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Metabolic enzyme activities of abyssal and hadal fishes: pressure effects and a re-evaluation of depth-related changes

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ABSTRACT

Metabolic enzyme activities of muscle tissue have been useful and widely-applied indicators of whole animal metabolic capacity, particularly in inaccessible systems such as the deep sea. Previous studies have been conducted at atmospheric pressure, regardless of organism habitat depth. However, maximum reaction rates of some of these enzymes are pressure dependent, complicating the use of metabolic enzyme activities as proxies of metabolic rates. Here, we show pressure-related rate changes in lactate and malate dehydrogenase (LDH, MDH) and pyruvate kinase (PK) in six fish species (2 hadal, 2 abyssal, 2 shallow). LDH maximal reaction rates decreased with pressure for the two shallow species, but, in contrast to previous findings, it increased for the four deep species, suggesting evolutionary changes in LDH reaction volumes. MDH maximal reaction rates increased with pressure in all species (up to 51 ± 10% at 60 MPa), including the tide pool snailfish, *Liparis florae* (activity increase at 60 MPa 44 ± 9%), suggesting an inherent negative volume change of the reaction. PK was inhibited by pressure in all species tested, including the hadal liparids (up to 34 ± 3% at 60 MPa), suggesting a positive volume change during the reaction. The addition of 400 mM TMAO counteracted this inhibition at both 0.5 and 2.0 mM ADP concentrations for the hadal liparid, *Notoliparis kermadecensis*. We revisit depth-related trends in metabolic enzyme activities according to these pressure-related rate changes and new data from seven abyssal and hadal species from the Kermadec and Mariana trenches. Results show that, with abyssal and hadal species, pressure-related rate changes are another variable to be considered in the use of enzyme activities as proxies for metabolic rate, in addition to factors such as temperature and body mass. Intraspecific increases in tricarboxylic acid cycle enzymes with depth of capture, independent of body mass, in two hadal snailfishes suggest improved nutritional condition for individuals deeper in the hadal zone, likely related to food availability. These new data inform the discussion of factors controlling metabolism in the deep sea, including the visual interactions hypothesis and extend published trends to the planet's deepest-living fishes.

1. Introduction

Certain citric acid cycle and glycolysis enzymes have been commonly used as proxies for whole-animal metabolic capacity and activity (Childress and Somero, 1979; Sullivan and Smith, 1982; Dickson et al., 1993; Vetter and Lynn, 1997; Hickey and Clements, 2003; Dahlhoff, 2004; Friedman et al., 2012; Torres et al., 2012; Ombres et al., 2011; Condon et al., 2012; Drazen et al., 2015; Saavedra et al., 2015). This technique has been particularly valuable in deep-sea systems, due to the logistical constraints of traditional measurements of metabolic rate, such as the monitoring of oxygen consumption, although a few of these data exist at great depths (e.g. Smith et al., 1978; Hughes et al., 2011; Drazen and Yeh, 2012). Four major metabolic enzyme activities (maximal reaction rate) are typically used to estimate metabolic rate, to estimate relative metabolic capacity, or as indices of metabolic capacity—lactate dehydrogenase (LDH), pyruvate kinase (PK), citrate synthase (CS), and malate dehydrogenase (MDH). LDH, which catalyzes the conversion of pyruvate to lactate during anaerobic glycolysis followed by fermentation and the reverse reaction of lactate to pyruvate during recovery from anaerobiosis, and PK, which catalyzes an ATP-yielding step in glycolysis, are used as proxies to indicate burst locomotory capability and anaerobic capacity (Childress and Somero, 1979; Dahlhoff, 2004). The activities of the tricarboxylic acid (TCA) cycle enzymes, CS and MDH, are applied as indicators of routine metabolic rate and aerobic activity (Somero and Childress, 1980; Childress and Thuesen, 1992; Thuesen and Childress, 1993).

The most common use of enzyme activities in deep sea animals has been to evaluate changes in metabolic capacity with depth (e.g.

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Childress and Somero, 1979; Sullivan and Somero, 1980; Siebenaller et al., 1982). Many taxa such as pelagic cephalopods, shrimps, and fishes, as well as benthic fishes, show declines in both measured respiration rates and metabolic enzyme activities in white and red muscle (e.g. Childress and Thuesen, 1992; Thuesen and Childress, 1993; Drazen et al., 2015). These declines are hypothesized to reflect a decrease in metabolic rate, which has been attributed to a reduction in food supply with depth (Smith et al., 1978; Siebenaller and Yancey, 1984) and/or reduced predator-prey interaction distances with declining light levels, known as the visual interactions hypothesis (Childress, 1995; Seibel and Drazen, 2007). The latter hypothesis suggests that in dark environments, where interaction distances are short, there is limited selective pressure for high locomotory capacities, explaining the declines in metabolic activities with depth that are not otherwise accounted for by temperature and body mass. This hypothesis was recently supported by an analysis of 61 species of benthic and benthopelagic fishes ranging from 50 to 3180 m depth, using a standardized methodology of measuring metabolic enzyme activities (Drazen et al., 2015).

Conclusions of these studies rely on the assumptions not only that metabolic enzyme activities are indeed indicators of metabolic capacity, but also that rates of these metabolic enzymes at atmospheric hydrostatic pressure reflect those at *in situ* pressures. However, the effects of pressure on enzyme catalysis can be non-linear and complex (reviewed by Mozhaev et al., 1996), calling the assumption that maximum reaction rates would not change with pressure into question. Half-saturation constants (K_m) for NADH of A_4-lactate dehydrogenase (originally termed M_4), which catalyzes the conversion of pyruvate to lactate to convert NADH to NAD^+ in anaerobic glycolysis and fermentation, have been shown in a number of deep-sea fish species to be either insensitive or less sensitive to pressure than orthologs from shallow species (Siebenaller and Somero, 1979; Somero and Siebenaller, 1979; Siebenaller, 1984; Dahlhoff et al., 1990; Brindley et al., 2008). A similar insensitivity was discovered in other important metabolic enzymes of deep-sea fishes—MDH (Dahlhoff and Somero, 1991) and phosphofructokinase (PFK; Moon et al., 1971a). These types of studies have suggested that pressure insensitivity in deep-sea species comes at the cost of a reduced catalytic efficiency (Somero and Siebenaller, 1979; Hennessey and Siebenaller, 1985). Enzyme concentration can be increased to offset the effects of lower catalytic efficiencies (capacity adaptations), so tissue-specific maximum reaction rate has not been hypothesized to change with pressure in fishes. However, at least 25 enzymes are known to exhibit increased maximum activity under pressure. Most of these have been isolated from piezophilic microbes (Eisenmenger and Reyes-De-Corcuera, 2009; Luong and Winter, 2015), but at least one animal enzyme has this property: a cellulase from the hadal amphipod *Hirondellea gigas* reportedly increased activity at 100 MPa (their habitat pressure in the Mariana Trench) relative to atmospheric pressure (Kobayashi et al., 2012).

In other contrasting studies, other enzymes appear to lack intrinsic pressure adaptations or are only partially adapted, and so may require protection from pressure by factors extrinsic to the protein, i.e., other cellular molecules. For example, K_m of ADP (but not maximum reaction rate, V_{max}) for PK in both shallow and deep-sea fish and anemones was found to be equally, and greatly, inhibited by pressure, such that higher ADP concentrations than in routine assay buffers are needed to achieve V_{max} (Yancey et al., 2001, 2004). However, in the presence of the osmolyte trimethylamine oxide (TMAO)—which is high in the deep-sea animals from which PK was tested (Kelly and Yancey 1999)—K_m of ADP was largely restored under pressure. TMAO was designated a 'piezolyte' ('pressure solute') for this property (Martin et al., 2002), which arises from TMAO's enhancing effects on water structure (reviewed by Yancey and Siebenaller. 2015). Unlike PK, LDHs appear to rely on both intrinsic and extrinsic adaptations. As noted earlier, K_m of NADH for LDH from many deep-sea fishes is more resistant to pressure than for shallow orthologs, but is still somewhat sensitive. However, full counteraction of this residual pressure inhibition was found with TMAO at *in situ* concentrations (Gillett et al., 1994; Yancey et al., 2004). Despite these and findings for other taxa, the effects of pressure on enzyme maximum reaction rates (as opposed to K_m) have been considered negligible in studies of metabolic rate. Moreover, enzyme kinetic responses to pressure in fishes at *in situ* habitat pressures greater than 40 MPa have not been explored.

To inform the discussion of metabolic capacity declines with depth, we use recent collections from the Mariana and Kermadec trenches to extend the published depth range of metabolic enzyme activities for fishes from ~3000 to almost 8000 m (*in situ* pressure ~80 MPa), approaching the likely depth limit for bony fishes (Yancey et al., 2014; Linley et al., 2016). The inclusion of hadal species in this analysis also allows the exploration of two additional factors that may affect metabolic rates besides light levels: food availability and hydrostatic pressure. In terms of food supply, although the deep sea is generally considered a food-limited environment, the topography of hadal trenches is hypothesized to facilitate the accumulation of organic matter (George and Higgins, 1979; Danovaro et al., 2003; Jamieson et al., 2011; Ichino et al., 2015). This is comparable to submarine canyons, which channel organic material, resulting in high faunal abundance, biomass and diversity (e.g. De Leo et al., 2010). In subducting trenches, downslope transport is enhanced by seismic activity and internal tides, resulting in the deposition of material into the trench (Itou et al., 2000; Oguri et al., 2013; Turnewitsch et al., 2014). The depositional characteristics of the hadal zone likely allow trenches to support higher biomass than the surrounding abyss (Wolff, 1970; Beliaev, 1989; Jamieson et al., 2010), as seen in increased amphipod (Jamieson, 2015) and meiofaunal (Danovaro et al., 2002; Itoh et al., 2011) abundances with depth and high rates of sediment community oxygen consumption (Glud et al., 2013; Wenzhöfer et al., 2016). This increased food availability may be a strong evolutionary driver to inhabit greater depths for a number of animals, particularly for the amphipod-feeding hadal snailfishes (Linley et al., 2017; Gerringer et al., 2017). According to previous analyses, neither food availability nor pressure is expected to affect metabolic rate in the deep sea interspecifically (reviewed by Seibel and Drazen, 2007). The hadal zone offers an ideal site to test the previously proposed hypotheses that neither pressure nor food availability will affect metabolic rates using a standardized protocol.

Here, we investigate pressure-related rate changes in maximal reaction rates of three metabolic enzymes from fast-glycolytic myotomal (white) muscle of deep- and shallow-adapted fishes. We then apply the pressure-related rate changes in metabolic enzyme activities to published and new results measured at atmospheric pressure, allowing a re-evaluation of the depth trends for metabolic proxies. This study extends a large existing dataset of metabolic enzyme activities to much greater depths with new data on abyssal and hadal species and elucidates depth-related trends in metabolic capacities in fishes in light of pressure-related changes in maximum enzyme reaction rates.

2. Materials and methods

2.1. Sample collection

Abyssal and hadal fishes were collected by free-vehicle trap baited with mackerel near and from the Kermadec (Apr–May, 2014) and Mariana trenches (Nov–Dec, 2014). Further details on collection sites and traps are provided by Linley et al. (2016). *Liparis florae*, the tidepool snailfish, was collected from Puget Sound near Friday Harbor, WA, by trawl and hand net (July 2014). A shallow-living, cold adapted species was also included, *Paraliparis devriesi,* collected by trawl from Antarctica (Andvord Bay, FjordEco Cruise), where it lives at a habitat temperature of ~−1°C, comparable to the hadal environment. Collected whole fish were kept on ice or in a cold room and processed as quickly as possible. White muscle samples were dissected from the