

# Metabolic enzyme activities in swimming muscle of medusae: is the scaling of glycolytic activity related to oxygen availability?

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This study compared the scaling of the glycolytic enzyme lactate dehydrogenase (LDH) and the Krebs cycle enzyme citrate synthase (CS) in the swimming muscle and tentacle tissue of the mesopelagic coronate scyphomedusa *Periphylla periphylla* in two populations living under different oxygen minimum layer conditions. The LDH and CS activities in these tissues of two other coronate scyphomedusae (*Paraphyllina ransoni* and *Periphyllopsis galathea*) and the bathypelagic narcomedusa *Aegina citrea* were also studied. The scaling of these two enzymes along with total protein was investigated in whole organism homogenates of the surface-living scyphomedusa *Aurelia labiata*. Mass-specific LDH activities in swimming muscle showed positive scaling in relation to body size in *Periphylla periphylla* collected off California and Hawaii. Mass-specific LDH activities in tentacle tissue increased with regards to increasing mass only in specimens of *P. periphylla* collected off California. The LDH values of the scaling coefficient,  $b$ , in swimming muscle and tentacle were significantly higher in *P. periphylla* collected in the low oxygen waters off California than from those collected off the Hawaiian Islands in a higher oxygen environment. The LDH showed a significant decrease with body size in *Aegina citrea* swimming muscle and in *Aurelia labiata* whole animal homogenates. The largest species in this study, *Periphyllopsis galathea*, had LDH activities similar to the smallest specimens of *Periphylla periphylla*. The results of this study suggest that the scaling of glycolytic activity is related to oxygen availability for *P. periphylla*. In *Aurelia labiata*, which is only exposed to episodic hypoxia, and *Aegina citrea*, scaling of glycolytic activity is not affected by oxygen availability.

## INTRODUCTION

Allometric scaling of glycolytic potential has attracted attention because larger animals often have larger mass-specific glycolytic enzyme activities (Somero & Childress, 1980; Somero & Childress, 1985, 1990; Childress & Somero, 1990; Hochachka et al., 1988; Goolish, 1989). This trend does not follow the well-known paradigm of aerobic metabolism whereby larger animals have lower mass-specific metabolic rates (Schmidt-Nielsen, 1984). In pelagic fish, the accepted explanation for positive scaling of glycolytic enzyme activities is to increase burst swimming abilities used in predator–prey interactions (Somero & Childress, 1990; Childress & Somero, 1990). Medusae have low burst swimming potential, and the positive scaling of LDH activity in whole animal homogenates of coronate scyphomedusae has been interpreted as an adaptation for vertical migration, an adaptation for life at reduced oxygen tensions in the oxygen minimum

layer, an adaptation in response to increased oxygen diffusion distances in tissues, or some combination of these three (Thuesen & Childress, 1994).

Hypoxic regions exist at intermediate depths throughout the Earth's oceans that are called oxygen minimum layers (OMLs), and although the oxygen partial pressures in these layers can be lower than 5.0 hPa, populations of metazoans do exist there (Childress & Seibel, 1998). Some shrimps are able to regulate oxygen consumption down to very low levels and live aerobically in the OML (Childress, 1971; Sanders & Childress, 1990), while other midwater crustaceans can survive complete anoxia for many hours in the laboratory (Childress, 1977) and presumably use anaerobic energy during their stay in low-oxygen water (Thuesen et al., 1998). Changes in glycolytic enzyme activity have also been suggested as a key adaptation that allows fish to live at low environmental oxygen concentrations (Yang et al., 1992). The OML is more pronounced below the eastern bound-

ary current off California ( $O_2 < 10.0$  hPa) than in the gyre waters near Hawaii ( $O_2 < 30.0$  hPa), and several comparative studies have successfully used these regions to elucidate the ecological physiology of OML animals (Sanders & Childress, 1990; Childress et al., 1990; Cowles et al., 1991; Seibel et al., 2000).

The current investigation was undertaken to elucidate the relationships between body size and glycolytic potential in invertebrates that lack advanced oxygen delivery systems. This study focused on lactate dehydrogenase (LDH) and citrate synthase (CS) as two enzymes representative of glycolytic potential and aerobic metabolic potential, respectively. The LDH is the terminal enzyme in glycolysis in most organisms, and although some anthozoan Cnidaria use various -opine dehydrogenases in anaerobic metabolism (Livingstone, 1991), we have previously been unable to detect -opine dehydrogenases in hydrozoan and scyphozoan Cnidaria indicating they are either absent or of much less importance in medusae (Thuesen & Childress, 1994). The CS is an important regulatory enzyme and functions in the first step of the citric acid cycle. Enzymatic activities in the scyphomedusa *Periphylla periphylla* (Péron & Lesueur, 1809) from California and Hawaii were compared, and enzymatic activities in other deep-sea medusae that live associated with the oxygen minimum layer were also studied. The scaling of metabolic enzymes in *Aurelia labiata* Chamisso & Eysenhardt 1821, a near-shore scyphomedusa that is known to live in regions which experience episodic near-bottom hypoxia (Thuesen et al., 2005b) and can survive in complete anoxia for up to six hours (Rutherford & Thuesen, 2005) was also investigated.

## MATERIALS AND METHODS

This study used a comparative approach to investigate if glycolytic enzyme activities in medusae are correlated with environmental oxygen levels. One species of medusa, *Periphylla periphylla* (Figure 1) that lives in the oxygen minimum zone off California and in the higher oxygen waters off Hawaii was the primary species investigated. Comparisons of *P. periphylla* were based on several hypotheses: (1) LDH activities are higher in individuals that live in lower oxygen environments; (2) allometric scaling of LDH in muscle tissue is more pronounced in the population that lives in lower oxygen; and (3) allometric scaling of LDH in muscle tissue is the same as allometric scaling of LDH in tentacle tissue. These studies on *P. periphylla* were augmented with opportunistic observations on other medusae in these two regions. In contrast to the studies on deep-sea species, enzymatic activities in the ner-

itic species *Aurelia labiata* were also investigated. If oxygen diffusion to tissues became limiting in larger specimens, larger specimens may have higher LDH activities than smaller specimens, and this hypothesis was tested on *A. labiata* in southern Puget Sound.

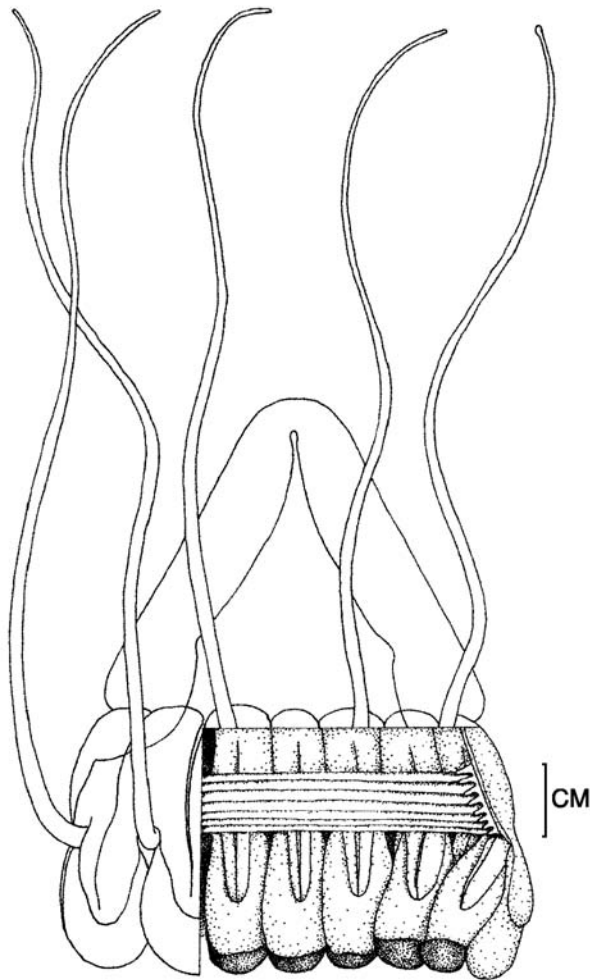
### *Collection of medusae*

The meso- and bathypelagic coronate scyphozoans *Periphylla periphylla*, *Paraphyllina ransoni* Russell, 1956, and *Periphyllopsis galathea* Kramp, 1959, and the bathypelagic narcomedusa *Aegina citrea* Eschscholtz, 1829 were collected using an opening-closing Mother Tucker trawl with a 10-m<sup>2</sup> mouth fitted with a closing 30-l insulated cod end (Childress et al., 1978). The cod end prevented injury to the animals from heat shock and turbulence while the net was being recovered at the surface. Animals were collected ~100 km off Point Conception, California in March 1993 and 10–50 km south-west of Oahu, Hawaii in April–May 1993 during oceanographic cruises of the Research Vessel 'New Horizon'. The ship's speed through the water was kept very low (0.5–1 knot) to reduce turbulence and damage in the net and to reduce the biomass of animals in the cod end. Animals were immediately transferred to 5°C seawater upon recovery and then held up to 24 h in 20-l containers at 5°C. Animals were weighed on a shipboard precision balance system (Childress & Mickel, 1980), then measured and dissected while on a steel tray that was nested in ice. The discrete coronal swimming muscle (Figure 1) and tentacles were removed from the coronate scyphozoans and immediately frozen in liquid nitrogen. The velum and tentacles were dissected from *A. citrea* and treated in the same manner. Tissue samples of some smaller specimens were dissected using a binocular microscope. Some tissue samples that were originally frozen in liquid nitrogen were stored up to four months in a -80°C freezer (Thuesen & Childress, 1994).

The epipelagic scyphomedusa *Aurelia labiata* was hand collected from dense aggregations in Budd Inlet, Puget Sound, Washington using a dip net and bucket from the yacht 'Fjord'. Since it is not possible to dissect discrete muscle tissue samples from *A. labiata*, these animals were frozen either whole or in one-quarter sections (measured by eye and inclusive of one set of gonads) in liquid nitrogen within three hours after capture.

### *Enzymatic activity measurements*

Tissue samples were weighed on a Mettler analytical balance while still frozen and homogenized by hand using Duall tissue grinders kept on ice. Dilutions from



**Figure 1.** Line drawing of *Periphylla periphylla* showing the subumbrellar coronal muscle (CM) that was used in this study.

1:1 to 1:199 parts mass/volume of  $0.01^{\circ}\text{M}$  tris buffer (pH 7.5 at  $10^{\circ}\text{C}$ ) were used for homogenization. Aliquots of homogenate were transferred to microfuge tubes and centrifuged at  $6600g$  for ten min at  $5^{\circ}\text{C}$ . All assays were performed within one hour of homogenization using either Shimadzu UV160U or Hewlett-Packard diode array spectrophotometers equipped with water-jacketed cuvette holders. Assays were done using  $100\ \mu\text{l}$  of sample supernatant. Measurements of enzymatic activity were made in 1 ml total volume in quartz cuvettes at  $20^{\circ}\text{C}$  under non-limiting conditions in order to estimate maximum metabolic potential and followed procedures essentially as those described previously (Thuesen & Childress, 1994; Childress & Somero, 1979). Enzyme activities are expressed as units ( $\mu\text{moles substrate converted to product min}^{-1}$ ) per gram wet mass of animal.

Citrate synthase (CS, E.C. 4.1.3.7) activity measurements were performed in a medium containing 50 mM imidazole/HCl buffer (pH 7.8 at  $20^{\circ}\text{C}$ ), 0.5 mM

oxaloacetate, 0.1 mM acetyl-CoA, 0.1 mM 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) and 1.5 mM  $\text{MgCl}_2$ . The increase in absorbance at 412 nm due to the reaction of the reduced coenzyme A generated by the enzymatic reaction with DTNB was recorded. Background activity before the addition of homogenate supernatant was recorded, and this background rate was later subtracted from the overall rate after the assay reaction was initiated by addition of the oxaloacetate.

Lactate dehydrogenase (LDH, E.C. 1.1.1.27) activity measurements were performed in a medium containing 80 mM tris/HCl buffer (pH 7.2 at  $20^{\circ}\text{C}$ ), 2 mM sodium pyruvate,  $150\ \mu\text{M}$  NADH, and 100 mM KCl. The assay reaction was started by the addition of sample supernatant, and the decrease in absorbance at 340 nm due to NADH oxidation was recorded.

Protein measurements of *Aurelia labiata* homogenates were performed using the Lowry method (Lowry et al., 1951) with BSA as a standard. Protein assays were not completed for the tissue-specific samples of *Periphylla periphylla* and *Aegina citrea* due to smaller sample sizes.

#### Data analyses

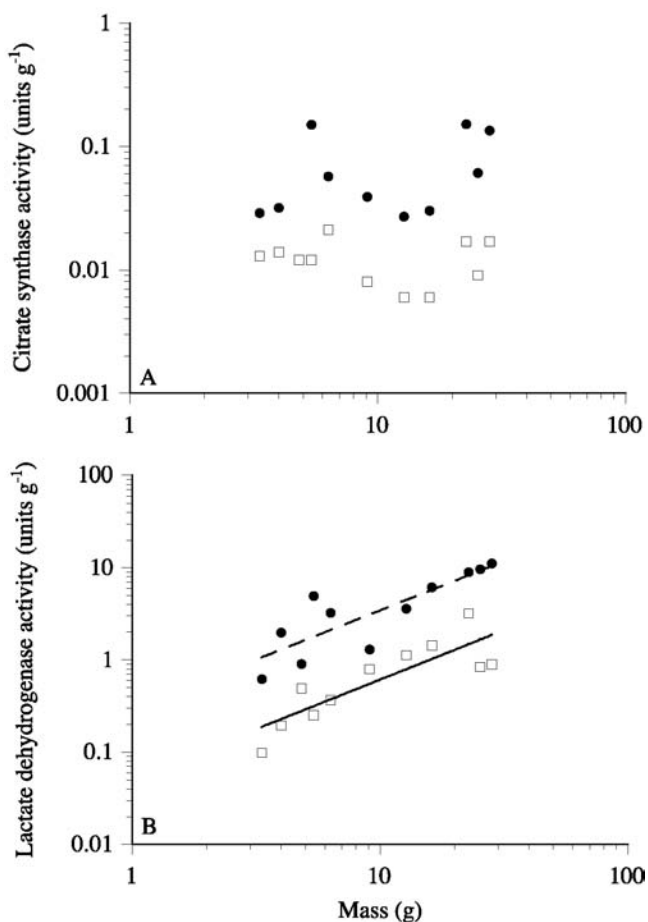
All statistical analyses were performed with Statview II or SuperANOVA (Abacus Concepts, Inc., Berkeley, CA). Enzymatic activities of medusan tissue were evaluated in relation to the total wet mass of the animals, and scaling coefficients were derived from the well-known allometric equation  $y = aM^b$ , where  $M$  is the wet mass of the animal,  $b$  is the scaling coefficient and  $a$  is a constant for the species at a given temperature (Schmidt-Nielsen, 1984). To compare differences in enzyme activities within tissues, two-tailed  $t$ -tests were used. Analysis of covariance (ANCOVA) was used to examine differences in enzymatic activities between Californian and Hawaiian populations of *P. periphylla*.

## RESULTS

Deep-water medusae were collected in very good condition and could be kept alive for many days on board ship. *Periphylla periphylla* is a powerful swimmer and can swim continuously for many hours in captivity. *Aegina citrea* and *Paraphyllina ransoni* are usually less active than *Periphylla periphylla* in large tanks. *Aurelia labiata* is well known for its sustained swimming abilities (Mackie et al., 1981; Hamner et al., 1994), and our observations of *A. labiata in situ* and in the laboratory agree with those made previously. Data collected on the two other coronate scyphozoans is included here to augment the information on *P. periphylla*.

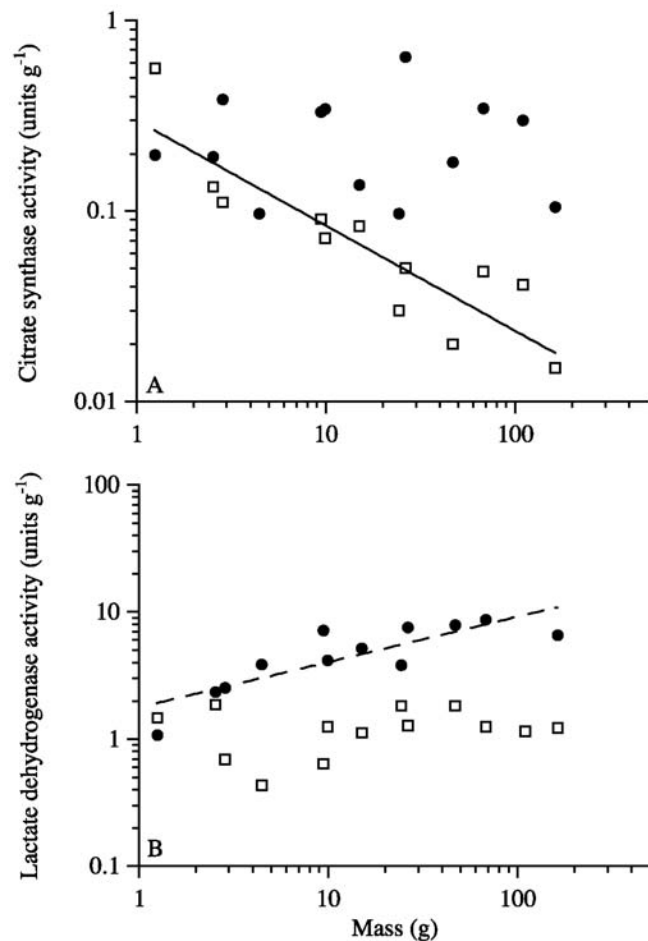
*Periphylla periphylla*

Specimens of *P. periphylla* collected offshore of the island of Oahu reached a much larger size (162.42 g) than those collected off California (28.4 g). Both coronal muscle and tentacle tissues are anaerobically poised with LDH activities being higher than CS activities in both Californian and Hawaiian animals (Figures 1 & 2). The CS activities are significantly higher in *P. periphylla* from Hawaii than from California (Table 1; two-tailed *t*-tests,  $P=0.002$  for muscle and  $P=0.05$  for tentacle). The activities of CS show no significant change with body size in coronal muscle tissue in either Californian or Hawaiian *P. periphylla*



**Figure 2.** (A) Citrate synthase and (B) lactate dehydrogenase activities in coronal swimming muscle (closed circles) and tentacle (open squares) tissue in the coronate scyphozoan *Periphylla periphylla* collected off California. Regression analysis showed no significant relationship between CS activities and wet mass ( $P>0.05$ ). The regression line describing the relationship between muscle tissue LDH activity and wet mass is  $y=0.293x^{1.068}$  ( $R=0.94$ ; F-test for regression coefficient  $P=0.001$ ). The regression line describing the relationship between tentacle tissue LDH activity and wet mass is  $y=0.051x^{1.075}$  ( $R=0.61$ ; F-test for regression coefficient  $P=0.001$ ).

(Figures 2A & 3A), and CS activities in tentacle tissue decrease significantly with increasing mass (Figure 3A). The three largest specimens of *P. periphylla* from California had the three highest LDH activities in our study (Figure 2B), however, LDH activities are not significantly different in either data set (two-tailed *t*-tests,  $P>0.20$ ). The activities of LDH in coronal muscle tissue increase with size in both Californian and Hawaiian *P. periphylla* (Figures 2B & 3B). The LDH activities of tentacle tissue increase significantly in specimens from California (Figure 2B). In contrast, tentacle tissue in Hawaiian *P. periphylla* shows no significant change of LDH activities with body size (Figure 3B). The scaling coefficient of coronal-muscle LDH activities is signifi-

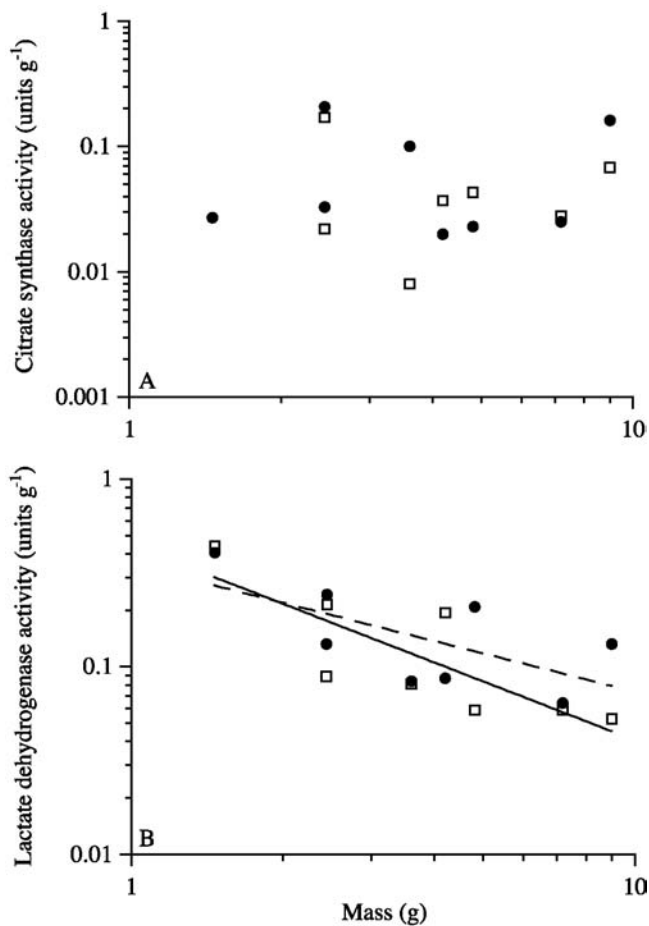


**Figure 3.** (A) Citrate synthase and (B) lactate dehydrogenase activities in coronal swimming muscle (closed circles) and tentacle (open squares) tissue in the coronate scyphozoan *Periphylla periphylla* collected off Hawaii. Regression analysis showed no significant relationship between CS activity of muscle and body size ( $P>0.05$ ). The regression line describing the relationship between CS activity in tentacle and wet mass is  $y=0.030x^{-0.55}$  ( $R=0.86$ ; F-test for regression coefficient  $P<0.001$ ). There is no significant relationship between tentacle LDH activity and body size. The regression line describing the relationship between muscle tissue LDH activity and wet mass is  $y=1.768x^{0.36}$  ( $R=0.73$ ; F-test for regression coefficient  $P<0.001$ ).

**Table 1.** Citrate synthase and lactate dehydrogenase activities in muscle tissue and tentacles of medusae collected off California, Hawaii and Washington.

Class	Location	Wet weight (g)		Enzymatic activity (units g <sup>-1</sup> )							
				CS-muscle		LDH-muscle		CS-tentacle		LDH-tentacle	
Genus and species	N	Range	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	
Hydrozoa											
<i>Aegina citrea</i>	CA	8	1.464–8.997	0.075	0.026	0.169	0.040	0.054	0.026	0.148	0.047
Scyphozoa											
<i>Aurelia labiata</i>	WA	16	0.100–174.360	*0.006	0.004	*0.006	0.008				
<i>Paraphyllina ransoni</i>	CA	4	13.662–1700.0	0.036	0.015	0.199	0.116	0.015	0.010	0.336	0.133
<i>Paraphyllina ransoni</i>	HI	1	78.43	0.014		0.251		0.010		0.874	
<i>Periphylla periphylla</i>	CA	11	3.347–28.359	0.071	0.017	4.776	1.129	0.012	0.001	0.879	0.262
<i>Periphylla periphylla</i>	HI	13	1.252–162.420	0.258	0.043	5.083	0.718	0.104	0.043	1.240	0.129
<i>Periphyllopsis galathea</i>	HI	1	6200.0	0.170		1.286		0.021		0.243	

CS, citrate synthase; LDH, lactate dehydrogenase; N, number; SE, standard error; CA, California; HI, Hawaii; WA, Washington; \*, Enzymatic activities were measured in whole animal homogenates of *Aurelia labiata*, not in discrete tissues.



**Figure 4.** (A) Citrate synthase and (B) lactate dehydrogenase activities in swimming muscle (closed circles) and tentacle (open squares) tissue in the hydrozoan *Aegina citrea* collected off California. Regression analysis showed no significant relationship between CS activities and body size ( $P > 0.05$ ). The regression line describing the relationship between muscle tissue LDH activity and wet mass is  $y = 0.349x^{-0.68}$  ( $R = 0.79$ ; F-test for regression coefficient  $P = 0.07$ ). The regression line describing the relationship between tentacle tissue LDH activity and wet mass is  $y = 0.444x^{-1.04}$  ( $R = 0.87$ ; F-test for regression coefficient  $P = 0.015$ ).

cantly higher in *P. periphylla* from California than from Hawaii (ANCOVA,  $P < 0.01$ ).

#### Other Coronatae

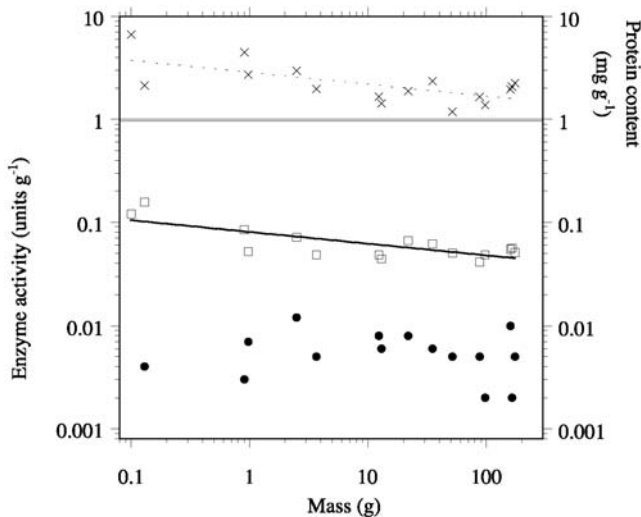
Four specimens of *Paraphyllina ransoni* were captured off California (Table 1). One individual of *P. ransoni* weighing 78.43 g was captured off Hawaii and had CS activities of 0.014 and 0.010 in muscle and tentacle, respectively, and LDH activities of 0.251 and 0.874 in muscle and tentacle, respectively. These activities are within the ranges of the California specimens. One individual of the rare giant coronate species *Periphyllopsis galathea* was also captured off Hawaii. It weighed 6.2 kg and had CS activities of 0.017 and 0.021 in muscle and tentacle, respectively, and LDH activities of 1.286 and 0.243 in muscle and tentacle, respectively. In all these coronate specimens, coronal muscle and tentacle tissues are anaerobically poised with LDH activities being higher than CS activities.

#### *Aegina citrea*

The size range of our specimens of *A. citrea* only encompasses one order of magnitude. There is no significant relationship between CS activity and body size in either swimming muscle (velum) or tentacle tissue in *A. citrea* (Figure 4A). The LDH activity decreases with increasing size in both swimming muscle and tentacle tissue (Figure 4B). The magnitudes of both enzymatic activities are similar in both tissues. Five of the eight individuals had higher LDH activities in tissues than CS activities.

#### *Aurelia labiata*

Like the other scyphozoans in this study, *A. labiata* has significantly higher LDH activities than CS activities. The CS in whole animal homogenates of *A. labiata*



**Figure 5.** Citrate synthase (closed circles) and lactate dehydrogenase (open squares) activities and protein contents (x) in whole animal homogenates of the epipelagic scyphozoan *Aurelia labiata* collected in southern Puget Sound, Washington. Regression analysis showed no significant relationship between CS activity and body size ( $P > 0.05$ ). The regression line describing the relationship between LDH activity and wet mass is  $y = 0.080x^{-0.11}$  ( $R = 0.82$ ; F-test for regression coefficient  $P < 0.001$ ). The regression line describing the relationship between protein content and wet mass is  $y = 2.867x^{-0.12}$  ( $R = 0.70$ ; F-test for regression coefficient  $P = 0.005$ ).

showed no significant relationship with body size (Figure 5), but LDH activities decreased significantly with increasing body size (Figure 5). Protein content also decreased with body size (Figure 5), and the decrease in LDH activity relative to the decrease in protein content was significantly correlated ( $y = 0.037x^{0.55}$ ;  $R = 0.93$ ; F-test for regression coefficient  $P = 0.0001$ ). There was no significant relationship between CS activity and protein content in whole animal homogenates of *A. labiata*.

## DISCUSSION

### *Role of LDH in promoting hypoxia tolerance*

#### *Oxygen minimum layer*

*Periphylla periphylla* off California undertakes daily vertical migrations and moves in and out of the oxygen minimum layer from above, while *Aegina citrea* lives below the centre of the oxygen minimum layer and is not considered to be a diel vertical migrator (Alvarino, 1967). In general, LDH activities are higher than CS activities in scyphomedusae, and CS activities are higher than LDH activities in hydromedusae

(Thuesen & Childress, 1994). The tissue-specific enzyme activities of only a few individuals of *A. citrea* in this study concurs with this difference. Glycolytic potential may not be important in hypoxia tolerance of hydromedusae, and it is not possible to state that LDH promotes tolerance to the oxygen minimum layer based on the comparison between *A. citrea* and *Periphylla periphylla*. Further investigations on *A. citrea* and other hydromedusae in the OML are needed.

*Paraphyllina ransoni* lives below the oxygen minimum layer off California (Thuesen & Childress, unpublished). If LDH is promoting hypoxia tolerance in *P. periphylla* compared to other coronates, we would expect LDH activities to be higher in both tentacle and muscle tissues of *P. periphylla* than in those tissues of *P. ransoni*, and they are higher. If LDH is used during diel vertical migrations, we would expect the activities of this enzyme to be especially higher in muscle tissues of *P. periphylla* than in those tissues of *P. ransoni*, and they are higher. The activities of CS and LDH are lower in tentacles than in swimming muscle, reflecting the lower overall metabolic potential of tentacles.

Based on these interspecies comparisons, it appears that LDH is used during sustained swimming during vertical migrations and that it also promotes tolerance to the low oxygen partial pressures of the oxygen minimum layer in *P. periphylla*. During vertical migrations in low oxygen, *P. periphylla* would be expected to require a shift to anaerobic metabolism faster off California than off Hawaii, and LDH activities might be expected to be higher in *P. periphylla* off California than off Hawaii. However, the hypothesis that LDH activities are higher in individuals that live in lower oxygen environments was not substantiated in this intraspecies comparison. If glycolytic activities are sufficient to sustain metabolic processes in both areas, an enhanced ability to buffer glycolytic end products might be more important in California OML populations of *P. periphylla* (Somero & Childress, 1990).

#### *Estuarine hypoxia*

Unlike the much larger scyphozoan *Pelagia colorata* Russell, 1964 (Thuesen & Childress, 1994), LDH activities are higher than CS activities in *Aurelia labiata*. This may have the intrinsic property of promoting survival during extreme episodic hypoxia, and *A. labiata* can survive up to six hours in anoxia (Rutherford & Thuesen, 2005). Overall, planktonic estuarine coelenterates rely primarily on aerobic metabolism to tolerate low oxygen conditions (Rutherford & Thuesen, 2005; Thuesen et al., 2005a,b), and the ability of LDH to support metabolism during anoxia may be an exaptation that contributes to the survival of *A. labiata* in severely eutrophic estuaries.

*Scaling of glycolytic activity*

Positive scaling of lactate dehydrogenase activities has been hypothesized to be a result of additional glycolytic potential needed to sustain burst swimming in pelagic fish (Somero & Childress, 1980). Larger fish need to accelerate proportionally faster than smaller fish during predator–prey interactions (Childress & Somero, 1990). Positive scaling of glycolytic potential has also been observed in a pelagic cephalopod (Seibel et al., 1998) and a dendrobranchiate crustacean (González & Quiñones, 2002). Aquatic animals that do not undertake sustained burst swimming generally do not display positive scaling of their glycolytic abilities (Vetter et al., 1994; Baldwin et al., 1995). In contrast with fish, locomotory musculature makes up a tiny fraction of the wet mass of medusae, and medusae generally have poor acceleration abilities (Daniel, 1984; Larson, 1987b). Therefore, the adaptive value of positive scaling proposed for LDH in pelagic fish is unlikely to be the same as that for medusae. It has previously been suggested that the higher LDH activities of whole animal homogenates in larger sized coronate scyphozoans may be a result of increased demand for glycolytic energy as oxygen diffusion distances increase in larger individuals (Thuesen & Childress, 1994), and those observations prompted this investigation.

If glycolytic potential in coronates is merely a function of size, the largest individual in this study would have the highest LDH activities. However, the 6.2 kg specimen of *Periphyllopsis galathea* has LDH activities similar to the smallest specimens of *Periphylla periphylla*. Increased diffusion distances *per se* do not appear to affect glycolytic activities in coronate medusae. The size range of *P. periphylla* found in Hawaii was greater than that off California, and it is tempting to state that the low PO<sub>2</sub> off California is responsible for the smaller sizes of the individuals. However, *P. periphylla* in Norwegian fjords reach sizes over 1 kg (Fosså, 1992), and the deep hypoxia of that region is clearly not limiting maximum size in that population. The large Norwegian specimens are thought to be over 30 years old (Jarms et al., 1999), and it may be that the lifespan of *P. periphylla* off Hawaii and Norway is greater than off California.

Although LDH activities themselves are not significantly higher in *P. periphylla* from the California OML than from those off Hawaii, the scaling coefficient of muscle LDH is significantly higher off California. The hypothesis that allometric scaling of LDH in muscle tissue is more pronounced in the population that lives in lower oxygen was shown to be correct. Furthermore, tentacle tissues have positive scaling of

LDH off California but not off Hawaii. This scaling substantiated the third hypothesis of this investigation for the California population, but not for the Hawaii population. Although the population differences in scaling coefficients are subtle, it appears that LDH does play a role in hypoxia tolerance during vertical migrations through the OML. The results presented in this paper are consistent with the hypothesis that low habitat oxygen concentration can influence the allometric scaling of glycolytic enzyme activities (Yang et al., 1992). González & Quiñones (2002) have suggested that the positive scaling of LDH in the crustacean *Euphausia mucronata* Sars, 1883 may be an adaptation that assists diel vertical migration between the surface waters of the Humboldt Current and the underlying OML. It appears that positive scaling of LDH in *P. periphylla* also assists diel vertical migration through the OML in the North Pacific.

The negative scaling of LDH activity in *Aegina citrea*, even over the small size range, indicates that LDH is not used to offset diffusion barriers in this hydrozoan. The negative allometric scaling of LDH in *Aurelia labiata* indicates that glycolytic demand is not affected by body size. The lack of allometric scaling of CS activities in *A. labiata* agrees with the lack of allometric scaling in metabolic rates reported previously for *A. labiata* (Larson, 1987a) and *A. aurita* (Linnaeus, 1746) (Kinoshita et al., 1997) and further substantiates the use of CS as an indicator of aerobic metabolism. Although LDH in *A. labiata* might promote hypoxia tolerance in general, there is no indication that larger *A. labiata* are more hypoxia tolerant than smaller individuals as a function of glycolytic activity.

The morphology of the coronal swimming muscle in *P. periphylla* (Figure 1) and some other scyphomedusae (Russell, 1970) whereby the muscle bands are freely exposed to the environment, rather than embedded directly in the mesoglea, may partially account for their ability to swim actively in low-oxygen environments, such as oxygen minimum zones and fjords. Regular pumping of seawater across the subumbrellar coronal muscle and movement of the tentacles during swimming must decrease boundary layers surrounding the muscle bands and promote oxygen diffusion at very low oxygen concentrations. Alternatively, differences in behaviour or the diel migration patterns between populations could also play a role in hypoxia tolerance (cf. Decker et al., 2003; Thuesen et al., 2005b).

Is the scaling of glycolytic activity related to oxygen availability? The results of this study certainly suggest that this is true for *P. periphylla* of the OML, but the smaller size range of *P. periphylla* from California prevents a definitive answer to this question. For the deep-

sea hydromedusa *Aegina citrea* and the neritic scyphomedusa *Aurelia labiata*, the results of this study suggest that glycolytic scaling does not play a role in hypoxia tolerance.

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## REFERENCES

- Alvarino, A., 1967. Bathymetric distribution of Chaetognatha, Siphonophorae, Medusae, and Ctenophorae off San Diego, California. *Pacific Science*, **21**, 474–485.
- Baldwin, J., Seymour, R.S. & Webb, G.J.W., 1995. Scaling of anaerobic metabolism during exercise in the estuarine crocodile (*Crocodylus porosus*). *Comparative Biochemistry and Physiology*, **112A**, 285–293.
- Childress, J.J., 1971. Respiratory adaptations to the oxygen minimum layer in the bathypelagic mysid *Gnathophausia ingens*. *Biological Bulletin. Marine Biological Laboratory, Woods Hole*, **141**, 109–121.
- Childress, J.J., 1977. Effects of pressure, temperature and oxygen on the oxygen consumption rate of the midwater copepod *Gaussia princeps*. *Marine Biology*, **39**, 19–24.
- Childress, J.J., Barnes, A.T., Quetin, L.B. & Robison, B.H., 1978. Thermally protecting cod ends for recovery of living deep-sea animals. *Deep-Sea Research*, **25**, 419–422.
- Childress, J.J. & Mickel, T.J., 1980. A motion compensated shipboard precision balance system. *Deep-Sea Research*, **27A**, 965–970.
- Childress, J.J., Price, M.H., Favuzzi, J.A. & Cowles, D.L., 1990. The chemical composition of midwater fishes as a function of depth of occurrence off the Hawaiian Islands: food availability as a selective factor? *Marine Biology*, **105**, 235–246.
- Childress, J.J. & Seibel, B.A., 1998. Life at stable low oxygen levels: adaptations of animals to oceanic oxygen minimum layers. *Journal of Experimental Biology*, **201**, 1223–1232.
- Childress, J.J. & Somero, G.N., 1979. Depth related enzymic activities in muscle, brain and heart of deep-living pelagic marine teleosts. *Marine Biology*, **52**, 273–283.
- Childress, J.J. & Somero, G.N., 1990. Metabolic scaling: a new perspective based on scaling of glycolytic enzyme activities. *American Zoologist*, **30**, 161–173.
- Cowles, D.L., Childress, J.J. & Wells, M.E., 1991. Metabolic rates of midwater crustaceans as a function of depth of occurrence off the Hawaiian Islands: food availability as a selective factor? *Marine Biology*, **110**, 75–83.
- Daniel, T.L., 1984. Unsteady aspects of aquatic locomotion. *American Zoologist*, **24**, 121–134.
- Decker, M.B., Breitburg, D.L. & Marcus, N.H., 2003. Geographical differences in the behavioral response of zooplankton to hypoxia: local adaptation to an anthropogenic stressor? *Ecological Applications*, **13**, 1104–1109.
- Fosså, J.H., 1992. Mass occurrence of *Periphylla periphylla* (Scyphozoa, Coronatae) in a Norwegian Fjord. *Sarsia*, **77**, 237–251.
- González, R.R. & Quiñones, R.A., 2002. Ldh activity in *Euphausia mucronata* and *Calanus chilensis*: implications for vertical migration behaviour. *Journal of Plankton Research*, **24**, 1349–1356.
- Goolish, E.M., 1989. The scaling of aerobic and anaerobic muscle power in rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology*, **147**, 493–505.
- Hamner, W.M., Hamner, P.P. & Strand, S.W., 1994. Sun compass migration by *Aurelia aurita* (Scyphozoa) - population retention and reproduction in Saanich Inlet, British Columbia. *Marine Biology*, **119**, 347–356.
- Hochachka, P.W., Emmett, B. & Suarez, R.K., 1988. Limits and constraints in the scaling of oxidative and glycolytic enzymes in homeotherms. *Canadian Journal of Zoology*, **66**, 1128–1138.
- Jarms, G., Båmstedt, U., Tiemann, H., Martinussen, M.B. & Fosså, J.H., 1999. The holopelagic life cycle of the deep-sea medusa *Periphylla periphylla* (Scyphozoa, Coronatae). *Sarsia*, **84**, 55–65.
- Kinoshita, J., Hiromi, J. & Kadota, S., 1997. Do respiratory metabolic rates of the scyphomedusa *Aurelia aurita* scale isometrically throughout ontogeny in a sexual generation? *Hydrobiologia*, **347**, 51–55.
- Larson, R.J., 1987a. Respiration and carbon turnover rates of medusae from the NE Pacific. *Comparative Biochemistry and Physiology*, **87A**, 93–100.
- Larson, R.J., 1987b. Costs of transport for the scyphomedusa *Stomolophus meleagris* L. Agassiz. *Canadian Journal of Zoology*, **65**, 2690–2695.
- Livingstone, D.R., 1991. Origins and evolution of pathways of anaerobic metabolism in the animal kingdom. *American Zoologist*, **31**, 522–534.
- Lowry, O.H., Roseborough, N.J., Farr, A.L. & Randall, R.J., 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265–275.
- Mackie, G.O., Larson, R.J., Larson, K.S. & Passano, L.M., 1981. Swimming and vertical migration of *Aurelia aurita* (L.) in a deep tank. *Marine Behaviour and Physiology*, **7**, 321–329.
- Russell, F.S., 1970. *The medusae of the British Isles. II. Pelagic Scyphozoa with a supplement to the first volume on Hydromedusae*. Cambridge: Cambridge University Press.
- Rutherford, L.D. & Thuesen, E.V., 2005. Metabolic performance and survival of medusae in estuarine hypoxia. *Marine Ecology Progress Series*, in press.
- Sanders, N.K. & Childress, J.J., 1990. Adaptations to the deep-sea oxygen minimum layer: oxygen binding by the hemocyanin of the bathypelagic mysid, *Gnathophausia ingens* Dohrn. *Biological Bulletin. Marine Biological Laboratory, Woods Hole*, **178**, 286–294.
- Schmidt-Nielsen, K., 1984. *Scaling: why is animal size so important?* Cambridge: Cambridge University Press.

- Seibel, B.A., Thuesen, E.V. & Childress, J.J., 1998. Flight of the vampire: ontogenetic gait-transition in *Vampyroteuthis infernalis* (Cephalopoda: Vampyromorpha). *Journal of Experimental Biology*, **201**, 2413–2424.
- Seibel, B.A., Thuesen, E.V. & Childress, J.J., 2000. Light-limitation on predator-prey interactions: consequences for metabolism and locomotion of deep-sea cephalopods. *Biological Bulletin. Marine Biological Laboratory, Woods Hole*, **198**, 284–298.
- Somero, G.N. & Childress, J.J., 1980. A violation of the metabolism-size scaling paradigm: activities of glycolytic enzymes in muscle increase in larger-size fish. *Physiological Zoology*, **53**, 322–337.
- Somero, G.N. & Childress, J.J., 1985. Scaling of oxidative and glycolytic enzyme activities in fish muscle. In *Circulation, respiration, and metabolism* (ed. R. Gilles), pp. 250–262. Berlin: Springer-Verlag.
- Somero, G.N. & Childress, J.J., 1990. Scaling of ATP-supplying enzymes, myofibrillar proteins and buffering capacity in fish muscle: relationship to locomotory habit. *Journal of Experimental Biology*, **149**, 319–333.
- Thuesen, E.V. & Childress, J.J., 1994. Oxygen consumption rates and metabolic enzyme activities of oceanic California medusae in relation to body size and habitat depth. *Biological Bulletin. Marine Biological Laboratory, Woods Hole*, **187**, 84–98.
- Thuesen, E.V., Miller, C.B. & Childress, J.J., 1998. Ecophysiological interpretation of oxygen consumption rates and enzymatic activities of deep-sea copepods. *Marine Ecology Progress Series*, **168**, 95–107.
- Thuesen, E.V., Rutherford, L.D. & Brommer, P.L., 2005a. The role of aerobic metabolism and intragel oxygen in hypoxia tolerance of three ctenophores: *Pleurobrachia bachei*, *Bolinopsis infundibulum* and *Mnemiopsis leidyi*. *Journal of the Marine Biological Association of the United Kingdom*, **85**, 627–633.
- Thuesen, E.V., Rutherford, L.D., Brommer, P.L., Garrison, K., Gutowska, M.A. & Towanda, T., 2005b. Intragel oxygen promotes hypoxia tolerance of scyphomedusae. *Journal of Experimental Biology*, in press.
- Vetter, R.D., Lynn, E.A., Garza, M. & Costa, A.S., 1994. Depth zonation and metabolic adaptation in Dover sole, *Microstomus pacificus*, and other deep-living flatfishes: factors that affect the sole. *Marine Biology*, **120**, 145–159.
- Yang, T.-H., Lai, N.C., Graham, J.B. & Somero, G.N., 1992. Respiratory, blood, and heart enzymatic adaptations of *Sebastes alascanus* (Scorpaenidae; Teleostei) to the oxygen minimum zone: a comparative study. *Biological Bulletin. Marine Biological Laboratory, Woods Hole*, **183**, 490–499.

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