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Gill surface area and metabolic enzyme activities of demersal fishes associated with the oxygen minimum zone off California

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Abstract

Metabolic enzyme activities and gill surface areas were measured across 10 species of demersal fishes from Monterey Canyon, California, which features a prominent oxygen minimum zone (OMZ). Comparisons were made between species living both within and outside of the OMZ. Enzyme activities showed no significant trend toward aerobic suppression or heightened reliance on anaerobic metabolism in response to the OMZ. While flatfish species living both within and outside of the OMZ had similarly low enzyme activities, the OMZ-dwelling *Microstomus pacificus* had 1.8–3 times larger gill surface area than comparably sized flatfishes from higher-oxygen waters, suggesting a morphological adaptation to low oxygen. In scorpaeniform fishes, high aerobic metabolism was accompanied by large gill surface areas in two routine-swimming OMZ-dwelling species (*Anoplopoma fimbria* and *Careproctus melanurus*). Low aerobic activities and small gills were found in two *Sebastolobus* species, suggesting a low oxygen demand resulting from a more sedentary behavior compared to other Scorpaeniformes. In gadiform fishes, no differences were measured in enzyme activity levels, but larger gill surface areas were measured in OMZ-dwelling *Nezumia liolepis*. These results indicate adaptation to low oxygen in a variety of ways that balance oxygen demand with supply, with no indication that these species rely on enhanced anaerobic metabolism. With both flatfishes and rattails, adaptation to OMZs is demonstrated through increased gill surface area.

Oxygen minimum zones (OMZs) are midwater regions (200–1000 m) where dissolved oxygen levels are reduced by an order of magnitude relative to waters above and below the OMZ core, defined by a concentration of > 0.5 mL O_2 L^{-1} seawater (Levin 2003). While anoxic and hypoxic conditions exist temporarily in coastal margins, OMZs differ because they are temporally stable and impinge onto the continental shelf and slope.

Recent data have indicated an ongoing expansion in several OMZs. Tropical OMZs have been simultaneously shoaling and deepening tens of meters over the past four decades (Stramma et al. 2010). Alaskan Gyre hypoxic waters (60 μmol L^{-1}) have shoaled from 400 m to 300 m within the last five decades (Whitney et al. 2007). Additionally, California Current system dissolved oxygen levels have dropped, with the hypoxic boundary shifting 90 m vertically in the last three decades (Bograd et al. 2008).

Given these recent expansions, there is a heightened importance in understanding how species living both within and nearby OMZs are adapted to low oxygen. Some benthic and midwater taxa associated with OMZ cores have been studied and exhibit adaptations to aerobically meet requirements for routine metabolism in low oxygen (Childress and Seibel 1998). However, little information is available to characterize how demersal fish species may react to changes in ambient oxygen concentrations.

Fishes can cope with persistently low oxygen through two theoretical mechanisms that are not mutually exclusive: increased oxygen extraction from the environment (permitting routine behavior) and reduced oxygen demand (resulting in either low locomotor capacity or high anaerobic metabolic activity; Childress and Seibel 1998). Aerobic metabolism is more energetically efficient than anaerobic pathways; thus, aerobic activity is preferred even when dissolved oxygen levels are low (Seibel 2011). Midwater fishes which are OMZ transients do not rely on anaerobic metabolism, likely because of the energetically costly accumulation of lactate (Childress and Seibel 1998); therefore, it is unlikely permanent that OMZ dwellers would do the same (Seibel 2011). As in situ monitoring of oxygen extraction and demand is difficult for deep-sea fishes, approximations for these parameters can be determined from gill surface area (Hughes and Iwai 1978) and white muscle enzyme activities (Childress and Somero 1979), respectively.

Metabolic enzyme assays can be split into two major functional groups: enzymes within the citric acid cycle requiring chemically reduced oxygen to generate energy (e.g., malate dehydrogenase [MDH] and citrate synthase [CS]) and glycolytic enzymes dealing with pyruvate production (pyruvate kinase [PK]) and fermentation (lactate dehydrogenase [LDH]), which requires little to no oxygen at the expense of being less efficient. CS has been shown to correlate well with mass-specific oxygen consumption (Seibel et al. 2000) and can therefore provide a reasonable approximation of aerobic metabolism. Investigating the ratio between aerobic and glycolytic enzymes can provide additional insight, as evidenced by the proportionally higher glycolytic enzyme activities in heart muscle for *Sebastolobus* species captured from environmental hypoxic conditions relative to normoxic conditions (Yang et al. 1992). This suggests a heightened reliance on glycolysis to assist with life under hypoxia.

In order to meet routine metabolic requirements, organisms can also increase oxygen uptake through enlarged gas exchange surfaces relative to those in normoxic conditions. In OMZs, midwater fishes and

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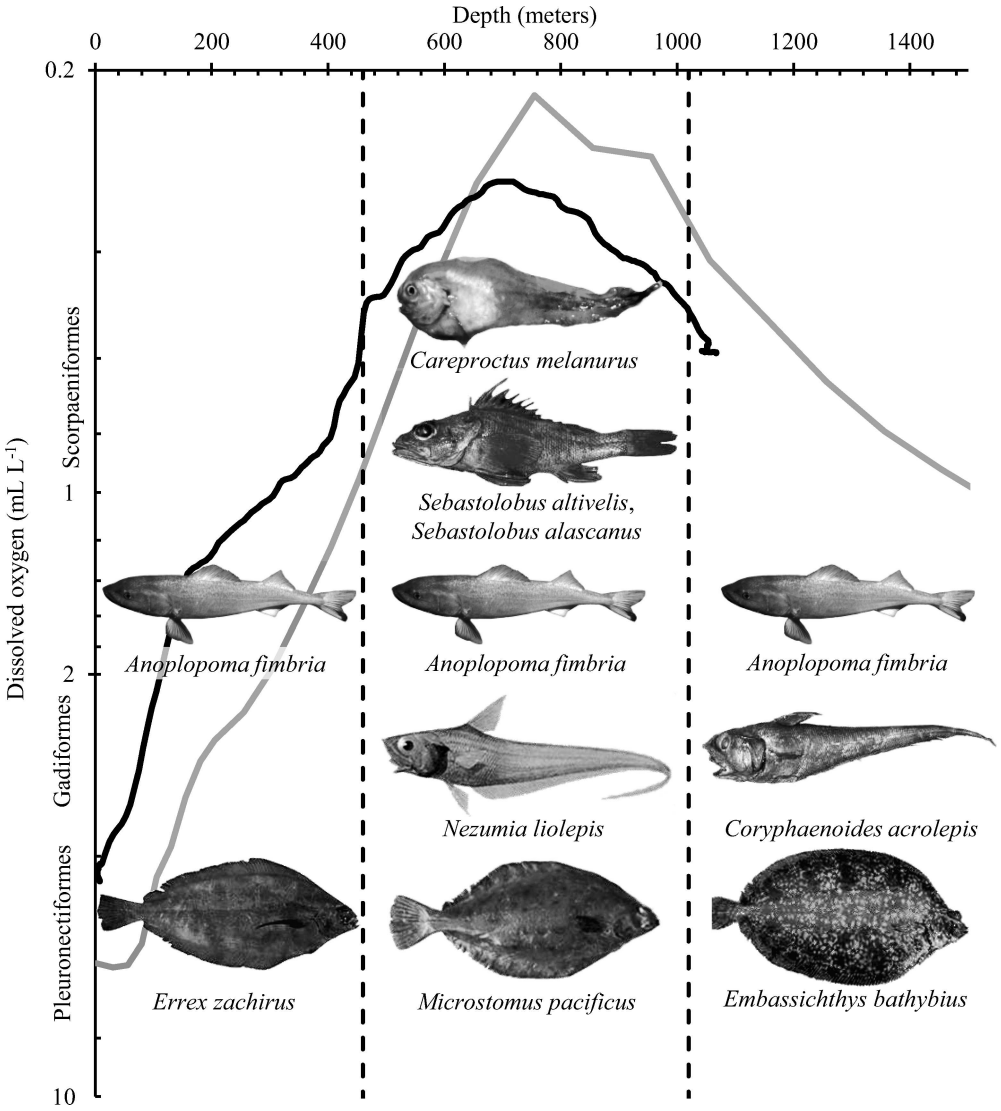

Fig. 1. Distribution of the fishes studied in relationship to oxygen concentration. The 10 species of fishes for analysis are presented figuratively against two oxygen profiles sampled in the California Current system. Depth profiles were collected in March 2009 in Monterey Canyon by MBARI's ROV *Ventana* (black line) and, in 1993, 340 km northwest of the canyon (gray line). Dashed lines represent the depths of the OMZ boundary from 2009 profile data. Fishes are characterized by their general habitat based on previous trawl records in addition to the present study efforts. (Images courtesy of National Oceanic and Atmospheric Administration National Marine Fisheries Service.

mysids have enlarged gill surface areas (Childress and Seibel 1998). Species of shallow water sculpin, which routinely experience hypoxia from tide-pool cycling, have larger mass-specific gill surface areas than similar species in more stable habitats (Mandic et al. 2009). Whether OMZ-dwelling demersal fishes have greater gill surface areas than related species living above or below the OMZ has not been investigated.

Here we present the results of gill surface area measurements and metabolic enzyme activities of 10 species of demersal fishes living in association with an OMZ off the Californian coast to identify potential adaptations to persistently low oxygen conditions.

Methods

Oxygen profile data were obtained by courtesy of the Monterey Bay Aquarium Research Institute (MBARI). Temperature, depth, and oxygen saturation measurements were taken one month before the initial sampling in April 2009 by the remotely operated vehicle (ROV) *Ventana* and confirm the presence of an OMZ between 460 m and 1020 m (Fig. 1). Fishes were collected from various depths within Monterey Bay in April and October 2009. Trawls fished along contour and were equipped with an acoustic pinger that enabled real-time depth recording and targeted three

specific depth ranges; shallower than OMZ (< 450 m), OMZ core (750 m), and deeper than OMZ (> 1100 m).

The distribution patterns of the fish species used in this study were derived from our trawl data and the Pacific West Coast Slope Trawl Survey (Lauth 2000). Species from each of the three orders (Pleuronectiformes, Scorpaeniformes, and Gadiformes) were categorized into three habitat bins, based on depth of capture with respect to OMZ boundary depths as described above. Two of the three orders of fishes examined in the present study have species living in all three of these regions.

Within the first order of investigated fishes, Scorpaeniformes, is *Anoplopoma fimbria*, which lives in all three depth classes in this study (Sullivan and Somero 1983); *Careproctus melanurus*, a small-bodied snailfish purported to be an OMZ specialist living exclusively in low-oxygen waters (Stein et al. 2006); *Sebastes diploproa*, a shallow rockfish living above the OMZ boundary (Vetter and Lynn 1997); *Sebastolobus alascanus*, a large-bodied thornyhead with an ontogenetic shift into the OMZ as individuals age (Yang et al. 1992); and *Sebastolobus altivelis*, a thornyhead fully restricted to OMZ waters throughout its development (Vetter and Lynn 1997).

The pleuronectiform fishes consisted of the following three species: *Errex zachirus* a shallow-living flatfish found in high-oxygen shallow water; *Microstomus pacificus*, a flatfish that undergoes ontogenetic migration with adults residing in the OMZ core; and *Embassichthys bathybius*, a flatfish typically living below OMZ waters (Vetter et al. 1994).

The gadiform fishes consisted of the following two species: *Nezumia liolepis*, a small-bodied grenadier with a vertical distribution limited to OMZ waters (Hoff et al. 2000), and *Coryphaenoides acrolepis*, a large-bodied grenadier with a peak abundance below the OMZ (Drazen 2007).

Enzyme activities—Fish were sorted and immediately placed on ice after each trawl. White muscle tissue samples were excised below the first dorsal ray, medial to the body. Samples were frozen in liquid nitrogen and stored at −80°C and processed within 18 months.

Enzyme activity levels were measured from the anaerobic (glycolysis and lactate fermentation) and aerobic (citric acid cycle) pathways for energy production. CS is the first enzyme within the citric acid cycle sequence (Childress and Somero 1979) and is an important indicator of aerobic metabolism and high activity levels are generally indicative of continuous swimming behavior (Dickson 1995). MDH is another enzyme of the citric acid cycle that could potentially be a rate limiting step (Childress and Somero 1979). Both aerobic indicators typically show negative correlations with increasing body size, similar to mass-specific oxygen consumption. LDH is an enzyme involved in the terminal reaction of fermentative adenosine triphosphate generation and is a proxy for maximum anaerobic capacity often used during burst locomotor activity or other situations in which the tissues are oxygen deprived (Childress and Somero 1979). PK is the last step in pyruvate formation and is a good overall indicator of anaerobic metabolic capacity.

Enzymatic analyses were conducted as in Drazen et al. (2011). Samples were homogenized in a 10:1 dilution using an ice cold titrated tris-buffer solution (pH of 7.55 at 10.0°C) with a motorized ground glass tissue homogenizer. CS was run first and without centrifuging the homogenate, as significant loss of activity (~ 30% reduction) was observed for spun samples (Condon et al. 2012). Subsequently, the homogenate was centrifuged (50,000 m s^{-2} for 5 min) prior to analysis of PK, LDH, and MDH. Samples were run in a thermally controlled spectrophotometer and measured the rate of change in products (CS) or substrates (LDH, PK, or MDH). In all cases, the maximal reaction velocity (Vmax) was measured under substrate saturated conditions. Enzyme activities are reported as units g^{-1} wet weight.

Individual enzyme activities were used to assess differences in proximate metabolism between species. The ratio of anaerobic to aerobic capacity as measured by enzymatic activity (glycolytic poise) was also compared between species to assess relative reliance of the two pathways (Yang et al. 1992) as ratios of the following: LDH:CS, LDH ×PK:CS ×MDH, and PK:CS.

Gill surface area—The right half of the gill basket was dissected from fishes and initially placed in 10% buffered formalin for storage. Prior to handling, all samples were transferred to 50% ethanol to accommodate measurement techniques. Gill half baskets were further dissected to separate and photograph the four individual arches. Images were used to determine the number and length of primary filaments, measuring every 10th filament for reasonable estimation purposes (Hughes 1984).

On the second arch, three primary filaments were sampled to measure secondary lamellae density and cross-sectional area. Each primary filament was photographed under magnification to estimate the density of secondary lamellae (number mm^{-1}). The primary filaments were then bisected into equal-length proximal and distal regions, as secondary lamellae cross-sectional area decreases distally along the primary filament axis (Hughes 1984). The primary filament regions were transferred into solutions of increasing ethanol concentration (sequential increases from 50%, 75%, 90%, and 100%), dehydrated with a repeated submersion in xylene, then embedded in molten paraffin blocks with the filaments vertically oriented for transverse 5-μm sectioning and staining (hemotoxylin and eosin). Secondary lamellae cross-sectional area was determined using a digital camera equipped stereomicroscope. Photographs were analyzed using ImageJ (Rasband 2011). Overall gill surface area was calculated from counts and measurements of both primary filaments and secondary lamellae after Hughes (1984).

Statistical tests—Statistical tests were performed using Statistica 7.1 (Statistica 2005). A Mann–Whitney *U*-test was performed to determine differences of enzymatic activities between species. Nonparametric tests were used because of small sample sizes, large variance, and in some cases nonnormal distributions. Interspecific comparisons of gill area used mass-standardized surface area and were tested using a Kruskal–Wallis analysis of variance.

Regressions were used to determine relationships among test variables, especially given the strong allometry of gill surface area metrics. All tests in this study were considered significant at $\alpha \leq 0.05$.

Results

Enzyme activities were measured for 88 fish specimens representing 10 species from three orders (Table 1). Sizes effects were examined and generally found to be insignificant because of high variance and small sample size. As a result, nonparametric tests were performed, with species exhibiting significant size scaling relationships being subdivided into size categories for interspecific comparisons.

Size-scaling effects—Species with significant scaling effects and greater masses than comparable species were split into size bins. *C. acrolepis* individuals were divided into two size classes with one group having similar mean body sizes to smaller-bodied *N. liolepis* (< 100 g). *A. fimbria* spanned a large size range but exhibited only a significant negative relationship between CS and mass and was split into two categories (< 1000 g, >1000 g) to facilitate comparisons to similarly sized fishes within the Scorpaeniformes, which were generally smaller bodied. The two size classes for *A. fimbria* were also based on the observation that individuals in OMZ depths are typically larger than 1000 g (Jacobson et al. 2001), which was confirmed by our trawl records.

Gill surface area measurements were performed on 52 individuals (Table 2). Total gill surface area scaled positively with increasing body mass, except for *N. liolepis* (two samples) and *S. altivelis* (minimal body range). Mass-specific gill surface area ($mm^2\ g^{-1}$ wet weight) did not correlate with increasing body mass (with the exception of *C. melanurus*) and was therefore used to assess OMZ adaptation across species. As this measurement was isometric, there was no need to split groups into size classes for interspecific comparisons as with enzyme activities.

CS activity levels correlated well ($p < 0.0001$) with mass-specific gill surface area (Fig. 2). Fishes living in OMZ waters did not have both consistently large gills and low aerobic enzyme activity, necessitating a taxonomic approach for comparisons.

Interspecific comparisons—Pleuronectiformes: The only significant interspecific difference in enzyme activity values observed amongst the three flatfish species was that *E. zachirus* had higher CS activity than *E. bathybius* ($p < 0.0214$). Glycolytic poise (Fig. 4) did not show any significant difference among the three flat fishes—all had an equal reliance of both aerobic and glycolytic capacities. The flatfishes had much larger ranges of variance for glycolytic poise ratios than those observed from species of other orders.

While enzyme activities showed little differentiation, each flatfish species differed significantly when comparing mass-specific gill surface areas (Fig. 5). The highest mean mass-specific value belonged to the OMZ-dwelling *M.*

Table 1. Summary of metabolic enzyme activities. Mean enzyme activities with one standard deviation are provided as units g^{-1} wet weight tissue of white muscle at 10°C. Both *C. acrolepis* (> 100 g and < 100 g) and *A. fimbria* (> 1000 g and < 1000 g) are split into two groups—large and small individuals—for more equivalent comparison across body sizes and taxa.

Species	*n*	Average mass (g)	Mass range (g)	Mean depth of capture (m)	Depth of capture range (m)	CS	MDH	PK	LDH
A. fimbria (small)	4	370.7	114.8–596.6	581	263.3–1084	2.26±0.82	25.6±7.9	42.9±10.9	131.9±30.0
A. fimbria (large)	3	2703.8	1689.5–3891.4	1000	513.5–1280	0.68±0.09	12.5±4.3	46.9±10.5	163.7±33.7
S. diploproa	10	306.8	21.6–611.3	386	136.5–513.5	1.31±1.33	15.7±10.2	23.9±13.6	65.5±35.9
S. alascanus	8	855.1	20.0–2318.6	823	327–1205	0.43±0.22	6.9±1.8	18.2±7.7	52.7±14.9
S. altivelis	11	200.1	36.4–265.9	990	488–1390	0.43±0.16	6.6±2.2	16.1±7.6	63.7±25.0
C. melanurus	7	86.1	13.1–193.2	906	488–1280	1.08±0.73	14.9±6.4	32.3±23.1	47.0±39.1
E. zachirus	8	174.3	15.4–486.9	308	88.25–513	0.63±0.31	9.9±3.7	34.1±10.7	99.0±38.0
M. pacificus	10	548.5	20.5–1622.4	687	115–1307	0.81±0.59	16.7±8.2	49.7±25.9	94.8±67.6
E. bathybius	11	642.4	87.5–1016.3	1223	793–1307	0.40±0.22	8.7±3.5	41.0±22.3	81.2±39.2
N. liolepis	5	51.2	7.4–80	732	585–793	1.42±0.84	13.4±0.4	22.1±9.2	36.5±18.2
C. acrolepis (small)	4	47.8	16.5–75.39	1607	1084–2110	1.36±0.23	15.3±1.9	14.5±5.6	17.9±7.1
C. acrolepis (large)	8	1133.6	442.6–2711.7	1592	1084–2110	0.72±0.39	15.1±2.3	16.6±3.6	133.8±95.0

pacificus (58.3 $mm^2\ g^{-1}$), followed by shallow-living *E. zachirus* (39.6 $mm^2\ g^{-1}$) and then the deepest species, *E. bathybius* (20.2 $mm^2\ g^{-1}$).

Scorpaeniformes: *A. fimbria* had the highest enzyme activity levels of any Scorpaeniform fishes (Fig. 3). OMZ-dwelling *Sebastolobus* species had much lower CS activities than the other Scorpaeniformes. MDH activities were higher in *A. fimbria* (< 1000 g) and *S. diploproa* as compared to *Sebastolobus* species. For anaerobic enzyme activities, *A. fimbria* (both size classes) had much higher activities than other Scorpaeniformes. No glycolytic poise differences were detected in any pairwise comparisons among these fishes (Fig. 4). The two *Sebastolobus* species were statistically insignificant from one another for all enzyme activities.

There were two distinct groups of species with respect to mass-specific gill surface area; *Sebastolobus altivelis* and *Sebastolobus alascanus* have roughly half the mass-specific gill surface area (78.9 $mm^2\ g^{-1}$ and 64.8 $mm^2\ g^{-1}$, respectively) of *A. fimbria*, *C. melanurus*, and the non-OMZ-dwelling *S. diploproa* (148.1 $mm^2\ g^{-1}$, 124.1 $mm^2\ g^{-1}$, and 131.0 $mm^2\ g^{-1}$, respectively; $p < 0.05$; Fig. 5).

Gadiformes: Small gadiform fishes (< 100 g) showed no significant differences in any of the four metabolic activities (Fig. 3). Significantly greater CS and lower LDH was found in *N. liolepis* compared to larger *C. acrolepis*. Significant differences existed between large and small *C. acrolepis* individuals. Similarly sized *N. liolepis* and *C. acrolepis* exhibited comparable glycolytic poise values (Fig. 4).

While similarly sized gadiform fishes had no enzymatic differences, the OMZ-living *N. liolepis* (220.1 $mm^2\ g^{-1}$) had roughly three times larger mass-specific gill surface area than *C. acrolepis* individuals (64.7 $mm^2\ g^{-1}$; Fig. 5).

Discussion

This study sought to determine the metabolic enzyme and gill surface area adaptations of demersal fishes in association with an OMZ off of California. By studying enzyme activities (a proxy for metabolic potential) and gill surface areas (relative index of oxygen extraction capability), a comprehensive examination of potential adaptations to OMZs was performed.

Metabolic enzyme activities did not correlate with ambient oxygen concentrations in a consistent manner. No evidence was found supporting either a convergent decrease in aerobic activities or an increased reliance on anaerobic activities in OMZ-core species, confirming that aerobic activity is neither suppressed outright nor significantly bolstered by anaerobic activity for overcoming persistently low oxygen levels (Childress and Seibel 1998). Furthermore, it appears that routine aerobic capacity can be supported in OMZ-core species by incorporating two to three times larger mass-specific gill surfaces relative to non-OMZ species.

Pleuronectiformes—Despite living across a broad range of dissolved oxygen habitats, enzyme activities were

Table 2. Summary of gill surface measurements. *C. acrolepis* is split into two groups to make direct comparisons to similarly sized *N. liolepis*. Regression equations took the form of gill surface area (mm^2) = α (body mass [g])$^{\beta}$ with the resulting R^2 values produced below.

Species	No. sampled	Average mass (g)	Mass range (g)	Mean depth of capture (m)	Depth of capture range (m)	Mean mass-specific gill surface area ($mm^2\ g^{-1}$)	α	β	R^2
A. fimbria	6	2148.9	566.2–3891.4	842	457.5–1280	148.05	559.6	0.83	0.968
S. diploproa	5	415.6	208.6–611.3	458	458	130.99	68	1.11	0.997
S. alascanus	4	1843	160.7–3615.7	1092	772–1307	64.81	133.6	0.9	0.986
S. altivelis	6	220.7	195.3–265.9	976	745–1390	78.88	—	—	—
C. melanurus	6	50.2	13.1–80.5	941	745–1307	124.11	52.2	1.22	0.993
E. zachirus	5	285	170.0–486.9	480	457–513	39.57	119.8	0.8	0.993
M. pacificus	6	675.2	260.9–1622.4	858	772–1205	58.29	229.1	0.79	0.951
E. bathybius	6	709.7	469.8–994.5	1252	1205–1307	20.2	0.442	1.58	0.813
N. liolepis	2	50.3	45.0–55.6	769	745–793	220.09	—	—	—
C. acrolepis	6	764.1	66.2–2232.9	1721	1390–2028	64.73	13.2	1.2	0.987

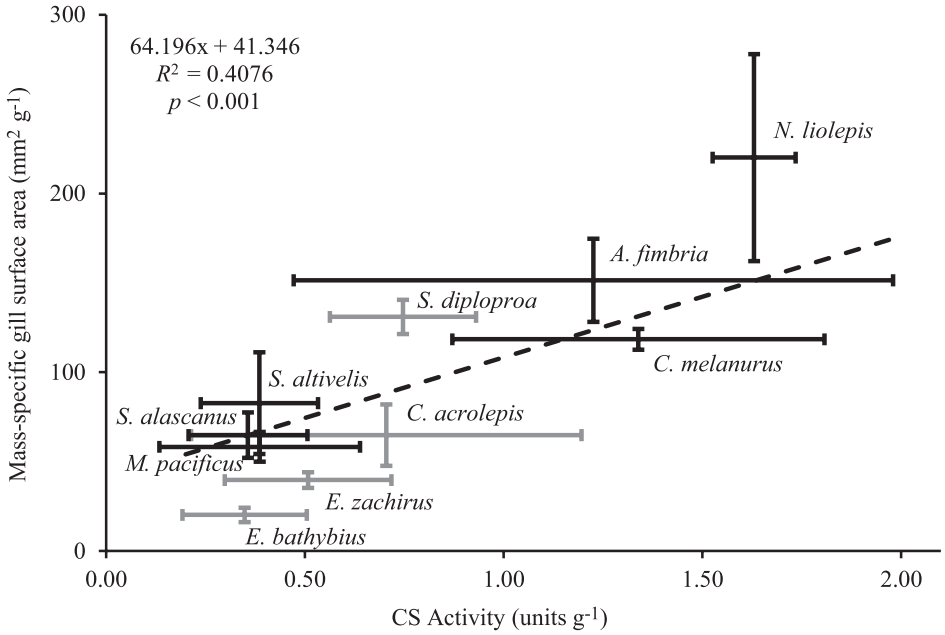

Fig. 2. Mass-specific gill surface area (mm^2 g^{-1}) regressed against CS activity levels (units g^{-1}). Data are plotted by individual species and consist of means with one standard deviation. Species living within OMZ depths are in black, while OMZ-excluded species are in gray.

indistinguishable amongst the flatfishes, likely because of similar body morphology, size, and swimming behavior. There was no reduction in the activity of aerobic enzymes or increased reliance on anaerobic pathways for the OMZ-dwelling *M. pacificus*. Previous work confirms equally low CS activities and LDH activities among the three species (Vetter et al. 1994). Compared to *Sebastolobus*, flatfishes had similarly low aerobic activities, which correspond well to sedentary behavior (Drazen and Seibel 2007).

Adaptation to low oxygen in flatfishes was evident in gill architecture. OMZ-dwelling *M. pacificus* had the highest mass-specific gill surface area of all the flatfishes, while *E. bathybius* had the smallest mass-specific gill surface area despite being found at an intermediary dissolved oxygen level (typically 0.75–1.25 mL O_2 L^{-1}). While *M. pacificus* likely has increased gill surface areas because of low ambient oxygen, *E. zachirus* (living in high-dissolved-oxygen waters) had larger mass-specific gill surface than *E. bathybius*, likely because of physiology. *E. bathybius* has high water content in white muscle, with a large subcutaneous gel layer (Vetter et al. 1994). Whole-body aerobic demand for *E. bathybius* is likely lower relative to an *E. zachirus* individual of comparable size, as more body mass is accounted for by metabolically inert gel tissue, explaining the relatively small mass-specific gill surface area values. Adult *M. pacificus* have similar water muscle content as *E. bathybius* (Hunter et al. 1990) with a less pronounced subcutaneous gel layer, suggesting that gill differences between itself and *E. zachirus* are even more substantial.

Scorpaeniformes—The Scorpaeniformes in this study exhibited many interspecific differences in both enzyme activities and gill morphometrics; however, these species vary in their body form and biology, making direct comparisons between species difficult. *Sebastolobus* species had significantly lower CS and MDH activities in addition to lower mass-specific gill values than the other scorpaeniforms. While other Scorpaeniforms are active swimmers, *Sebastolobus* species are generally sedentary fishes observed sitting on the seafloor with minimal avoidance responses from towed cameras, indicating a reduced need for large gills or high aerobic enzyme activities (Vetter and Lynn 1997; Lauth et al. 2004). Direct measurements of oxygen consumption rates also suggest they have three to four times lower oxygen demand than *Sebastes* and *A. fimbria* (Drazen and Seibel 2007). Even in high-oxygen waters, *Sebastolobus* species not considered in this study exhibit minimal swimming activity (Watanabe et al. 2004), indicating that *S. altivelis* and *alascanus* may have small gills and low aerobic enzyme activities from phylogeny rather than as an adaptation to inhabit the OMZ.

All the remaining Scorpaeniformes, OMZ-dwelling *C. melanurus* and *A. fimbria*, in addition to the OMZ-excluded *S. diploproa*, all had similarly high mass-specific gill surface areas in addition to high aerobic enzyme activities. *C. melanurus* is a purported OMZ specialist based on distribution data and has been observed swimming continuously above the benthos (Stein et al. 2006), which is corroborated by high aerobic enzymatic activities. Likewise, *A. fimbria* is a highly capable swimmer with high enzyme activities (Sullivan and Somero 1983). Both of these species balance high aerobic demand for swimming in low-oxygen environments with large mass-specific gill surface areas. In contrast, *S. diploproa* had similar enzyme activities and gill surface areas but is excluded from OMZ depths, likely because of factors not examined in the current study.

We included scorpaeniform fishes in our study because of their ecological and commercial importance on the

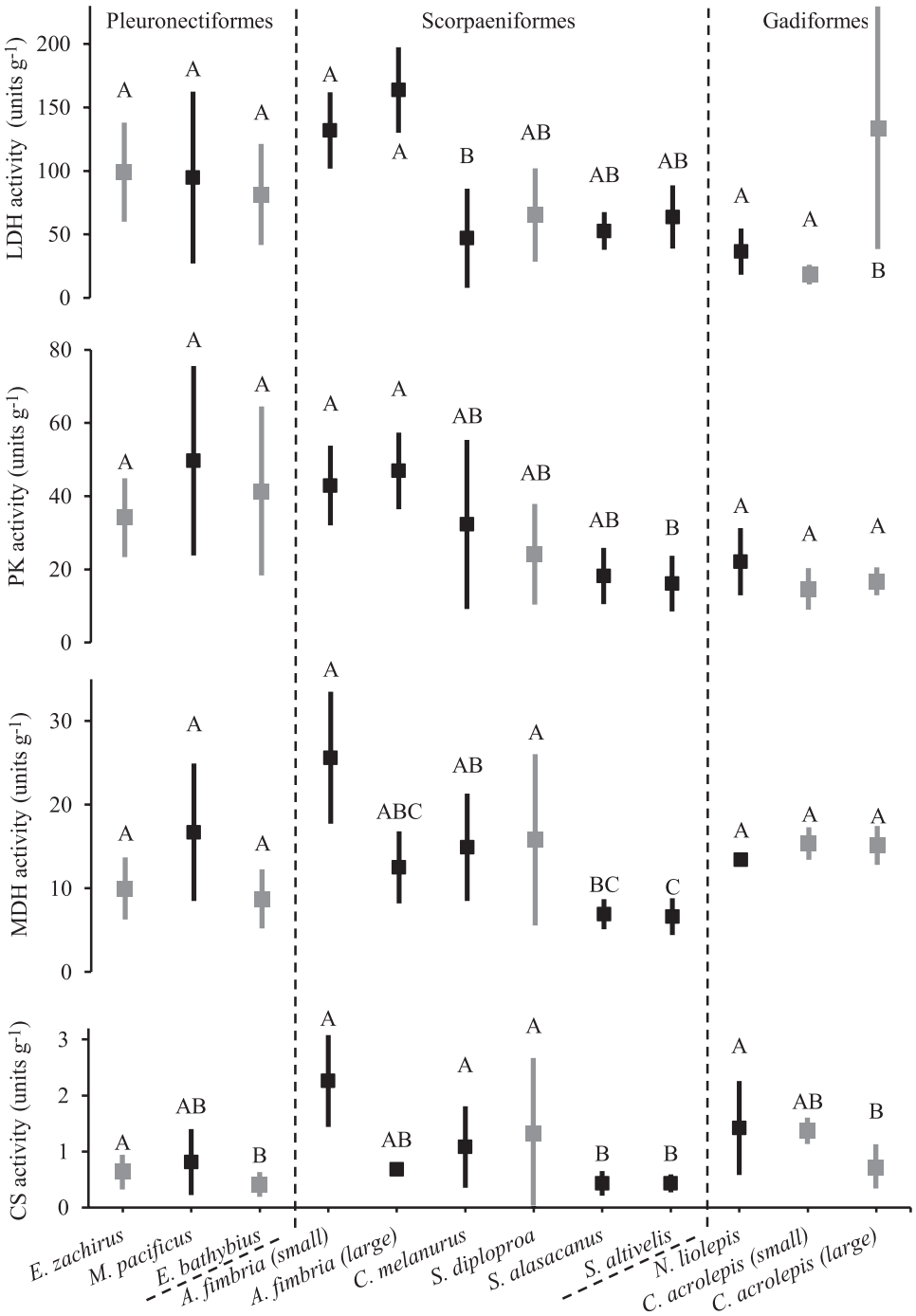

Fig. 3. Metabolic enzyme activities for all species assayed. Means are presented with one standard deviation in units g^{-1} wet weight of white muscle at 10°C. Letters indicate differences tested with analysis of variance (ANOVA) within each order of fishes ($p < 0.0500$, Kruskal–Wallis ANOVA). Orders are separated by the vertical dashed lines. Aerobic enzyme activities are represented by CS and MDH, while anaerobic pathways are represented by PK and LDH. Species living within OMZ depths are in black, while OMZ-excluded species are in gray.

California shelf and slope; however, conclusions about their adaptations must be drawn more carefully. Ideally, congeners living inside and outside the OMZ should be compared, but they were not available for this study. Generally, the data for this group would suggest that scorpaeniform fishes are either unaffected by OMZs because of sedentary lifestyles (*Sebastolobus*) or require large gill surface areas to continue routine swimming behavior (*Anoplopoma* and *Careproctus*).

Gadiformes—Of the two gadiforms studied, *N. liolepis* is adapted to life in the OMZ through large gill surface area. Virtually all *N. liolepis* samples come from OMZ-core

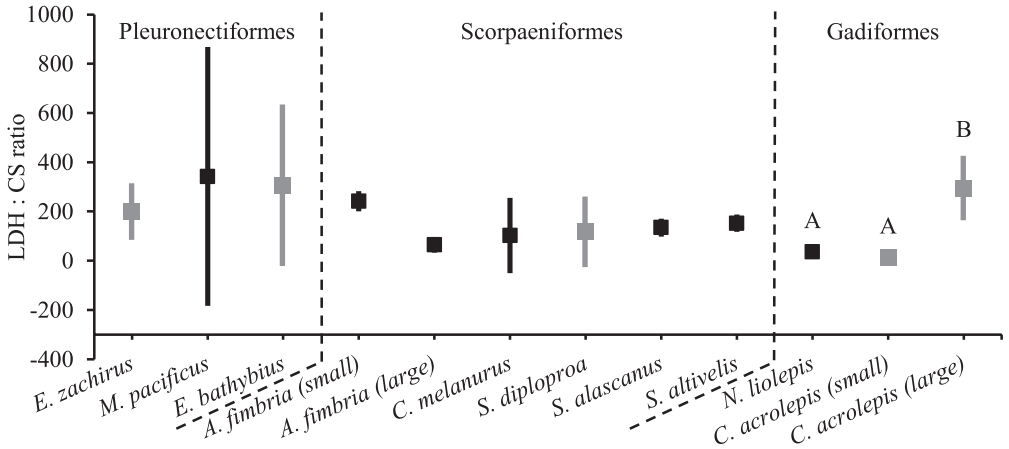

Fig. 4. Glycolytic poise as the ratio of LDH activity to CS activity. Means are presented with one standard deviation. Orders are separated by the vertical dashed lines. Because of high degrees of variance, most interspecific comparisons were insignificant ($p > 0.200$; Kruskal–Wallis ANOVA). The only significant differences existed between size classes of *C. acrolepis* and *N. liolepis* ($p < 0.0250$; Kruskal–Wallis ANOVA). Tests performed on other glycolytic poise index ratios showed no significant differences between species. Species living within OMZ depths are in black, while OMZ-excluded species are in gray.

depths in the California Current system (Hoff et al. 2000), indicating a narrow habitat specificity. While there were no observed metabolic enzyme activity differences between similarly sized Gadiformes, *N. liolepis* had significantly higher mass-specific gill surface area than *C. acrolepis.* Indeed, it was the highest mass-specific gill surface area in this study, comparable to the shallow-living, migratory steelhead trout, *Salmo gairdneri* (Hughes 1984), and approximately twice as large as previously estimated deep-sea species (Hughes and Iwai 1978).

Future considerations

This study has identified ways in which demersal fishes inhabiting OMZs are adapted to their environment at present. We have not determined the capability for these organisms to change within an evolving ecosystem. Temporary and reversible modifications in gill surface area have been observed in carp, permitting survival in nearly hypoxic conditions (Sollid et al. 2003). These types of studies should be conducted on marine fishes living at OMZ

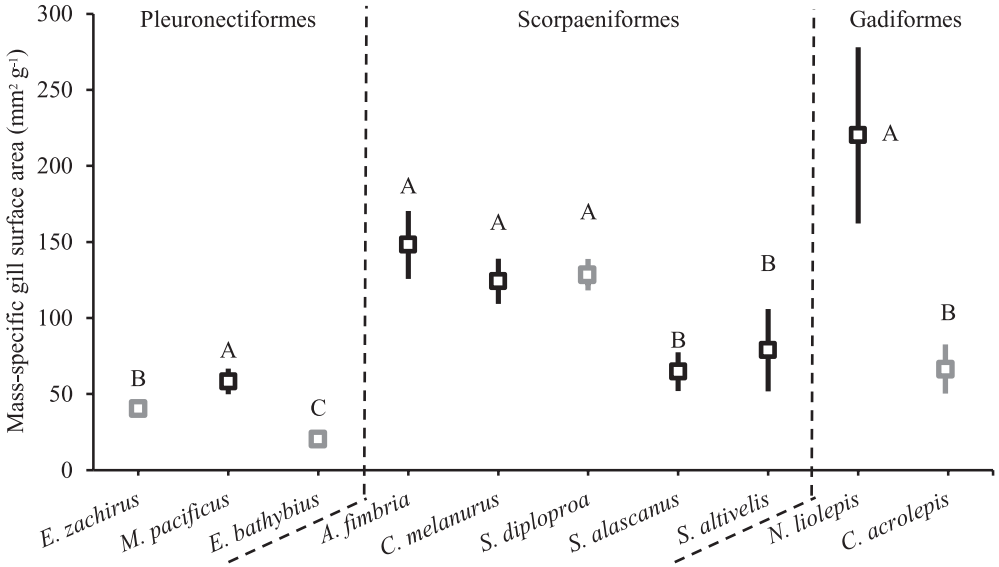

Fig. 5. Mass-specific gill surface area and count of primary filaments presented by species. Means are presented with one standard deviation. Significant differences are denoted by different letters ($p < 0.0500$; Kruskal–Wallis ANOVA), contrasts are kept within orders. Because of the small sample size for Gadiformes, the comparison was conducted with a Mann–Whitney *U*-test ($p < 0.0455$). Species living within OMZ depths are in black, while OMZ-excluded species are in gray.

boundaries. Some species studied herein experience dramatic distribution changes in response to temporary reductions in oxygen levels. For example, *Sebastes* species are not seen on ROV transects in temporary anoxic events, where they would otherwise be plentiful (Chan et al. 2008), while the abundance of several benthic fish species declines sharply during continental hypoxic events (Keller et al. 2010). These observations indicate that compensation may not be rapid or is less energetically favorable than simply leaving a habitat altogether; however, more information about these species morphological and physiological plasticity is necessary in predicting future distribution changes.

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