.35	213
14.5	26.8	−2.3	0.43	0.27	7.35	215
14.8	28.6	−1.9	0.43	0.25	7.35	212
16.2	37.7	−2.3	0.43	0.28	7.35	202
16.6	40.7	−1.8	0.43	0.27	7.35	199
16.1	37.0	−1.5	0.43	0.24	7.35	203
16.9	43.0	−1.8	0.43	0.27	7.35	197
17.4	47.0	−2.2	0.43	0.27	7.35	194
18	52.2	−2.9	0.31	0.26	7.95	198
67	2989	−0.8	1.54	0.2	1.80	63
26	162	−1	1.54	0.2	1.80	107
75	4231	−0.1	1.54	0.16	1.80	59
35	405	−1.9	1.54	0.25	1.80	90
34	370	−2.3	1.54	0.26	1.80	92
40	610	−0.8	1.54	0.2	1.80	84
18	52.2	−2.2	0.31	0.25	7.95	198
55	1628	−1.7	1.54	0.24	1.80	70
53	1452	−0.9	1.54	0.2	1.80	72

is expressed as the mass-specific oxygen consumption rate. The M_{oto} term increased significantly with mass-specific oxygen consumption (Fig. 2). Our regression trend indicated a positive and linear relationship between the M_{oto} term and oxygen consumption, but gave an unrealistic M_{oto} value (0.20) when the oxygen consumption was close to zero (Fig. 2). Considering that the M_{oto} term is constrained by both upper (~0.5) (Table 1) and lower boundaries (0), this may imply that the relationship is not a simple linear regression (Kalish 1991*a*), but an exponential decay model in increasing form (Chung *et al.* 2019). It is critical that the relationship between M_{oto} values and oxygen consumption should be widely investigated, especially across species. The functional form, including the upper limit of M_{oto} values, may vary between species according to their life history traits and physiological regulations. Nevertheless, it is believed that the relationship between M_{oto} and oxygen consumption rate among species will provide valuable information that will enhance progress in the research field of fish physiological ecology.

Further development based on the $\delta^{13}C_{oto}$ metabolic proxy

Knowledge of fish energy allocation between metabolic compartments (SMR, SDA and activity) may increase our understanding of their behavioural adaptation to environmental changes. The use of the otolith metabolic proxy could be instrumental in gaining this knowledge. For example, Sherwood and Rose (2003) found that $\delta^{13}C_{oto}$ values relate to the aspect

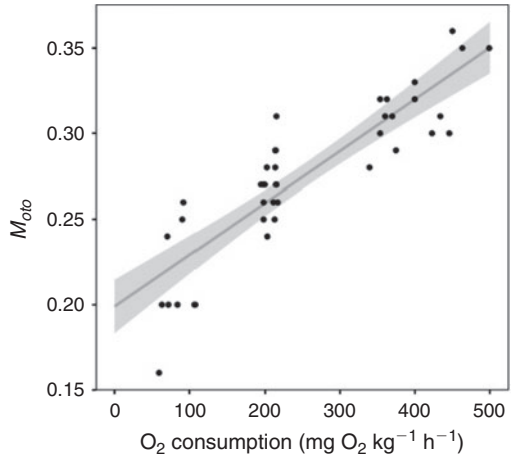

Fig. 2. Relationship between the proportion of metabolically derived carbon (M_{oto}) and reconstructed oxygen consumption rate of Atlantic cod (*Gadus morhua*; $y = 0.20+3.03 \times 10^{-4}x$; $n = 39$, $t = 11.17$, $P < 0.01$). M_{oto} values were extracted from Jamieson (2001). Oxygen consumption is theoretically estimated with known body mass and environmental temperature according to a bioenergetics model, and the calculations and parameter values follow Deslauriers *et al.* (2017). Individual information and data used in the relationship are given in Table 2.

ratios of the caudal fin of fish, which is associated with swimming form and activity. Solomon *et al.* (2006) further analysed these data to provide a regressed trend of M_{oto} values with the aspect ratios of the caudal fin of fish:

$$M_{oto} = 0.025 + 0.066 \times K_{caud} \quad (7)$$

where K_{caud} is the aspect ratio of the caudal fin. The relationship revealed the potential of using M_{oto} values to evaluate activity but without a link to fish swimming speeds and oxygen consumption rate. Thus, an experimental design in which the activity level of fish is manipulated (e.g. by enforcing different swimming speeds) may give direct evidence of the effect of activity metabolism on M_{oto} variations.

Otolith accretion and opacity are regulated by metabolic processes. The otolith annual pattern with alternating opaque and translucent bands is likely synchronised with energy acquisition and usage (Grønkjær 2016). At the microstructural level, increment widths have been found to relate linearly to SDA (Armstrong *et al.* 2004). As a general assumption, SDA is proportional to energy intake, and corresponds to 0.1- to 0.4-fold the total assimilated energy (Jobling 1981; Soofiani and Hawkins 1982; Kiørboe *et al.* 1987; Wieser and Medgyesy 1990). However, in wild fishes, it is difficult to determine SDA owing to uncertainties in meal size, feeding frequencies and postprandial durations. As an alternative, otolith increment analysis combined with the otolith metabolic proxy may make SDA determination possible. Furthermore, a modelling framework based on Dynamic Energy Budget (DEB) theory can be used to try to reconstruct individual and otolith growth history with known temperature and otolith opacity patterns (Fablet *et al.* 2011; Pecquerie *et al.* 2012). In this modelling framework, otolith growth and opacity are defined by two energy fluxes in the metabolism (i.e. maintenance and fish growth; Fablet *et al.* 2011). This means that the metabolic information of SMR and SDA, which is associated with maintenance and growth energy fluxes in the DEB model, can be acquired by analysing the optical properties of the otolith microstructure. Multiple approaches combining the $\delta^{13}C_{oto}$ metabolic proxy, otolith $\delta^{18}O$ analyses, microstructure analyses and the DEB model hold great potential when it comes to investigating and reconstructing individual life history in response to environmental changes.

Conclusion

In this paper we have illustrated three perspectives on otolith $\delta^{13}C$ metabolic proxy: (1) how to obtain the parameters used to estimate M_{oto} values according to a two-component mixing model; (2) the several unanswered questions that should be considered when using the otolith metabolic proxy; and (3) the great potential of using the otolith $\delta^{13}C$ metabolic proxy to study fish physiological ecology in combination with other valuable and well-established approaches. Despite the considerable efforts needed to acquire the necessary parameter values across species, the novel approach of the $\delta^{13}C_{oto}$ metabolic proxy shows great promise with regard to allowing us to track the ontogenetic and environmental effects on individual fish physiology, and thereby removes a major obstacle to understanding and predicting the performance of free-ranging wild fish.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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