The Journal of Experimental Biology 215, 1905-1914

doi:10.1242/jeb.060236

RESEARCH ARTICLE

Aerobic and anaerobic metabolism in oxygen minimum layer fishes: the role of alcohol dehydrogenase

Joseph J. Torres[1,*], Michelle D. Grigsby[1] and M. Elizabeth Clarke[2]

[1]College of Marine Science, University of South Florida, 140 Seventh Avenue South, St Petersburg, FL 33701, USA and [2]Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard, Seattle, WA 98112, USA

*Author for correspondence (jjtorres@usf.edu)

Accepted 24 January 2012

SUMMARY

Zones of minimum oxygen form at intermediate depth in all the world's oceans as a result of global circulation patterns that keep the water at oceanic mid-depths out of contact with the atmosphere for hundreds of years. In areas where primary production is very high, the microbial oxidation of sinking organic matter results in very low oxygen concentrations at mid-depths. Such is the case with the Arabian Sea, with O_2 concentrations reaching zero at 200 m and remaining very low (<0.1 ml O_2 l^{-1}) for hundreds of meters below this depth, and in the California borderland, where oxygen levels reach 0.2 ml O_2 l^{-1} at 700 m with severely hypoxic (<1.0 ml O_2 $l^{-1)}$ waters at depths 300 m above and below that. Despite the very low oxygen, mesopelagic fishes (primarily lanternfishes: Mytophidae) inhabiting the Arabian Sea and California borderland perform a daily vertical migration into the low-oxygen layer, spending daylight hours in the oxygen minimum zone and migrating upward into normoxic waters at night. To find out how fishes were able to survive their daily sojourns into the minimum zone, we tested the activity of four enzymes, one (lactate dehydrogenase, LDH) that served as a proxy for anaerobic glycolysis with a conventional lactate endpoint, a second (citrate synthase, CS) that is indicative of aerobic metabolism, a third (malate dehydrogenase) that functions in the Krebs' cycle and as a bridge linking mitochondrion and cytosol, and a fourth (alcohol dehydrogenase, ADH) that catalyzes the final reaction in a pathway where pyruvate is reduced to ethanol. Ethanol is a metabolic product easily excreted by fish, preventing lactate accumulation. The ADH pathway is rarely very active in vertebrate muscle; activity has previously been seen only in goldfish and other cyprinids capable of prolonged anaerobiosis. Activity of the enzyme suite in Arabian Sea and California fishes was compared with that of ecological analogs in the same family and with the same lifestyle but living in systems with much higher oxygen concentrations: the Gulf of Mexico and the Southern Ocean. ADH activities in the Arabian Sea fishes were similar to those of goldfish, far higher than those of confamilials from the less severe minimum in the Gulf of Mexico, suggesting that the Arabian Sea fishes are capable of exploiting the novel ethanol endpoint to become competent anaerobes. In turn, the fishes of California exhibited a higher ADH activity than their Antarctic relatives. It was concluded that ADH activity is more widespread in fishes than previously believed and that it may play a role in allowing vertically migrating fishes to exploit the safe haven afforded by severe oxygen minima.

Key words: oxygen minimum, mesopelagic fishes, enzyme activity, alcohol dehydrogenase.

INTRODUCTION

At all depths of the ocean, oxygen is removed from the water column as organisms respire and organic matter is biochemically degraded. Wave action, mixing and photosynthetic processes replenish the lost oxygen in the upper mixed layer, but a zone of minimum oxygen forms at intermediate depth in all the world's oceans (Richards, 1965; Sewell and Fage, 1948). The severity of oxygen minimum zones varies considerably (Schmidt, 1925; Sewell and Fage, 1948), with values of dissolved oxygen ranging from 0 ml l^{-1} in the Arabian Sea (Hitchcock et al., 1997) to about 4 ml l^{-1} in the Antarctic (Smith et al., 1999).

The major factors contributing to the persistence of oxygen minimum zones are global circulation patterns that result in the water at mid-depths being out of contact with the atmosphere for hundreds of years (Schmidt, 1925; Stramma et al., 2008). In areas where the water column is well stratified and rates of primary production are high, oxygen minima are especially severe. Dissolved oxygen values reach 0 ml l^{-1} and stay near zero for hundreds of meters of water depth. Examples of such regions are the Arabian Sea, Bay of Bengal, the California Borderland and the eastern tropical Pacific. Hypoxic conditions in those areas extend from the bottom of the mixed layer to about 1500 m depth (Kamykowski and Zentara, 1990).

Although the oxygen concentration in oxygen minimum zones can be less than 0.2 ml l^{-1}, large populations of organisms often reside there (Schmidt, 1925; Childress, 1971; Morrison et al., 1999). Those organisms include decapods (Pearcy et al., 1977; Krygier and Pearcy, 1981), mysids (Childress, 1971) and copepods (Longhurst, 1967). Oxygen minimum layer invertebrates often co-exist with large populations of mid-water fishes (Gjøsaeter, 1984; Kinzer et al., 1993; Nair et al., 1999; Luo et al., 2000). In the Arabian Sea, the fish biomass in the oxygen minimum zone is dominated by lanternfishes (family Myctophidae), which are sufficiently abundant to form a 'deep-scattering layer', an obvious echo return at intermediate depth well above the sea bottom on the ship's echosounder. During the day, most of the fishes are found in the oxygen minimum zone below

250–350 m depth, while at night most migrate up to about 100 m into normoxic waters (Gjøsaeter, 1984).

Organisms that inhabit oxygen minima exhibit a range of adaptations for dealing with hypoxic conditions. Those adaptations include behavior, such as diel vertical migration out of the layer, in addition to unusual morphological and physiological characteristics (Wishner et al., 2000). Childress and Seibel (Childress and Seibel, 1998) have proposed three modes of adaptation to the oxygen minimum: (1) development of mechanisms for efficient removal of oxygen from water, (2) reduction of metabolic rate, and (3) use of anaerobic metabolism to make up the difference between aerobic metabolism and total metabolic needs. The use of anaerobic metabolism may occur on a sustained basis, during periods of high metabolic demand, or during transient periods spent in the oxygen minimum layer by vertical migrators.

The lowered food energy available in the deep sea (Vinogradov, 1970) favors an aerobic existence where there is sufficient oxygen to be utilized. However, an oxygen minimum such as that in the Arabian Sea, where oxygen drops to zero, constrains species to an anaerobic existence while resident within it. Most vertebrates, including fish, rely almost exclusively on aerobic metabolism, only switching to locally active anaerobic pathways during periods of increased activity or ambient low oxygen levels. Tuna white muscle, for example, is very well adapted for anaerobiosis, which is used during burst swimming (Hochachka, 1980). Even accomplished breath-hold divers such as seals and marine turtles stay within aerobic dive limits during the vast majority of their excursions (Hochachka and Somero, 2002).

At least three exceptional anaerobes exist within the vertebrate realm: hibernating pond turtles (Warren and Jackson, 2008) and the two Cyprinid fishes, crucian carp (*Carassius carassius*) and goldfish (*Carassius auratus*) (Vornanen et al., 2009). *Carassius carassius* can survive for months during winter when lakes freeze over and become anoxic (Blazka, 1958; Vornanen et al., 2009). Those fishes have found an alternative to lactate accumulation by reducing pyruvate to ethanol, a molecule that is easily excreted by fish (Shoubridge and Hochachka, 1980). The possibility that the ethanol dehydrogenase pathway might be utilized by mesopelagic fishes inhabiting areas of severe oxygen minima was a major thrust of the present research.

The present study explored the anaerobic capabilities of mesopelagic fishes from the oxygen minimum layer of the Arabian Sea, comparing them with confamilials from the California Current, the Gulf of Mexico and the Antarctic. The four regions were chosen for the varying degrees of severity of their oxygen minima. Based on available information, two major questions arose. (1) How does anaerobic capability scale with the severity of the oxygen minima in fishes of the same families and life habits? (2) If fish are constrained to use anaerobiosis by the severity of the minimum, what metabolic pathway is employed? To answer those questions, enzyme activities were used as proxies for the activity of metabolic pathways. Lactate dehydrogenase (LDH) was used as a proxy for glycolytic activity; citrate synthase (CS) was used for Krebs' cycle activity; malate dehydrogenase (MDH) serves a bridging function between glycolysis and electron transport in addition to its role as the final step in the Krebs' cycle; and alcohol (ethanol) dehydrogenase (ADH) was used as a proxy for the ability to generate ethanol.

MATERIALS AND METHODS

Collection of specimens

Twenty-one species representing one family of mesopelagic fishes, the Myctophidae, were used in the study. The fishes varied in mass from less than 50 mg to nearly 40 g (Table 1) and were collected from between 0 and 1000 m depth. Fishes were collected from the Arabian Sea during two GLOBEC (Global Ocean Ecosystem Dynamics)-sponsored cruises aboard the R/V Malcolm Baldridge in spring and summer 1995 using 1 and 10 m^2 MOCNESS (multiple opening and closing net and environmental sampling system), and surface Neuston net tows. Fishes from the Antarctic were collected with a 10 m^2 MOCNESS during two GLOBEC-sponsored cruises aboard the R/V Laurence M. Gould and two aboard the R/V Nathaniel B. Palmer during austral autumn and winter of 2001 and 2002. Samples from the California Current were collected on the R/V Point Sur in the San Clemente Basin during the spring of 1994. Fishes from the Gulf of Mexico were collected aboard the R/V Suncoaster during summer 2000 using a 9 m^2 Tucker trawl fitted with a thermally protecting cod end (Childress et al., 1978). All fish were frozen with liquid nitrogen immediately after capture and kept frozen at –80°C until ready for analysis. Arabian Sea specimens were stored at –80°C for 6 years prior to processing. The high enzyme activity levels obtained from Arabian Sea specimens as well as previous research on the effects of storage time (Geiger et al., 2001) suggest that storage had a minimal effect on enzyme activities.

Homogenate preparation

Fishes were measured, weighed and dissected while frozen. Length measurements were taken as standard length to the nearest millimeter. Epaxial white muscle was dissected from immediately behind the skull on the dorsal side of the body and homogenized in Milli-Q filtered water (10- to 100-fold dilution) for approximately 10 s using an ultrasonic homogenizer. Homogenates were centrifuged at 11,750 ***g*** for 30 s and the supernatant used for analysis. Homogenates were kept cold throughout analysis.

All homogenates were assayed for the activities of three enzymes of intermediary metabolism: LDH, MDH and CS. Part-way through the study, ADH was added to the suite of enzyme assays to add another dimension to the analysis.

Enzyme assays

LDH, CS and MDH activities were measured in 178 specimens from the Arabian Sea, the Gulf of Mexico, the California Current and the Antarctic. ADH activities were measured in 149 of the fishes. All individuals were vertically migrating lanternfishes (Pisces: Myctophidae) with night-time vertical distributions in the upper 150 m (Table 1). All enzymes were assayed in a UV-visible spectrophotometer. Assays on samples from Antarctica and California were run at 10°C; samples from the Arabian Sea and the Gulf of Mexico were run at 20°C except for the ADH assay, which was run at 15°C. For consistency, ADH values reported in Table 1 for Arabian Sea and Gulf of Mexico fishes were temperature corrected to 20°C using a Q_{10} of 2.0. The concentrations of substrate and cofactor used in each assay yielded the maximum reaction velocity of the enzyme, so the activities represent the maximum potential activity of the white muscle. The methods below are slight modifications of those previously published for LDH and CS (Torres and Somero, 1988b), MDH (Somero and Childress, 1980) and ADH (Shoubridge and Hochachka, 1980).

LDH (EC 1.1.1.27; oxidoreductase)

LDH activity (pyruvate + NADH ↔ lactate + NAD^+) was assayed in a medium containing 80 mmol l^{-1} imidazole buffer (pH 7.2 at 20°C), 162 μmol l^{-1} NADH and 5 mmol l^{-1} sodium pyruvate. A 10 μl sample of tissue homogenate was added to 1 ml of the assay medium, and the reaction was monitored for 1 min. The reaction rate was

Table 1. Standard length, wet mass and enzyme activities for all myctophid species studied

Species (*N*)	Length (cm)	Mass (g)	LDH activity	CS activity	MDH activity	ADH activity
Antarctic						
Electrona antarctica (22)	7.47±0.38	6.65±1.18	109.63±21.53	3.15±0.17	30.85±2.35	4.03±0.97
50 m	(4.84–9.17)	(1.39–14.93)	(27.7–204.5)	(2.5–4.1)	(21.6–42.2)	(1.0–10.3)
Gymnoscopelus braueri (9)	10.35±0.83	11.20±3.29	30.11±9.9	1.82±0.33	22.14±2.3	19.8±13.1
150 m	(8.84–12.43)	(5.01–20.27)	(10.8–106.5)	(1.1–2.5)	(16.0–26.0)	(5.0–55.8)
Gymnoscopelus nicholsi (9)	14.98±1.03	37.50±7.14	32.54±10.7	3.82±0.83	39.54±13.5	8.31±3.08
100 m	(11.23–16.9)	(13.28–47.16)	(14.7–58.2),8	(1.9–5.8)	(16.2–88.5)	(4.9–31.4),8
Gymnoscopelus opisthopterus (3)	15.57±0.24	36.72±2.75	21.2±13.03	0.67±0.26	51.5±43.6	24.9±11.5
150 m	(15.4–15.8)	(33.99–38.66)	(13.2–34.4)	(0.4–0.8)	(32.1–184.9)	(17.2–36.4)
Arabian Sea						
Benthosema pterotum (23)	2.05±0.35	0.1825±0.0930	126.0±34.9	3.61±0.89	73.7±23.3	36.3±12.1
D 130–300 m	(0.99–3.61),19	(0.0133–0.7144),19	(24.3–296.2)	(1.16–8.80)	(3.5–238.9)	(23.6–57.5),5
N 10–200 m (25 m)						
Diaphus dumerilii (4)	3.38±0.12	0.5286±0.0221	126.1±50.1	4.63±1.58	62.7±21.8	
D 225–750 m	(3.25–3.54)	(0.4955–0.5451)	(64.8–189.9)	(2.56–6.47)	(36.0–89.2)	
N upper 125 m (75 m)						
Diaphus suborbitalis (2)	1.74±0.18	0.0885±0.0361	17.4±5.3	3.35±0.69	26.7±12.3	111.3±113.2
	(1.61–1.86)	(0.0629–0.114)	(14.7–20.1)	(3.02–3.68)	(20.4–33)	(53.6–169.1)
Lampanyctus sp. (2)	1.78±0.00	0.0429±0.0078	73.9	4.25±2.25		345.2±100.98
		(0.0373–0.0484)	n=1	(3.14–5.4)		(293.7–396.7)
Nannobrachium ater (8)	2.10±0.215	0.082±0.029	40.96±14.9	2.84±0.52	28.8±15.1	114.4±65.6
D 680–1200 m	(1.81–2.82)	(0.0423–0.1723)	(15.1–165.3),7	(1.77–3.77)	(13.9–80.7)	(19.4–240.3),7
N 50–925 m (50 m)						
Nannobrachium lineatum (3)	2.59±0.54	0.1950±0.0231	54.4±27	2.83±0.53	21.4±11.3	
D 650–1000 m	(2.05–2.96)	(0.1748–0.2156)	(27.4–72.8)	(2.27–3.22)	(12.2–32.1)	
N 60–225 m (100 m)						
Triphotorus microchir (2)	1.91±0.30	0.0563±0.0430	65.4±23.5	3.24±1.27	50±48.2	224.1±21.5
	(1.69–2.12)	(0.0340–0.0786)	(53.4–77.4)	(2.57–3.91)	(25.4–74.6)	(213.1–235.1)
Gulf of Mexico						
Benthosema suborbitale (13)	2.67±0.14	0.27±0.04	34.4±10.6	6.21±0.70	15.0±5.3	66.7
D 400–600 m	(2.16–3.08)	(0.1501–0.4069)	(17.1–69.8)	(4.68–8.28)	(8.9–45.0)	n=1
N 30–105 m (30 m)						
Diaphus dumerilii (12)	3.89±0.61	0.88±0.32	122.5±36.2	8.78±0.84	45.7±19.8	104.4±53.2
D 300–600 m	(1.98–5.31)	(0.0862–1.4927)	(66.4–250.7)	(6.47–11.34)	(14.0–120.4)	(17.8–121.4)
N 50–155 m (75 m)						
Diaphus lucidus (9)	5.35±1.19	3.19±2.1	35.06±6.1	7.25±1.33	40.3±4.8	9.84±5.9
D 450–1000 m	(3.15–8.54)	(0.4755–9.8242)	(27.1–53.1)	(2.91–9.30)	(31.6–51.7)	(2.5–25.80)
N 60–300 m (75 m)						
Diaphus splendidus (4)	6.37±0.7	3.69±1.28	52.8±17.6	5.37±1.82	30.3±9.2	6.8±3.2
D 300–600 m	(5.47–7.22)	(2.22–5.39)	(28.2–71.3)	(3.98–8.07)	(24.3–44.3)	(3.7–11.5)
N 50–110 m (75 m)						
Lampanyctus alatus (7)	3.7±0.34	0.48±0.16	39.1±12	5.65±1.00	33.5±9.3	51.2±11.0
D 350–900 m	(2.98–4.14)	(0.21–0.74)	(16.3–60.6)	(3.10–6.94)	(18.8–57.2)	(32.1–72.3)
N 75–155 m (100 m)						
Nannobrachium lineatum (11)	7.6±1.3	3.59±1.62	12.8±4.0	1.12±0.18	17.6±11.5	11.7±4.4
D 600–1000 m	(4.75–12.02)	(0.75–10.14)	(3.8–26.4)	(0.73–1.73)	(7.1–75.2)	(3.5–23.5)
N 110–300 m (100 m)						
California						
Nannobrachium ritteri (13)	6.21±0.71	2.457±0.884	20.32±7.32	1.49±0.0.15	17.25±4.59	23.9±7.1
75 m	(4.19–9.17)	(0.6093–6.364)	(5.9–54.3)	(1.0–1.9)	(6.3–32.6)	(1.9–40.0),11
Stenobrachius leucopsaurus (8)	4.72±0.85	1.32±0.79	38.6±15.2	2.4±0.7	34.9±9.2	25.5±9.6
25 m	(3.67–7.11)	(0.54–3.76)	(14.3–83.5)	(1.4–4.6)	(16.9–53.1)	(6.3–51.1)
Tarletonbeania crenularis (5)	4.16±1.33	0.89±0.78	82.8±18.1	5.6±1.8	71.4±31.5	29.3±24.9
0 m	(2.90–6.30)	(0.25–2.27)	(54.0–107.1)	(3.4–7.7)	(41.4–129.2)	(7.0–77.9)
Triphotorus mexicanus (5)	5.97±0.83	1.86±0.63	47.7±15.4	4.8±3.1	39.1±19.4	17.5±8.4
25 m	(4.67–7.15)	(0.93–2.72)	(61.0–151.6)	(1.9–9.4)	(20.7–70.1)	(5.8–30.9)

LDH, lactate dehydrogenase; CS, citrate synthase; MDH, malate dehydrogenase; ADH, alcohol dehydrogenase. Activities are expressed as micromoles of substrate converted to product per minute per gram wet mass ($U\,g^{-1}$ wet mass).

All values are means ±95% confidence interval. Values are reported for Antarctic and California fishes at 10°C, Arabian Sea and Gulf of Mexico fishes at 20°C (assay temperatures). Numbers in parentheses are ranges, followed by sample size if it differs from that given in parentheses by the species name.

D and N denote vertical distribution for day and night, respectively. Vertical distribution is given under the species name for Arabian Sea fishes (Smith and Heemstra, 1991) and Gulf of Mexico fishes (Gartner et al., 1987); minimum depth of occurrence follows the night value (Macdonald 1998). Vertical distributions for Antarctic and California species are minimum depths of occurrence (Torres and Somero, 1988; Childress and Nygaard, 1973).

calculated from the slope of a line tracing the decrease in absorbance of NADH at 340 nm.

CS [EC 4.1.3.7; citrate: oxaloacetate lyase (CoA-acetylating)]

CS activity (acetyl-CoA + oxaloacetate + H_2O ↔ citrate + HS-CoA + H^+) was assayed in a medium containing 42.5 mmol l^{-1} imidazole buffer (pH 8.0 at 10°C), 0.2 mmol l^{-1} 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB), 1.5 mmol l^{-1} $MgCl_2 \cdot 6H_2O$ and 124 μmol l^{-1} acetyl-CoA. A 40 μl sample of tissue homogenate was added to 1 ml of the assay medium, and the rate of absorbance increase at 412 nm was examined until the background level was zero. The enzyme reaction was then initiated by adding 12.5 μl of 40 mmol l^{-1} oxaloacetate and monitored for 4 min. The reaction rate was calculated from the slope of a line tracing the increase in absorbance at 412 nm due to the reaction of reduced acetyl-CoA with DTNB.

MDH (EC 1.1.1.37; L-malate: NAD^+ oxidoreductase; malic dehydrogenase)

MDH activity (oxaloacetate + NADH ↔ malate + NAD^+) was assayed in a medium containing 40 mmol l^{-1} buffer (200 mmol l^{-1} Tris, 200 mmol l^{-1} $MgCl_2 \cdot 6H_2O$, pH 7.5 at 20°C), 162 μmol l^{-1} NADH and 200 μmol l^{-1} oxaloacetate. A 10 μl sample of tissue homogenate was added to 1 ml of the assay medium, and the reaction was monitored for 1 min. The reaction rate was calculated from the slope of a line tracing the decrease in absorbance of NADH at 340 nm.

ADH (ADH, EC 1.1.1.1; alcohol: NAD^+ oxidoreductase)

ADH activity (acetaldehyde + NADH ↔ ethanol + NAD^+) was assayed in a medium containing 100 mmol l^{-1} potassium phosphate buffer (pH 7.0), 8.9 mmol l^{-1} acetaldehyde, 1 mmol l^{-1} glutathione (reduced form) and 0.2 mmol l^{-1} NADH. A 10 μl sample of tissue homogenate was added to 1 ml of the assay medium, and the reaction was monitored for 1 min. The reaction rate was calculated from the slope of a line tracing the decrease in absorbance of NADH at 340 nm.

Statistical analysis and system comparisons

All statistical analyses were conducted using Statistica versions 7.1 and 8.0. Initially, the entire data set was subjected to principal components analysis (PCA) to discern major sources of variability. Variables examined were region, species, minimum depth of occurrence, temperature, minimum dissolved oxygen and individual mass. The data set was then examined for variability in enzyme activities between regions. Between-region variability was assessed between each pair of systems most similar to one another in temperature using ANCOVA with species mass as a covariate: Arabian Sea and Gulf of Mexico, and Antarctic and California. ANCOVA were run using the entire data set from each system, i.e. each individual value was given equal weight. Values for all species within each system were combined to produce a global mean for each. Numbers of individuals for each species are given in Table 1. A second inter-regional ANCOVA was performed using the mean values for each species (i.e. giving each species equal weight) to evaluate the influence of individual species on the overall trends observed between regions.

Cut-off for statistical significance was at the $P>0.05$ level.

Oxygen and temperature profiles

Oxygen and temperature profiles for each of the study regions were generated using published information (Arabian Sea, California Borderland, Gulf of Mexico) or cruise data (Antarctic). They are presented in Fig. 1 for reference on the character of the oxygen minima in the different regions. For each region, fish vertical distributions were determined from published information (Arabian Sea, California Borderland, Gulf of Mexico) or from a combination of published data and cruise data (Antarctic) (Table 1). Literature citations can be found in the legends of Fig. 1 and Table 1.

RESULTS

Oxygen and temperature profiles and fish distributions

Oxygen profiles differed considerably in the four study regions (Fig. 1). Two (Antarctic and Gulf of Mexico) may be considered typical or normoxic for open ocean systems, showing an oxygen concentration of about 50% of surface (air-saturated) values between 200 and 1000 m depth. The two other oxygen minima were considerably more severe. In the Arabian Sea, oxygen concentrations of 0 ml l^{-1} characterized the entire depth stratum between 200 and 1000 m. In the California Borderland, the oxygen profile reached 1.0 ml l^{-1} at 400 m depth, declined to a value of 0.2 ml l^{-1} (about 3% of air saturation) at a depth of 700 m, and rose again to 1.0 ml l^{-1} at 1000 m.

Three of the four systems (Arabian Sea, California Borderland, Gulf of Mexico) exhibited a highly stratified thermal profile with a well-developed permanent thermocline resulting in declining temperatures with increasing depth. In contrast, temperature changed little with depth (about 2°C) in the Antarctic region with lower (–0.5°C) temperatures at the surface than at 200 m (1.5°C).

Fish vertical distributions are presented in Table 1. All were lanternfishes (Myctophidae) exhibiting a night-time vertical distribution in the upper 150 m and a center of daytime distribution between 300 and 1000 m, making them strong diel vertical migrators. The daytime distributions of all species overlapped the oxygen minimum layer in their respective system.

PCA

The first two axes of the principal components analysis explained 77% (60.4% for axis 1 and 16.2% for axis 2) of the variability in the environmental data set, with 'region' and dissolved oxygen at the core of the oxygen minimum ('DO-MIN') acting as the two most important variables, each accounting for about 20% of the total variability in the first axis (Table 2). 'Species' and environmental temperature at the minimum depth of occurrence ('TMDO') accounted for 18% and 17%, respectively, while species mass ('mass') and minimum depth of occurrence ('MDO') were least important at 16% and 9%, respectively. Based on the results of PCA and established principles of temperature adaptation, it was deemed most sensible to compare enzyme activities in the two pairs of regions most similar in temperature: the Arabian Sea and Gulf of Mexico, and the Antarctic and California Borderland. The pairs kept system temperatures and species masses most similar while contrasting normoxic systems with severely hypoxic ones.

Enzyme activities

Mean enzyme activities for all species are shown in Table 1. Enzyme activities are reported at assay temperature for each pair of systems: 20°C for the Arabian Sea and Gulf of Mexico and 10°C for the Antarctic and California Borderland. In three out of the four systems the assay temperature represented an intermediate temperature within the normal range of the vertical migrators that were our experimental subjects (Fig. 1, Table 1). The exception was the Antarctic system, where the assay temperature was outside that encountered by the fishes (maximum temperature 2°C) (Klinck et al., 2004), though well within the temperature capabilities of the enzymes (Torres and Somero, 1988b; Simoniello, 2003).

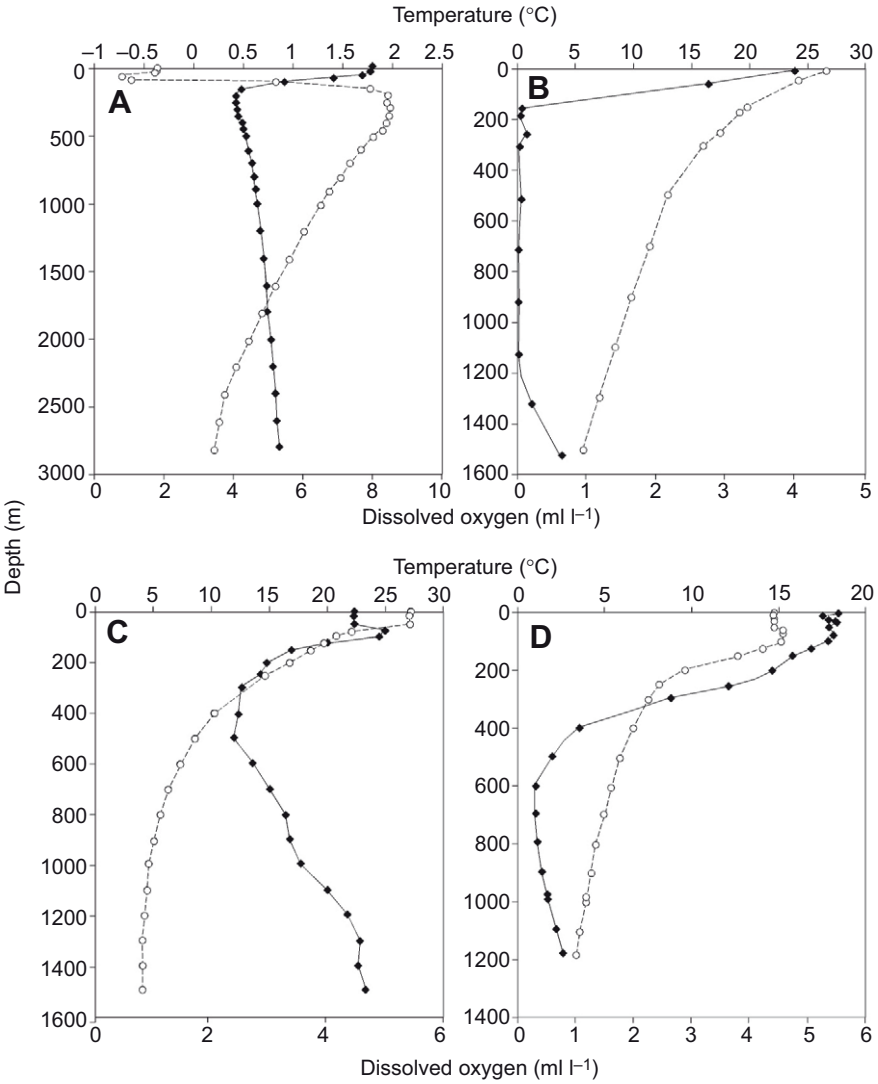

Fig. 1. Dissolved oxygen (solid line) and temperature (dashed line) *vs* depth in the Antarctic (A), Arabian Sea (B), Gulf of Mexico (C) and California Current (D). Antarctic temperature–oxygen profile is a composite from NBP cruise 02-04, US Southern Ocean GLOBEC program, vic. Palmer Peninsula shelf. Arabian Sea data from Hitchcock et al. (Hitchcock et al., 1997). Gulf of Mexico data from Emilsson (Emilsson, 1975). California Current data from CalCOFI (Scripps Institution of Oceanography, 1960).

Comparisons between regions

Arabian Sea *vs* Gulf of Mexico

Fig. 2 shows a composite of four ANCOVA with each panel comparing a different enzyme between the two systems. The ANCOVA technique employed was a one-way ANOVA, taking into account variability in species mass as a covariate. The comparison between the Arabian Sea and the Gulf of Mexico was a near-ideal one to show the effects of environmental oxygen on enzyme activities. Temperature profiles were very similar in each system, and the species composition contained many genera common to both (Table 1). The main difference between the systems was the radically different oxygen profile. Oxygen availability showed a profound influence on the activities of the four enzymes assayed in the two warm water systems. LDH, the terminal step in anaerobic glycolysis, showed very much higher activity in the Arabian Sea than the Gulf of Mexico (P<0.05), suggesting a well-developed anaerobic capability in the Arabian Sea fishes. The result was corroborated by the relatively high activity of ADH in Arabian Sea fishes, being approximately 3.5 times that of fishes from the Gulf of Mexico (Fig. 2), which produced a highly significant (P<0.05) difference between the two regions. CS activity was over twice as high in Gulf of Mexico fishes as it was in Arabian Sea fishes (Fig. 2), suggesting a very pronounced aerobic poise in the migrators dwelling in the Gulf of Mexico. CS catalyzes the first and rate-limiting step in the Krebs' cycle. As such, its activity is a good proxy for activity of the aerobic pathway. MDH was significantly higher (about 1.5 times; P<0.05) in Arabian Sea fishes than in those from the Gulf of Mexico. The activity of MDH in Arabian Sea fishes mirrors the high activity of the glycolytic pathway indicated by the LDH results.

Antarctic *vs* California Current

Enzyme activities in fishes from the Antarctic and California Current showed effects of both temperature and oxygen, making comparisons between the systems less straightforward. The four ANCOVA shown in Fig. 3 represent enzyme activities at the common assay temperature of 10°C. Thus, for Antarctic fishes, the activities are about twice what would be expected at environmental temperature (0°C), assuming a Q_{10} of 2.0.

LDH activity was significantly higher in Antarctic than California fishes (P<0.05, ANCOVA) at the common assay temperature of 10°C. The high LDH activity observed in Antarctic fishes was due largely to the contribution by *Electrona antarctica* (Table 1), the most active species, which had the highest LDH activity, the highest number of fish analyzed and the greatest degree of temperature adaptation (Torres and Somero, 1988b). The activities of MDH and CS in Antarctic fishes were not significantly different from those of California species when assayed at the same temperature (Fig. 3), though the mean MDH and CS activities were slightly higher in

Table 2. Contributions to data variability in the first two axes of the principal components analysis

	Factor 1	Factor 2
Region	0.1963	0.2276
Species	0.1777	0.2865
MDO	0.0937	0.3187
TMDO	0.1707	0.0181
DO-MIN	0.2029	0.0219
Mass	0.1587	0.1272

MDO, minimum depth of occurrence; TMDO, temperature at minimum depth of occurrence; DO-MIN, dissolved oxygen at the core of the oxygen minimum in each region; Mass, mass of individual fishes analyzed in each region. See Results for details.

California species. In the last and most important comparison, ADH activity was significantly higher in California than in Antarctic fishes (Fig. 3; $P<0.05$, ANCOVA) despite the influence of assay temperature, suggesting that a higher activity of ADH may confer an advantage in the low-oxygen concentrations ($0.2\,ml\,l^{-1}$) of the California Borderland.

To evaluate the influence of *Electrona*, a second inter-regional ANCOVA was run for all enzymes, with each species weighted equally; that is, comparing only the mean values for each species. In that analysis, Antarctic and California species' LDH values were nearly identical ($P>0.05$, ANCOVA), with the remaining enzyme comparisons showing little change from the trends observed in Fig. 3. A similar ANCOVA comparing mean values for the suite of Arabian Sea and Gulf of Mexico species showed no change in the trends described above.

DISCUSSION

The lanternfish species from the Arabian Sea and their confamilials from the California Current, the Gulf of Mexico and the Antarctic are strong vertical migrators, moving from mid-water depths (200–1000 m), where they spend daylight hours, to near-surface waters at night where they feed, most often on zooplankton (Pearcy et al., 1977; Gjøsaeter, 1984; Torres and Somero, 1988a; Lancraft et al., 1989; Hopkins and Gartner, 1992; Luo et al., 2000). Vertical migration is a foraging strategy used by lanternfishes all over the world (Robinson et al., 2010), making the four regions of the present study natural experimental systems that differed markedly in their dissolved oxygen and temperature profiles, but very little in the daily habits of their common species. Thus, the experimental subjects for all four regions were ecological analogs. Physiological responses to two important physical variables, oxygen and temperature, were in play.

Within the Arabian Sea and Gulf of Mexico, oxygen was clearly the physical variable with the most influence. Temperature profiles in the two systems were virtually identical (Fig. 1) but the oxygen profiles differed radically. In the Arabian Sea system, oxygen disappeared at a depth of 200 m and remained at zero to a depth of 1000 m, whereas in the Gulf of Mexico, oxygen dropped to about half of surface (air-saturation) values over the same range (Fig. 1). In both systems, fishes resided in the oxygen minimum during daylight hours. However, in the Arabian Sea the myctophids would have been constrained to anaerobiosis but in the Gulf of Mexico oxygen concentrations were well within the myctophid's normoxic range (Donnelly and Torres, 1988). The species' biochemical strategies for dealing with their respective oxygen profiles are reflected in their enzymatic activities. LDH, the terminal enzyme in the glycolytic pathway during anaerobiosis, is about three times higher in Arabian Sea fishes than in those from the Gulf of Mexico, suggesting a strong anaerobic capability (Fig. 2). In contrast, the much higher activity of CS in the Gulf of Mexico fishes indicates a highly aerobic strategy, made possible by the mild oxygen minimum found in the Gulf of Mexico. ADH activity in Arabian Sea fishes was also about three times that observed in fishes from the Gulf of Mexico, giving the fishes the biochemical machinery to convert lactate, which cannot be excreted at the gills, to ethanol, which can (Shoubridge and Hochachka, 1980; Vornanen et al., 2009).

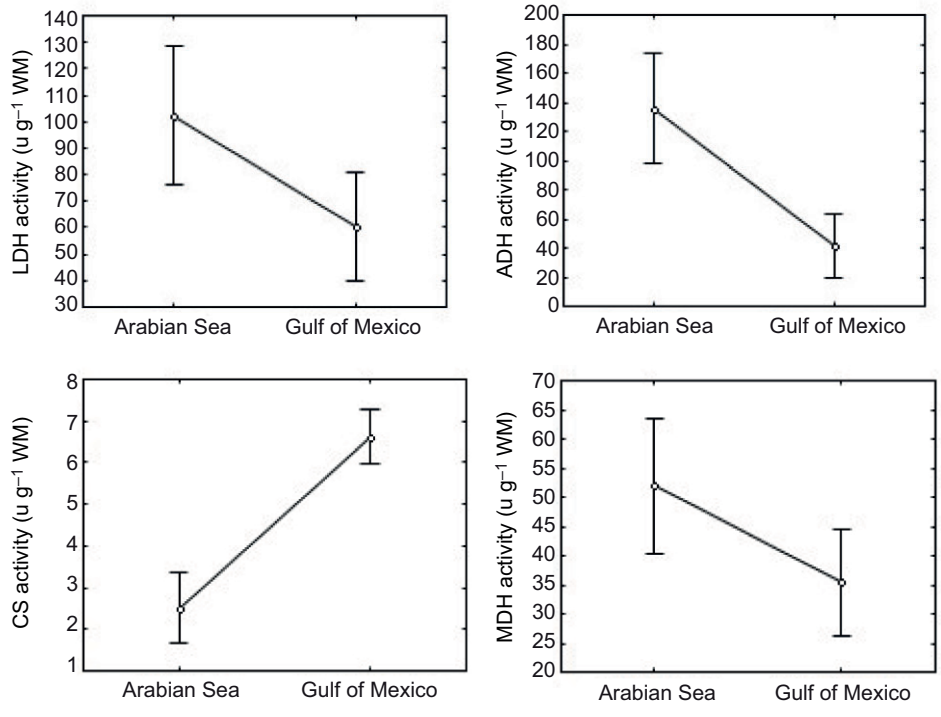

Fig. 2. ANCOVA comparing activities of lactate dehydrogenase (LDH), alcohol dehydrogenase (ADH), citrate synthase (CS) and malate dehydrogenase (MDH) in lanternfishes from the Arabian Sea and Gulf of Mexico. Mean values are shown ±95% confidence limits. WM, wet mass. All means are significantly different between the two regions ($P<0.05$, ANCOVA). See Results for details.

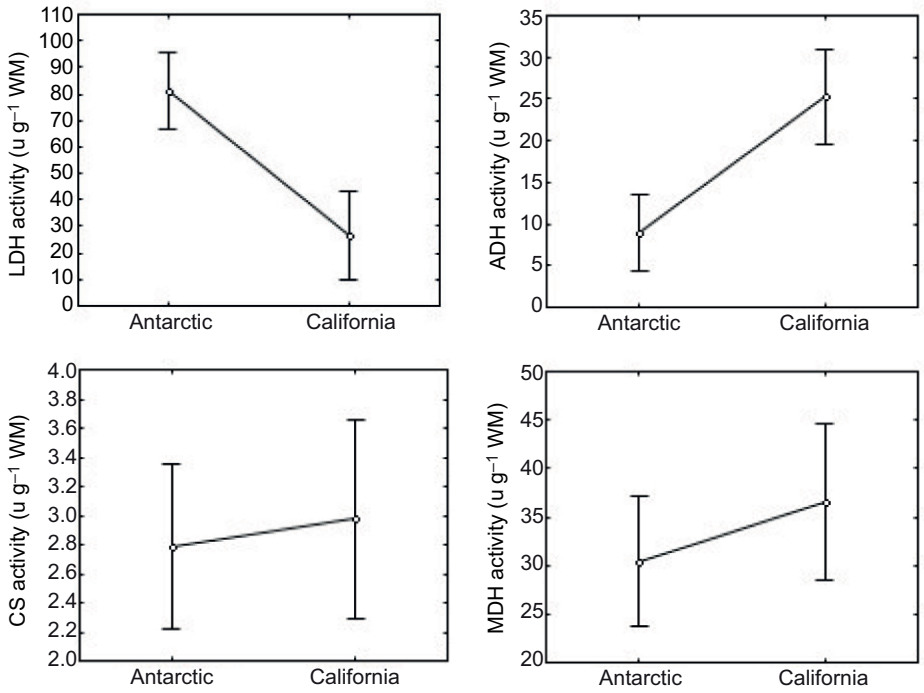

Fig. 3. ANCOVA comparing activities of LDH, ADH, CS and MDH in lanternfishes from the Antarctic and California Borderland. Mean values are shown ±95% confidence limits. Means are significantly different between the two regions (P<0.05, ANCOVA) for LDH and ADH, but not for CS and MDH. See Results for details.

The current model for conversion of lactate to ethanol in goldfish and carp (e.g. Shoubridge and Hochachka, 1980; Vornanen et al., 2009) requires first that lactate be oxidized to pyruvate in the presence of NADH, a thermodynamically uphill step ($\Delta G'^{o}$=+25.1 kJ mol^{-1}) (Lehninger, 1970). Pyruvate is subsequently converted to acetaldehyde within the mitochondrion by the pyruvate dehydrogenase complex, with the release of CO_2. Acetaldehyde is then reduced to ethanol by ADH in the presence of NADH (Fig. 4). The biochemical machinery for the formation of ethanol is located exclusively in the swimming muscle, which processes not only the lactate generated within the muscle but also that produced by other tissues such as the brain, liver and heart and delivered to the muscle *via* the bloodstream (Shoubridge and Hochachka, 1980; Vornanen et al., 2009) (Fig. 4). The activity of ADH found in the muscle of the Arabian Sea fishes suggests a similar strategy.

Skeletal muscle of fishes plays an important role in the post-exercise processing of lactate through the gluconeogenic and glycogenic pathways, a situation very different from that in mammals, where the enzymatic machinery for regenerating glucose is primarily found in the liver (Suarez et al., 1986; Moon, 1988; Gleeson, 1996). The muscle of fish is thus more multi-functional than that of mammals, possessing a wider array of enzymes and greater inherent metabolic flexibility than mammalian muscle, including a greater potential for biosynthesis (Gleeson, 1996). In fact, fish skeletal muscle has been shown to sequester lactate post-exercise (Gleeson, 1996), facilitating the regeneration of glucose within the muscle and enhancing post-exercise recovery. In the case of the goldfish, crucian carp and now the lanternfishes, the metabolic flexibility of the muscle enzyme suite allows for novel biochemical solutions to the problem of anaerobiosis.

MDH activity was also significantly higher in Arabian Sea fishes than in those from the Gulf of Mexico, but the multiple roles of MDH in the cell make the explanation for this considerably less straightforward. Muscle tissue was homogenized with ground glass homogenizers, which would allow both cytosolic and mitochondrial MDH to be active in the assay cocktail. The activities reported in Table 1 were therefore a combination of MDH activity from the two cellular compartments. In aerobic metabolism, MDH is clearly important as the enzyme catalyzing the formation of oxaloacetate in the Krebs' cycle. In addition, during aerobic conditions, cytosolic and mitochondrial MDH work in tandem to shuttle reducing equivalents produced by glycolysis into the mitochondrion for processing in the electron transport system, a necessity due to the impermeability of the mitochondrial membrane to cytosolic NADH. A third role of MDH is in catalyzing an important step in the conventional gluconeogenic pathway. As it happens, in fish muscle, gluconeogenesis is believed to proceed by a nearly direct reversal of glycolysis (Suarez et al., 1986; Moon, 1988; Gleeson, 1996) and MDH is not an important participant.

What is most likely is that MDH helps in maintaining redox balance within the cell during periods of transition from normoxic to anoxic conditions as the fishes migrate downward into the oxygen minimum during daylight hours and again as the fishes migrate upwards toward the higher oxygen of surface waters at night. The malate shuttle can move NADH either into or out of the mitochondrion depending on the cellular needs for reducing power.

Lanternfish species of the California Borderland and the Antarctic showed similar trends to their confamilials of the Arabian Sea and Gulf of Mexico, with the caveat that temperature as well as oxygen influenced the results. *Electrona antarctica*, the strongest and most active vertical migrator among the Antarctic species also showed the highest LDH activity, giving the Antarctic species as a whole a significantly higher value than those from California at the same assay temperature. The influence of temperature adaptation in a robust active fish (Torres and Somero, 1988b; Yang and Somero, 1993; Vetter and Lynn, 1997) is the most likely explanation for its high value. If the LDH activity of *Electrona* is considered at its environmental temperature of 0°C, or half of the value in Table 1 (54 U g^{-1} wet mass), it is squarely in the mid-range of values for the California species at 10°C, underscoring the upward influence of temperature adaptation on the LDH activity in *Electrona*. Neither CS nor MDH activity showed significant differences between

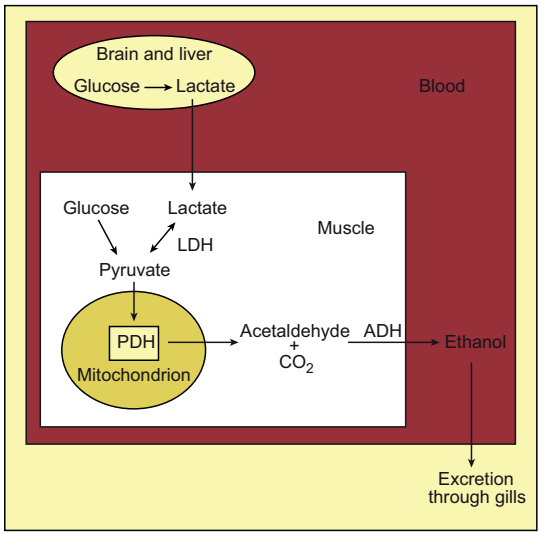

Fig. 4. The ADH pathway in goldfish and crucian carp. Skeletal muscle acts as a clearing house for lactate produced in brain, heart and liver during anaerobiosis. Lactate is taken up by muscle and converted to pyruvate by LDH. Pyruvate reaching the muscle *via* the bloodstream and that produced during glycolytic activity in the muscle is processed by the pyruvate dehydrogenase (PDH) pathway within the mitochondrion to produce acetaldehyde and CO_2; acetaldehyde is converted to ethanol within the cytosol, and ethanol can then diffuse out of the cell to be excreted at the gills. Model adapted from Shoubridge and Hochachka (Shoubridge and Hochachka, 1980); figure after Vornanen et al. (Vornanen et al., 2009) with permission from Elsevier.

California and Antarctic species at the assay temperature of 10°C, though mean values for California species were higher in both cases.

ADH activity was significantly higher in the California species despite the influence of assay temperature, which, as with LDH, would have tended to skew the Antarctic values upward. With ADH, the most likely influence on overall enzyme activities was the California oxygen minimum, which at its core (700 m; Fig. 1) exhibits a value of 0.2 ml l^{-1}. Vertical migrations of California lanternfishes would bring them to daytime oxygen values ranging between 0.5 and 1.0 ml l^{-1} (16–32 Torr, ~2.1–4.2 kPa), well below their abilities to efficiently extract oxygen (cf. Torres et al., 1979; Donnelly and Torres, 1988).

Lanternfishes from the Arabian Sea and the California Borderland encounter zero to near-zero oxygen concentrations at their daytime depths, after a swim of at least 5000 body lengths during their downward excursion (assuming a minimum swim of 300 m and a body length of about 5 cm; Table 1). Their most energetically efficient recourse would be to keep swimming activity within the oxygen minimum to the lowest possible level so as to minimize the need for anaerobiosis. Most facultative anaerobes (e.g. bivalves) (Hochachka, 1980; Hochachka and Somero, 2002) exhibit a marked downturn in activity during anaerobiosis as a result of the high energy cost of even the most efficient anaerobic pathways. Minimal activity minimizes the use of stored energy. When coupled with use of the ADH pathway, end-product accumulation would also be minimized by the excretion of ethanol at the gills, all the while maintaining redox balance within the muscle cells. The excursion upward at dusk into normoxic waters can then be initiated with a relatively clean 'metabolic slate' with the malate shuttle aiding in the transition as described above.

Diel vertical migration is an adaptation displayed by many species of zooplankton and micronekton in addition to the lanternfishes (Hopkins et al., 1994; Hopkins et al., 1996; Robinson et al., 2010), presumably to minimize visual predation. As visual acuity and risk of attack by visual predators are greatly increased during daylight hours, many animals only enter the food-rich surface waters at night, migrating to the darker depths at dawn. Species residing within oxygen minima, or migrating into them, are coping with a stable physical feature (Stramma et al., 2008) that can be addressed through physiological and biochemical adaptation. In the case of the lanternfishes studied in the present work, the oxygen minimum provides a refugium from pelagic predators that would have the same difficulty hunting in the oxygen minima of the Arabian Sea or California Borderland as they do in the dead zones of the coastal ocean.

Mid-water fishes of the open ocean differ considerably from coastal and freshwater fishes in the timing and character of their exposure to low or zero oxygen. The most accomplished piscine anaerobe (and best studied), the crucian carp, undergoes a predictable sojourn at zero oxygen during the winter months in its native habitat of central Asia and Europe (Vornanen and Paajanen, 2004). Individuals inhabiting small eutrophic ponds over-winter under ice and snow cover that preclude atmospheric exchange, resulting in periods of anoxia that can last 90 days or more (Piironen and Holopainen, 1986). The fact that exposure to anoxia is a seasonal event allows for metabolic and morphological adjustment to take place prior to the drop in environmental oxygen. Metabolic adjustment includes the accumulation of glycogen in liver, muscle, brain and heart to fuel the need for anaerobic activity. Morphological adjustments include an increase in the number of gill lamellae, resulting in a 7-fold increase in gill surface area (Vornanen et al., 2009) to facilitate oxygen exchange. Perhaps most astonishing is the fact that heart and brain of crucian carp remain fully functional during anoxia, a strategy that is unique among the vertebrates, allowing the ethanol flushed from muscle tissues to be delivered for excretion at the gills, and the distribution of glucose from liver stores to sites in need (Vornanen et al., 2009).

Coastal marine fishes encounter hypoxia in a variety of natural and anthropogenically influenced situations. Tidal pools or channels in areas that have decaying vegetation, such as in mangrove stands, or in intertidal areas with organically rich mud, can go anoxic at low tide, sometimes exposing native species to sulfide (Bagarinao and Vetter, 1989; Graham, 1997; Geiger et al., 2000). In those fishes that are equipped to do so, air breathing is a viable option to minimize the effects of anoxia and sulfide exposure (Graham, 1997; Geiger et al., 2000). Others exploit the biochemical option of mitochondrial oxidation of the sulfide to minimize its toxic effects, similar to species at the hydrothermal vents (Bagarinao and Vetter, 1989). In most naturally occurring hypoxic systems, excursions to zero oxygen are either episodic, as in mangrove tidal pools, or occur regularly at low tide during the warmer months of the year. Those situations are predictable and often enough to be accommodated by physiological adaptation. Species that live in environments subject to natural hypoxia are physiologically poised to deal with it.

Anthropogenically induced or enhanced hypoxic events, or dead zones, are occurring with greater regularity in the coastal oceans and bays of North America, Europe and Asia (Diaz and Rosenberg, 2008). Dead zones are usually associated with nutrient run-off and summer stratification in shallow marine systems (Diaz and Breitburg, 2009; Rabalais et al., 2002). Nutrient loading accelerates production, and mixing down of atmospheric oxygen is inhibited by water column stratification. Below the mixed layer, microbial degradation of sinking organic matter severely depletes the oxygen, in some cases removing all value from the bottom and near-bottom

habitat (Diaz and Breitburg, 2009). Survival of fishes in hypoxic zones is determined by species' effectiveness in removing oxygen at reduced P_{O_2} (Chapman and McKenzie, 2009; Perry et al., 2009; Richards, 2009), which varies considerably across taxa (Richards, 2009). In no case is prolonged anaerobiosis used by fishes as a strategy for coping with dead zones.

If there are small but consistent amounts of oxygen in the water, such as in an oxygen minimum zone, animals have developed mechanisms for extracting and using it. Three mechanisms are increased gill surface area, an efficient circulatory system and a high affinity blood pigment. Large gill surface areas have been measured in fishes that inhabit the California Current oxygen minimum layer (Ebeling and Weed, 1963; Gibbs and Hurwitz, 1967). *Gnathophausia ingens*, a lophogastrid crustacean resident in this area, in addition to having highly developed gills and an efficient circulatory system (Childress, 1971; Belman and Childress, 1976), has a high-affinity hemocyanin with a large positive Bohr effect and high co-operativity. It is able to load oxygen at a partial pressure of 6 Torr (~0.8 kPa); its high co-operativity allows the pigment to unload oxygen at the tissues across a very small diffusion gradient (Sanders and Childress, 1990).

The lanternfishes comprise a speciose, pan-global pelagic fish family, the great majority of which are vertical migrators (Nelson, 2006; Smith and Heemstra, 1991). Their geographical distributions include all the severe oceanic oxygen minima, two of which were treated in the present study. We propose that, rather than utilizing the strategy of those species that remain permanently within the severest portions of the oxygen minimum layer, which requires a considerable physiological and morphological investment (Childress and Seibel, 1998), they are capable of using the ADH pathway to minimize end-product accumulation while using anaerobiosis for part of the day to cope with the demands of the oxygen minimum. The anaerobic strategy is reminiscent of that used by intertidal species during low tides (cf. Hochachka, 1980; Torres et al., 1977), but with the advantage of a more predictable and controllable entry into low oxygen. This is the first report of ADH activity in the muscle of fishes other than the goldfish and carp, a largely unlooked-for anaerobic strategy that may be more widespread.

ACKNOWLEDGEMENTS

This paper is dedicated to the memory of Peter W. Hochachka. The captains and crews of the Pelican, Suncoaster and Malcolm Baldridge helped make the research possible. Joe Donnelly and Joel Bellucci, our colleagues at sea and in the lab, were always there for help and good cheer. Thanks to Steve Geiger for helping us bring the ADH assay up to speed in the lab. Thanks to Sharon Smith for providing hydrographic data and cruise reports. Many thanks to Carlie Williams and Chad Emisten for their help in creating figures. Thanks most of all to George N. Somero for introducing us to the many creative pathways used by animals faced with low oxygen.

FUNDING

This research was supported by the National Science Foundation [grant number OPP 9910100 and OCE 0727883 to J.J.T.].

REFERENCES

Bagarinao, T. and Vetter, R. D. (1989). Sulfide tolerance and detoxification in shallow-water marine fishes. *Mar. Biol.* **103**, 251-262.
Belman, B. W. and Childress, J. J. (1976). Circulatory adaptations to the oxygen minimum layer in the bathypelagic mysid *Gnathophausia ingens*. *Biol. Bull.* **150**, 15-37.
Blazka, B. (1958). The anaerobic metabolism of fish. *Physiol. Zool.* **31**, 117-128.
Chapman, L. J. and McKenzie, D. J. (2009). Behavioral responses and ecological consequences. In *Fish Physiology*, Vol. 27, *Hypoxia* (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 26-79. Amsterdam: Academic Press.
Childress, J. J. (1971). Respiratory adaptations to the oxygen minimum layer in the bathypelagic mysid *Gnathophausia ingens*. *Biol. Bull.* **141**, 109-121.
Childress, J. J. and Nygaard, M. H. (1973). The chemical composition of midwater fishes as a function of depth of occurrence off southern California. *Deep Sea Res.* **20**, 1093-1109.
Childress, J. J. and Seibel, B. A. (1998). Life at stable low oxygen levels: adaptations of animals to oceanic oxygen minimum layers. *J. Exp. Biol.* **201**, 1223-1232.
Childress, J. J., Barnes, A. T., Quetin, L. B. and Robison, B. H. (1978). Thermally protecting cod ends for the recovery of living deep-sea animals. *Deep Sea Res.* **25**, 419-422.
Diaz, R. J. and Breitburg, D. L. (2009). The hypoxic environment. In *Fish Physiology*, Vol. 27, *Hypoxia* (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 2-25. Amsterdam: Academic Press.
Diaz, R. J. and Rosenberg, R. (2008). Spreading dead zones and consequences for marine ecosystems. *Science* **321**, 926-929.
Donnelly, J. and Torres, J. J. (1988). Oxygen consumption of midwater fishes and crustaceans from the eastern Gulf of Mexico. *Mar. Biol.* **97**, 483-494.
Ebeling, A. W. and Weed, W. H. (1963). Melamphaidae III. Systematics and distribution of the species in the bathypelagic fish genus *Scopelogadus* Vaillant. *Dana Reports* **60**, 1-58.
Emilsson, I., Alatorre, M. A., Ruiz, F. and Vasquez, A. (1975). Programa cicar datos oceanograficos del Golfo de Mexico crucero 70-12 del B/Oc Cadete Virgilio Uribe Oct-Nov 1970. *Datos Geograficos Serie A*, *Oceanografia 4*. Instituto de Geofisica, Universidad Nacional Autonoma de Mexico.
Gartner, J. V., Hopkins, T. L., Baird, R. C. and Milliken, D. M. (1987). Ecology of the lanternfishes (Pisces: Myctophidae) of the eastern Gulf of Mexico. *Fish. Bull.* **85**, 81-98.
Geiger, S. P., Kawall, H. G. and Torres, J. J. (2001). The effect of the receding ice edge on the condition of copepods in the northwestern Weddell Sea: results from biochemical assays. *Hydrobiologia* **453/454**, 79-90.
Geiger, S. P., Torres, J. J. and Crabtree, R. E. (2000). Air breathing and gill ventilation frequencies in juvenile tarpon, *Megalops atlanticus*: responses to changes in dissolved oxygen, hydrogen sulfide, and pH. *Environ. Biol. Fishes*. **59**, 181-190.
Gibbs, R. H. J. and Hurwitz, B. A. (1967). Systematics and zoogeography of the stomiatoid fishes, *Chauliodus pammelas* and *C. sloani*, of the Indian Ocean. *Copeia* **4**, 798-805.
Gjosaeter, J. (1984). Mesopelagic fish: a large potential resource in the Arabian Sea. *Deep Sea Res.* **31**, 1019-1035.
Gleeson, T. T. (1996). Post-exercise lactate metabolism: a comparative review of sites, pathways, and regulation. *Ann. Rev. Physiol.* **58**, 565-581.
Graham, J. B. (1997). Air breathing fishes-evolution, diversity, and adaptation. San Diego: Academic Press.
Hitchcock, G. L., Wiebinga, C. and Ortner, P. B. (1997). CTD hydrographic data from the Global Ocean Ecosystem Dynamics (GLOBEC) Indian Ocean cruises. Technical Report 97-006, pp. 1-69, University of Miami.
Hochachka, P. W. (1980). *Living Without Oxygen*. Cambridge, MA: Harvard University Press.
Hochachka, P. W. and Somero, G. N. (2002). *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. Oxford: Oxford University Press.
Hopkins, T. L. and Gartner, J. V. (1992). Resource-partitioning and predation impact of a low-latitude myctophid community. *Mar. Biol.* **114**, 185-197.
Hopkins, T. L., Flock, M. E., Gartner, J. V. and Torres, J. J. (1994). Structure and trophic ecology of a low latitude midwater decapod and mysid assemblage. *Mar. Ecol. Prog. Ser.* **109**, 143-156.
Hopkins, T. L., Sutton, T. T. and Lancraft, T. M. (1996). The trophic structure and predation impact of a low latitude midwater fish assemblage. *Prog. Oceanogr.* **38**, 205-239.
Kamykowski, D. and Zentara, S. (1990). Hypoxia in the world ocean as recorded in the historical data set. *Deep Sea Res.* **37**, 1861-1874.
Kinzer, J., Bottger-Schnack, R. and Schulz, K. (1993). Aspects of horizontal distribution and diet of myctophid fish in the Arabian Sea with reference to the deep water oxygen deficiency. *Deep Sea Res. II* **40**, 783-800.
Klinck, J. M., Hofmann, E. E., Beardsley, R. C., Salihoglu, B. and Howard, S. (2004). Water mass properties and circulation on the west Antarctic Peninsula continental shelf in Austral fall and winter 2001. *Deep Sea Res. II* **51**, 1925-1946.
Krygier, E. E. and Pearcy, W. G. (1981). Vertical distribution and biology of pelagic decapod crustaceans off Oregon. *J. Crustacean Biol.* **1**, 70-95.
Lancraft, T. M., Torres, J. J. and Hopkins, T. L. (1989). Micronekton and macrozooplankton in the open waters near Antarctic ice edge zones (AMERIEZ 1983 and 1986). *Polar Biol.* **9**, 225-233.
Lehninger, A. L. (1970). *Biochemistry*. New York: Worth Publishers.
Longhurst, A. R. (1967). Vertical distribution of zooplankton in relation to the eastern Pacific oxygen minimum. *Deep Sea Res.* **14**, 51-63.
Luo, J., Ortner, P. B., Forcucci, D. and Cummings, S. R. (2000). Diel vertical migration of zooplankton and mesopelagic fish in the Arabian Sea. *Deep Sea Res. II* **47**, 1451-1473.
MacDonald, T. C. (1998). *The Influence of Moonlight, Temperature, Hydrostatic Pressure and Zooplankton Abundance Upon the Vertical Distribution and Relative Abundance of Micronekton in the Epipelagic Zone at Night*. MSc thesis, University of South Florida, Tampa, FL.
Moon, T. W. (1988). Adaptation, constraint, and the function of the gluconeogenic pathway. *Can. J. Zool.* **66**, 1059-1068.
Morrison, J. M., Codispoti, L. A., Smith, S. L., Wishner, K., Flagg, C., Gardner, W. D., Gaurin, S., Naqvi, S. W. A., Manghnani, V., Prosperi, L. et al. (1999). The oxygen minimum zone in the Arabian Sea during 1995. *Deep Sea Res. II* **46**, 1903-1931.
Nair, K. K. C., Madhupratrap, M., Gopalakrishnan, T. C., Haridas, P. and Gauns, M. (1999). The Arabian Sea: physical environment, zooplankton and mytophid abundance. *Indian J. Mar. Sci.* **28**, 138-145.
Nelson, J. F. (2006). *Fishes of the World*. Hoboken: John Wiley and Sons.
Pearcy, W. G., Krygier, E. E., Mesecar, R. and Ramsey, F. (1977). Vertical distribution and migration of oceanic micronekton off Oregon. *Deep Sea Res.* **24**, 223-245.
Perry, S. F., Jonz, M. G. and Gilmour, K. M. (2009). Oxygen sensing and the hypoxic ventilatory response. In *Fish Physiology*, Vol. **27**, *Hypoxia* (ed. J. G.

Richards, A. P. Farrell and C. J. Brauner), pp. 194-253. Amsterdam: Academic Press.
Piironen, J. and Holopainen, I. J. (1986). A note on seasonality in anoxia tolerance of crucian carp (*Carassius carassius* L.) in the laboratory. *Ann. Zool. Fennici.* **23**, 335-338.
Rabalais, N. N., Turner, R. E. and Wiseman, W. J. (2002). Gulf of Mexico hypoxia, aka the dead zone. *Ann. Rev. Ecol. Systematics* **33**, 235-263.
Richards, F. A. (1965). Anoxic basins and fjords. In *Chemical Oceanography*, Vol. 1 (ed. J. P. Riley and G. Skirrow), pp. 611-645. London: Academic Press.
Richards, J. G. (2009). Metabolic and molecular responses of fish to hypoxia. In *Fish Physiology*, Vol. 27, *Hypoxia* (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 444-487. Amsterdam: Academic Press.
Robinson, C., Steinberg, D. K., Anderson, T. R., Aristegui, J., Carlson, C. A., Frost, J. R., Ghiglione, J.-F., Hernandez-Leon, S., Jackson, G. A., Koppelmann, R. et al. (2010). Mesopelagic zone ecology and biogeochemistry – a synthesis. *Deep Sea Res. II* **57**, 1504-1518.
Sanders, N. K. and Childress, J. J. (1990). Adaptations to the deep-sea oxygen minimum layer: oxygen binding by the hemocyanin of the bathypelagic mysid, *Gnathophausia ingens* Dohrn. *Biol. Bull.* **178**, 286-294.
Schmidt, J. (1925). On the contents of oxygen in the ocean on both sides of Panama. *Science* **61**, 592-593.
Scripps Institution of Oceanography (1960). *Oceanic Observations of the Pacific, 1950*. Berkeley, CA: University of California Press.
Sewell, R. B. S. and Fage, L. (1948). Minimum oxygen layer in the ocean. *Nature* **162**, 949-951.
Shoubridge, E. A. and Hochachka, P. W. (1980). Ethanol: novel end product of vertebrate anaerobic metabolism. *Science* **209**, 308-309.
Simoniello, C. (2003). The effect of temperature on metabolic characteristics and biochemical structure: indices of thermal sensitivity in mesopelagic fishes. In *Marine Science*, pp. 138. St Petersburg: University of South Florida.
Smith, D. A., Hofmann, E. E., Klinck, J. M. and Lascara, C. M. (1999). Hydrography and circulation of the West Antarctic Peninsula continental shelf. *Deep Sea Res. I* **46**, 925-949.
Smith, M. M. and Heemstra, P. C. (1991). *Smith's Sea Fishes*. Johannesburg: Southern Book Publishers.
Somero, G. N. and Childress, J. J. (1980). A violation of the metabolism-size scaling paradigm: activities of glycolytic enzymes in muscle increase in larger-sized fish. *Physiol. Zool.* **53**, 322-337.
Stramma, L., Johnson, G. C., Sprintall, J. and Mohrholz, V. (2008). Expanding oxygen-minimum zones in the tropical oceans. *Science* **320**, 655-658.
Suarez, R. K., Mallet, M. D., Daxboeck, C. and Hochachka, P. W. (1986). Enzymes of energy metabolism and gluconeogenesis in the Pacific blue marlin, *Makaira nigricans*. *Can. J. Zool.* **64**, 694-697.
Torres, J. J. and Somero, G. N. (1988a). Vertical distribution and metabolism in Antarctic mesopelagic fishes. *Comp. Biochem. Physiol.* **90B**, 521-528.
Torres, J. J. and Somero, G. N. (1988b). Metabolism, enzymic activities, and cold adaptation in Antarctic mesopelagic fishes. *Mar. Biol.* **98**, 169-180.
Torres, J. J., Gluck, D. L. and Childress, J. J. (1977). Activity and physiological significance of the pleopods in the respiration of *Callinassa californiensis* (Dana) (Crustacea: Thalassinidea). *Biol. Bull.* **152**, 134-146.
Torres, J. J., Belman, B. W. and Childress, J. J. (1979). Oxygen consumption rates of midwater fishes as a function of depth of occurrence. *Deep Sea Res.* **26**, 185-197.
Vetter, R. D. and Lynn, E. A. (1997). Bathymetric demography, enzyme activity patterns, and bioenergetics of deep-living scorpaenid fishes (genera *Sebastes* and *Sebastolobus*): paradigms revisited. *Mar. Ecol. Prog. Ser.* **155**, 173-188.
Vinogradov, M. E. (1970). *Vertical Distribution of the Oceanic Zooplankton*, pp. 1-339. Jerusalem: Israel Program for Scientific Translations.
Vornanen, M. and Paajanen, V. (2004). Seasonality of dihydropyridine receptor binding in the heart of an anoxia-tolerant vertebrate, the crucian carp (*Carassius carassius* L.). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287**, R1263-R1269.
Vornanen, M., Steyck, J. A. W. and Nilsson, G. E. (2009). The anoxia-tolerant crucian carp (*Carassius carassius* L.). In *Fish Physiology, Vol. 27: Hypoxia* (ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp. 398-443. Amsterdam: Academic Press.
Warren, D. and Jackson, D. (2008). Lactate metabolism in anoxic turtles: an integrative review. *J. Comp. Physiol. B* **178**, 133-148.
Wishner, K. F., Gowing, M. M. and Gelfman, C. (2000). Living in suboxia: ecology of an Arabian Sea copepod. *Limnol. Oceanogr.* **45**, 1576-1593.
Yang, T.-H. and Somero, G. N. (1993). Effects of feeding and food deprivation on oxygen consumption, muscle protein concentration, and activities of energy metabolism enzymes in muscle and brain of shallow-living (*Scorpaena guttata*) and deep-living (*Sebastolobus alascanus*) scorpaenid fishes. *J. Exp. Biol.* **181**, 213-232.