Deep-Sea Research I 100 (2015) 117–126

Contents lists available at ScienceDirect

Deep-Sea Research I

journal homepage: www.elsevier.com/locate/dsri

Enzyme activities of demersal fishes from the shelf to the abyssal plain

Jeffrey C. Drazen [a,*], Jason R. Friedman [a], Nicole E. Condon [a], Erica J. Aus [a], Mackenzie E. Gerringer [a], Aimee A. Keller [b], M. Elizabeth Clarke [b]

[a] University of Hawai'i, Department of Oceanography, 1000 Pope Rd., Honolulu, HI 96822, USA
[b] Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard East, Seattle, WA 98112, USA

ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form
18 February 2015
Accepted 22 February 2015
Available online 7 March 2015

Keywords:
Metabolism
Visual interactions hypothesis
Enzymes
Locomotory mode

ABSTRACT

The present study examined metabolic enzyme activities of 61 species of demersal fishes (331 individuals) trawled from a 3000 m depth range. Citrate synthase, lactate dehydrogenase, malate dehydrogenase, and pyruvate kinase activities were measured as proxies for aerobic and anaerobic activity and metabolic rate. Fishes were classified according to locomotory mode, either benthic or benthopelagic. Fishes with these two locomotory modes were found to exhibit differences in metabolic enzyme activity. This was particularly clear in the overall activity of citrate synthase, which had higher activity in benthopelagic fishes. Confirming earlier, less comprehensive studies, enzyme activities declined with depth in benthopelagic fishes. For the first time, patterns in benthic species could be explored and these fishes also exhibited depth-related declines in enzyme activity, contrary to expectations of the visual interactions hypothesis. Trends were significant when using depth parameters taken from the literature as well as from the present trawl information, suggesting a robust pattern regardless of the depth metric used. Potential explanations for the depth trends are discussed, but clearly metabolic rate does not vary simply as a function of mass and habitat temperature in fishes as shown by the substantial depth-related changes in enzymatic activities.

1. Introduction

The enormity and inaccessibility of the deep-sea habitat hampers controlled, live-animal experiments like those required for routine measurement of metabolic rate. Despite these difficulties, a number of direct laboratory measurements have been made on deep-sea animals collected mostly from trawls (Ikeda, 2013; Ikeda et al., 2006; Seibel and Drazen, 2007; Seibel et al., 2000; Torres et al., 1979), but also from gentler submersible or ROV capture (Wilson et al., 2013), and a few in situ measurements (Bailey et al., 2002; Drazen and Yeh, 2012; Hughes et al., 2011; Smith, 1978). These few direct measurements of metabolic rate suggest that the rates of some deeper-living animals are lower than those in shallow water and do not conform to predictions based on mass and temperature alone. The observed pattern is an exponential decline in metabolic rates with depth, leveling off at about 1000 m. This trend only occurs in visual taxa (fishes, crustaceans, cephalopods) and does not correlate with food supply. The visual interactions hypothesis (Childress, 1995; Seibel and Drazen, 2007) suggests that metabolism declines with decreasing light levels which result in declining distances over which visually orienting predators and prey can rapidly interact with one another. At the surface, where reaction distances are large, animals maintain high locomotory capability to escape or chase. In darkness, long chases or evasions do not occur and many animals lack streamlining and robust musculature, thus reducing metabolic "overhead."

Given the difficulties of measuring direct metabolic rate in the deep sea, biochemical proxies serve as important estimates. The activities of enzymes in glycolysis and the tricarboxylic acid (TCA) cycle, responsible for the generation of adenosine triphosphate (ATP), approximate the potential metabolic rate of an organism (Childress and Somero, 1979; Dalhoff, 2004; Ombres et al., 2011). Some of these enzymatic activities have correlated directly with metabolic rate or mitochondrial density (Burness et al., 1999; Hochachka and Somero, 2002). Importantly, measurement of their activity can be performed on tissues from recently deceased animals. While these enzyme activities do not provide direct metabolic measurements, they can provide points of interspecific comparison. The use of such proxies is even more valuable for deep-sea fishes, as many have gas bladders that expand, killing fish retrieved from even modest depths.

A comprehensive study of enzyme activities in demersal fishes has not been conducted. Sullivan and Somero (1980) provided the first analysis, showing depth-related declines in enzyme activities down to ~600 m. This analysis, while important, combined benthic and pelagic species and probably does not reflect the complexity of the relationship for demersal animals (e.g. comparing shallow water tuna to deep-sea eelpout). Several other studies focused on specific

* Corresponding author. Tel.: +1 808 956 6567; fax: +1 808 956 9225.
E-mail address: jdrazen@hawaii.edu (J.C. Drazen).

http://dx.doi.org/10.1016/j.dsr.2015.02.013
0967-0637/

families or small groups of species and/or depth ranges (Drazen et al., 2011; Siebenaller et al., 1982; Vetter and Lynn, 1997; Vetter et al., 1994). These often found depth-related trends but not in all cases, perhaps due to a lack of broad depth coverage or low sample sizes (sometimes only one deep and one shallow species; e.g. Treberg et al., 2003; Yang et al., 1992). Important findings from these various studies include similarities in enzyme activities between species of similar foraging habits and locomotory modes. These data were assembled in a meta-analytical approach, though data were limited, particularly for benthic fishes (Drazen and Seibel, 2007), those which spend considerable time resting on the seafloor, such as flatfish. Benthopelagic species, those fishes that are associated with the benthos but spend most of their time swimming in the water column, showed clear depth-related declines in enzyme activities, however trends for benthic species were less certain. Interpretation was complicated because data were acquired from multiple oceans, studies, assay temperatures, and laboratories, each with slightly differing protocols. To provide a stronger evaluation of the factors affecting metabolic enzymatic activity in demersal fishes and reduce uncontrolled variation, a more cohesive data set was needed. Here we present an analysis of enzymatic activity across a broad diversity of 61 species of fish from 50 to 3180 m depth off the central California coast, with a consistent methodology to robustly test whether there are depth-related declines in metabolic enzyme activities.

2. Methods

Demersal fishes were collected primarily by trawl during two oceanographic research expeditions off Monterey Bay, California in 2009. Trawls were conducted between ~50 and 3180 m. Additional specimens were obtained from National Oceanic and Atmospheric Administration trawls in 2009 off the central Oregon coast. The mass and length of the fish were measured. The depths of occurrence of each species were recorded from this sampling effort and also from the literature. Deep-sea fishes display a variety of locomotory modes in the demersal environment and have been very broadly classified as either benthic or benthopelagic (Drazen and Seibel, 2007), the latter term referring to species which spend most of their time swimming in the water column rather than resting on the seafloor. This anecdotal classification is cautiously employed in this study in the absence of any other information.

2.1. Enzymatic assays

White muscle was taken from below the first dorsal fin and inspected carefully to ensure no red muscle was inadvertently sampled. All tissues were frozen in cryovials in liquid nitrogen aboard ship and later stored at −80 °C until the preparation of homogenates for assays. Tissue samples of approximately 0.1 g were homogenized in 1 mL of 0 °C 10 mM Tris/HCl buffer (pH 7.55 at 10 °C). Each fish was examined through the duplicate testing of two independent homogenates.

The maximal activities of four enzymes were assayed using the protocols detailed in Condon et al. (2012). Citrate synthase (CS) catalyzes the first step in the TCA cycle, serves an important role in oxidative metabolism (Somero and Childress, 1980) and correlates with mitochondrial density (Moyes et al., 1992). Malate dehydrogenase (MDH) is a part of the TCA cycle, but also serves to maintain redox balance between the mitochondria and cytoplasm (Gelpi et al., 1992; Ombres et al., 2011; Siebenaller et al., 1982). Lactate dehydrogenase (LDH) is the final enzyme of anaerobic glycolysis, resulting in the production of lactate. Thus, its activity is indicative of anaerobic capacity and, in white muscle, of interspecific burst locomotory capacity (Childress and Somero, 1979; Dalhoff, 2004). Pyruvate kinase (PK) catalyzes the last reaction in glycolysis, producing pyruvate. This substrate can either be used in the mitochondrial TCA cycle or shuttled to anaerobic glycolysis. The rapid flux of pyruvate during anaerobic metabolism suggests that activities of PK more likely indicate anaerobic capacity of the tissue and studies have found positive correlations between PK and LDH in white muscle (Childress and Somero, 1979; Ombres et al., 2011; Sullivan and Somero, 1980).

In assays of these four enzymes the amount of homogenate used was varied to ensure that concentrations of substrates were not limiting. Measurements were made in a spectrophotometer externally cooled to 10 °C. Activity levels are expressed as μmoles product generated per minute (SI unit) per gram wet weight of tissue). Specific reaction conditions may be referenced from Condon et al. (2012).

2.2. Water content

Subsamples of white muscle taken during initial collection were used to conduct water content assays. Tissue samples were initially weighed, dried for 24 h in a 60 °C furnace, and then weighed again. Care was taken to constrain condensation or rehydration. Each fish was tested in triplicate, and the resulting mean difference in weight was attributed to the water content of the tissue and recorded as a percentage relative to the overall tissue composition.

2.3. Depth parameterization

To evaluate depth patterns, mean enzymatic activities of each species were regressed against depth. For pelagic species which undergo diel vertical migration, minimum depth of occurrence has been used in previous studies. This characterization of species habitat was chosen because the minimum depth represents the extreme end of the individual's daily range in food and light levels. Many demersal fishes exhibit ontogenetic downslope migrations (Jacobson et al., 2001; Polloni et al., 1979; Yeh and Drazen, 2011). Little is known about whether diel vertical migration occurs up- and down-slope. Therefore, depth ranges (for adults) often represent a broader range than for pelagic species, one that individual fish likely do not use on a daily basis. Further, the minimum depth of occurrence has been argued to represent only a small portion of a demersal species population, perhaps making median depth of occurrence a more appropriate parameter (Condon et al., 2012). For this study we decided to conduct analyses on minimum, median, and maximum depths of occurrence to fully evaluate habitat influences on metabolic enzyme activities. Further we used our own trawl capture information and known depth ranges from the literature which can encompass a broader range than any single sampling effort.

2.4. Size effects

Body mass affects metabolic enzymatic activities in fishes and other animals (Childress and Somero, 1990; Seibel, 2007; Somero and Childress, 1980). These relationships are usually power functions. Thus, we examined the relationship between body mass and enzyme activity for each species using linear regression after first performing natural log transformations of the data. This was done for all species where there were more than 4 individuals. With the great diversity of species examined there was a wide range of body sizes, and the size ranges of many species did not overlap (Table 1). This made the use of intraspecific enzyme-body mass regressions to standardize enzyme activities to a mean size difficult to justify for interspecific comparisons. Even when significant relationships were found, they would require extrapolation of enzyme activities to outside the natural range of body mass for many species, certainly outside the size range of specimens from the present analysis.