Blood and Tissue Characteristics of *Hemisquilla californiensis* (Crustacea: Stomatopoda), A Burrow-Dwelling Mantis Shrimp Which Routinely Encounters Hypoxia

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Abstract

Hemisquilla californiensis is a burrow-dwelling mantis shrimp which routinely encounters hypoxic conditions and can survive up to 48 hours or more in complete anoxia. We compared the properties of this species' tissue and hemolymph to those of the spot shrimp *Pandalus platyceros* (Caridea: Pandalidae), which inhabits an aerobic environment and dies within a few hours of anoxia, to find the biochemical adaptations that allow *Hemisquilla* to survive in low oxygen. Both species accumulated lactate under anaerobic conditions. *Hemisquilla* accumulated lactate more slowly than did *Pandalus* and tolerated a much higher lactate concentration in the blood before death. The buffering capacity of abdominal muscle and hemolymph in *Hemisquilla* is no greater than that in *Pandalus*, providing no added protection against acidification during anaerobiosis. However, *Hemisquilla* tolerates a two-fold greater decrease in hemolymph pH before death than does *Pandalus*. The capacity for survival in anaerobic conditions shown by *Hemisquilla* is due both to a mechanism for reducing the rate of lactate buildup in the blood and to an increased tolerance for lactate and acidosis.

Introduction

Most animals can withstand only brief periods without oxygen, but some species are surprisingly tolerant to anaerobic conditions (Hochachka, 1982). Environmental anaerobiosis can occur regularly for some marine species, especially those who may be trapped by the changing tides or live in the sediment. The most common anaerobic pathway utilized converts the glycolytic product pyruvate to lactate via fermentation. This results in significantly lower ATP yields than aerobic metabolism and also results in a potential accumulation of toxic byproducts such as lactate and H^+ ions. Because of this, few animals that utilize fermentation use the process for an extended period of time.

Arthropods are highly mobile and either avoid anoxic environments or simply leave when oxygen levels decrease. As a result, most crustaceans have a lower tolerance to anoxia or hypoxia as compared to many facultative anaerobic bivalves and mollusks (Zebe, 1991). Energy production in Crustaceans appears to proceed only by the glycolytic degradation of glycogen and no special metabolic pathways seem to be utilized (Zebe, 1991).

Hemisquilla californiensis (Stomatopoda: Hemisquillidae) (Owen, 1832) is a burrow-dwelling crustacean that resides off the southern coast of California to Panama (Basch and Engle, 1989) (Figure 1). Their blind-ended burrows, usually about 1 m long, are constructed in stable mud-sand habitats at depths ranging from 4-90 m. While active, animals may sit at the burrow entrance or move around the surrounding area. During inactive periods, individuals will cap their burrow with a sand plug and remain inside for extended periods of time. Within the sealed burrow, oxygen levels rapidly deplete within 2-3 hours while activity levels, such as movement and pleopod beating, remain constant and high (Cowles, 2004). Studies have shown that *Hemisquilla* is a poor oxyregulator, but can survive at least 52 hours of anoxia (Peters, 1997).

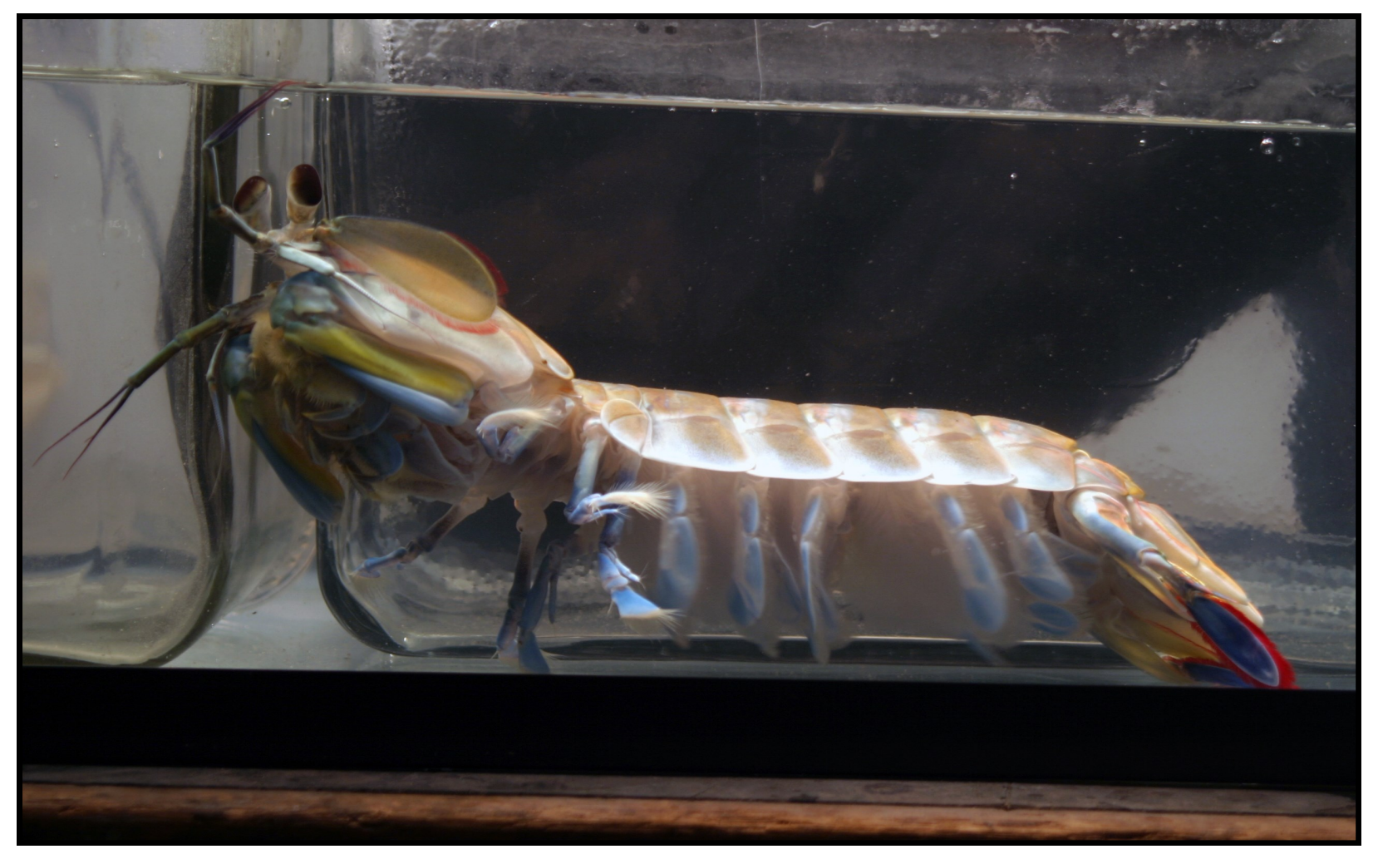


Figure 1. *Hemisquilla californiensis*. Photo by Ruwan Randeniya

The focus of this research was to determine the biochemical pathways that allow *Hemisquilla californiensis* to sustain high metabolic activity under anaerobic conditions.

Methods

Animal Collection and Maintenance

Hemisquilla individuals were captured by otter trawl at 30 m depth off Santa Barbara, California in September 2003. All individuals were maintained in a recirculating salt water aquarium with artificial seawater. All animals were kept separate from each other and given a PVC pipe to simulate a burrow. Water salinity was kept between 32-35 ppt, pH was maintained between 8.0-8.2, and nitrate levels were controlled via carbon filtration and water exchange. The tank was kept at 16°C on a 14:10 light:dark cycle to simulate their natural habitat. Individuals were fed shrimp or salmon three times a week and were all allowed to acclimate for at least 10 days prior to any testing. *Pandalus platyceros*, the spot shrimp, which is of similar size but does not live in burrows, will be used as a control. *P. platyceros* specimens were captured at 80 m depth by otter trawl off San Juan Island, WA, and maintained at the ambient temperature of 11°C in seawater tables at the Walla Walla College Marine Station. *P. platyceros* is an epibenthic species and so is unlikely to encounter anoxia.

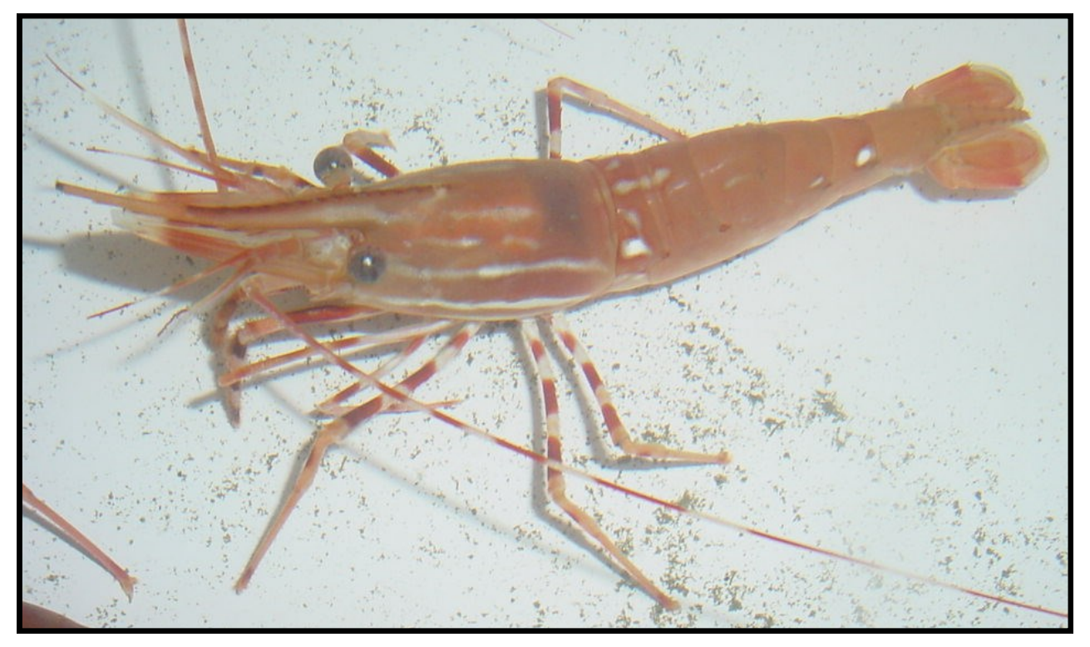


Figure 2. Photograph of the spot shrimp *Pandalus platyceros*. Note the spots on its abdomen that inspire its name.

Experimental Methods

For each experiment a single subject was placed into a sealed, temperature-controlled respirometry chamber and their rate of aerobic metabolism was monitored by oxygen electrode (Cowles, 2004). At least three animals of each species were allowed to deplete the oxygen down to sharply hypoxic conditions (<6 mmHg, <4% saturation), three were allowed to continue into anoxia until death or having spent at least 48h in anoxia, and three were allowed to remain in normoxic conditions. After each experiment the animal was removed from the chamber, a hemolymph sample was extracted, and the animal was flash frozen in liquid nitrogen for biochemical analysis. Frozen tissue was harvested from several key regions, including the hepatopancreas, abdominal muscle, leg muscle, eye, and hemolymph and kept in liquid nitrogen or at −70°C until analysis.

Biochemical Analysis

Lactate - Samples were homogenized and deproteinized with 1.0M ice-cold perchloric acid, followed by neutralization with 5M potassium carbonate. For analysis, 0.20 ml of sample homogenate was added to 2.5 ml hydrazine/glycine buffer (0.5M glycine, 0.4M hydrazine; pH 9.0) and 0.20 ml 40mM β-NAD^+. Twelve units of LDH was added to initiate the reaction. β-NAD^+ reduction was read at 340 nm after two hours of incubation at 37°C (adapted from Iain Ridgway, personal communication).

Lactate Dehydrogenase (LDH) - LDH is the terminal enzyme in anaerobic glycolysis in many crustaceans and therefore provides a good index of a tissue's metabolic capacity during anaerobiosis. LDH activity was measured in a medium containing 80 mM Tris/Hcl buffer (pH 7.2 at 20°C), 0.15 mM NADH, 5.0 mM Na-pyruvate, and 100 mM KCl. The decrease in absorbance at 340 nm due to the oxidation of NADH was recorded (Childress and Somero, 1979).

Buffering Capacity - The buffering capacity of all sample tissues was analyzed as described in Castellini and Somero (1981). Briefly, samples were homogenized in 0.9% NaCl and pH levels were measured. The homogenate was acidified with 0.02-0.2M HCl to lower the pH to ~5.0. Samples were titrated at 25°C with 0.02-0.2M NaOH. The buffering capacity (in slykes, β) was determined by examination of the titration curve at ±0.5 pH units around the initial tissue pH.

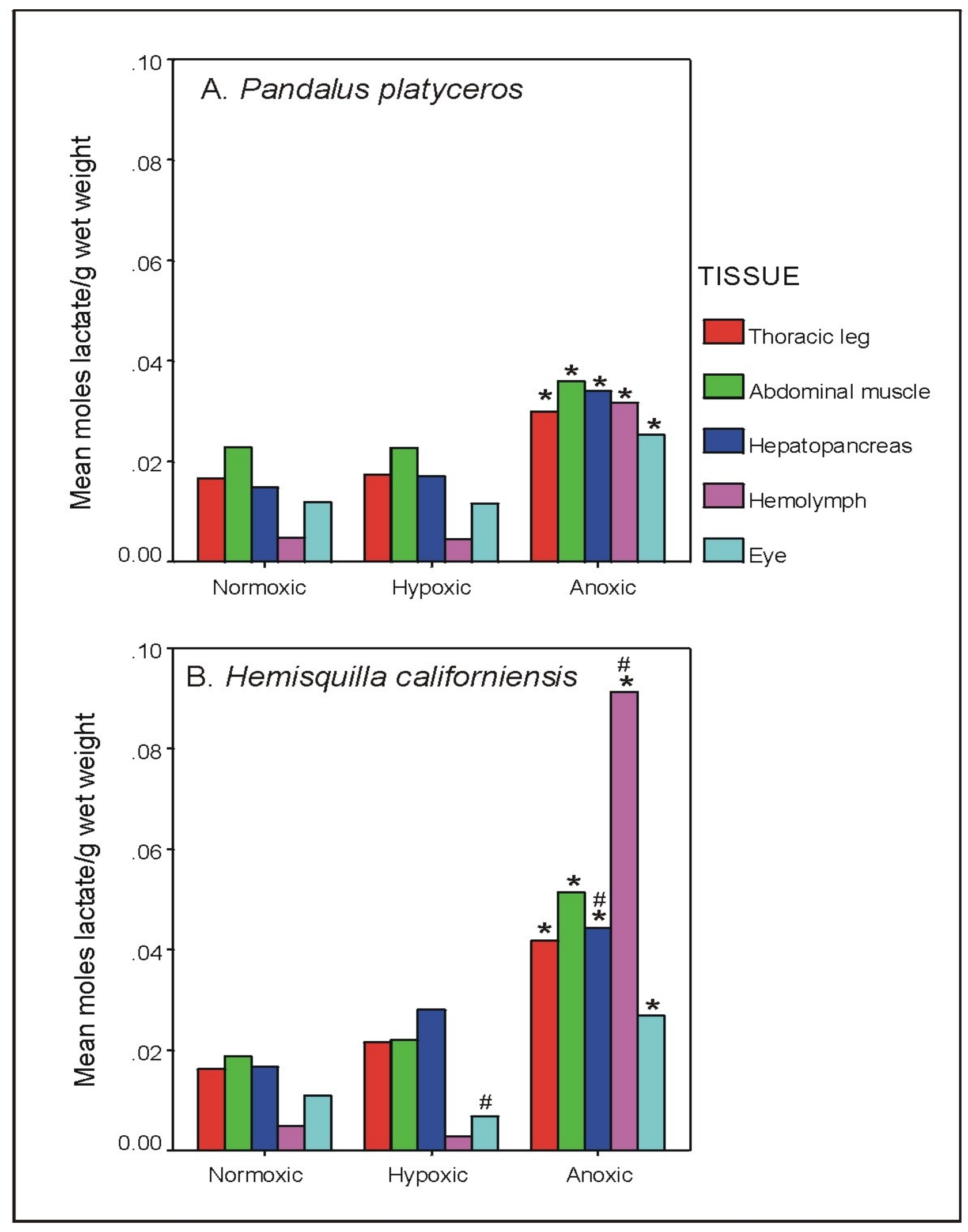


Figure 3. Increase of lactate concentration due to anaerobiosis in various tissues of (A) *Pandalus platyceros* and (B) *Hemisquilla californiensis*.
* - value is significantly different from normoxic value of the same tissue, same species
\# - *Hemisquilla* is significantly different from *Pandalus* for that value

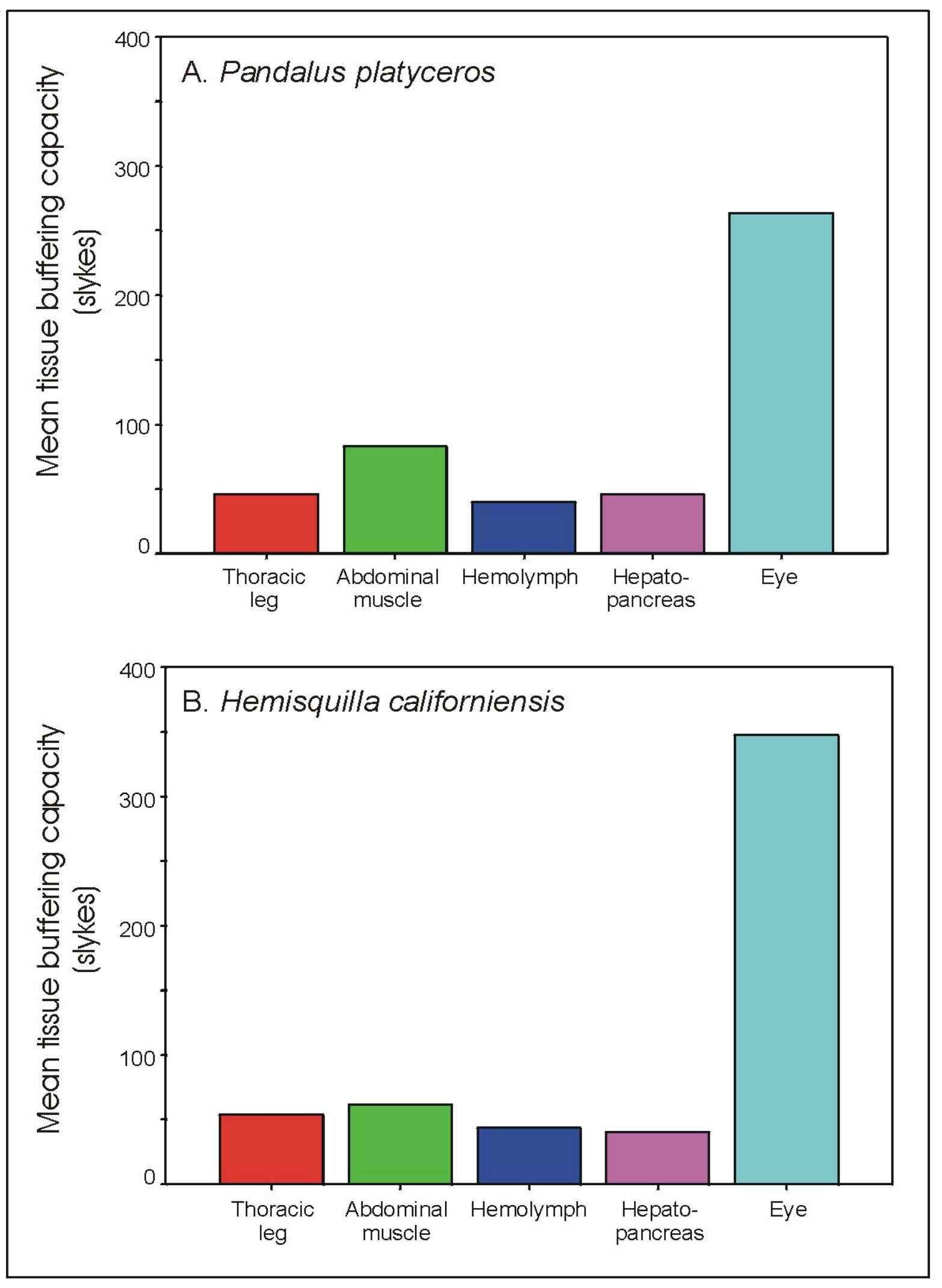


Figure 5. Buffering capacity (slykes) of various tissues of (A) *Pandalus platyceros* and (B) *Hemisquilla californiensis*.

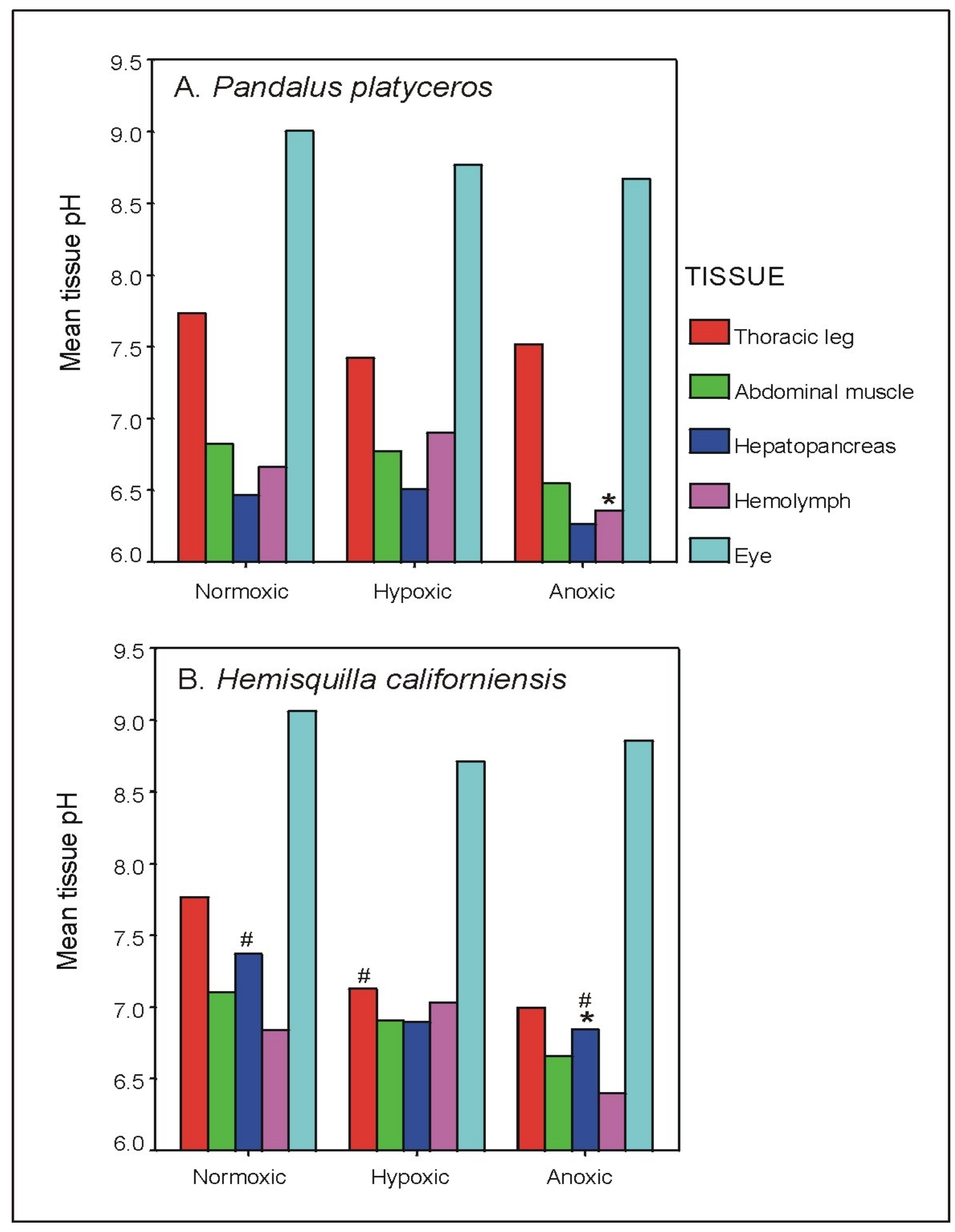


Figure 4. Change in tissue pH due to anaerobiosis in various tissues of (A) *Pandalus platyceros* and (B) *Hemisquilla californiensis*.
*- value is significantly different from normoxic value of the same tissue, same species
\# - *Hemisquilla* is significantly different from *Pandalus* for that value

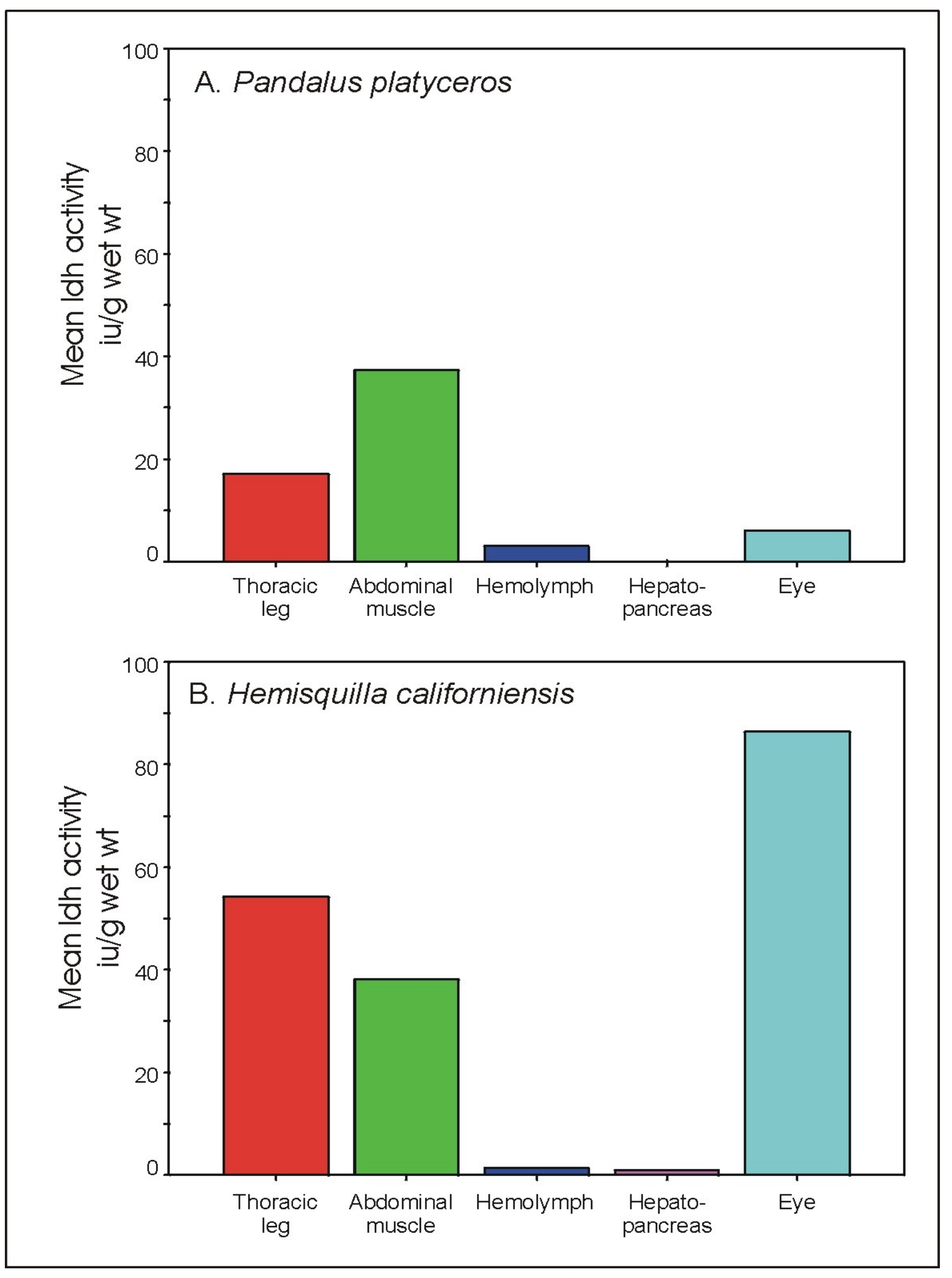


Figure 6. Lactate Dehydrogenase activity (iu/g) of various tissues of (A) *Pandalus platyceros* and (B) *Hemisquilla californiensis*.

Results

Lactate Concentrations - Lactate concentrations increased with anoxia in all tissues for both *Pandalus* and *Hemisquilla* (Figure 3) (Two-Way ANOVA, P=0.008 for *Pandalus*, 0.014 for *Hemisquilla*). While lactate increased in hypoxia for both species, the most marked increase was in anoxia. Lactate in *Pandalus* increased by a factor of nearly two, while in *Hemisquilla* it increased more than 2.5-fold. Lactate concentrations in the eye, as an example of neural tissue, were the lowest in anoxia, while abdominal muscle had slightly higher concentrations than most other tissues (Figure 3). Lactate accumulations in the hepatopancreas and in the hemolymph of *Hemisquilla* during anoxia were significantly greater than those in similar tissues of *Pandalus*. This is partly to be expected since *Pandalus* invariably died within a short time of anoxia while *Hemisquilla* lived much longer, with several individuals surviving 48h.

Buffering Capacity & pH - As expected from the buildup of lactate, tissue pH of both species dropped slightly in hypoxia and anoxia though this change was significant in only a few cases (Figure 4). Buffering capacity for the two species was similar in all tissues (Figure 5). In both species the eye was markedly different from the rest of the tissue, having a sharply higher pH and higher buffering capacity (Figures 4 & 5). This difference was especially pronounced in *Hemisquilla*.

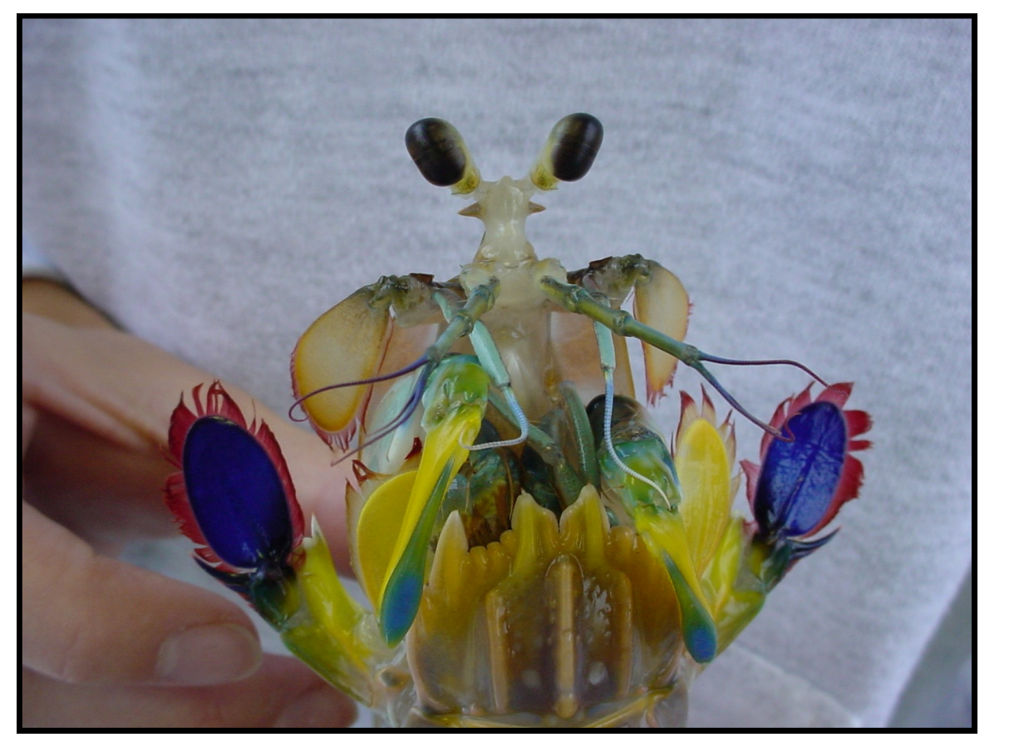
Lactate Dehydrogenase Activity - For both species, LDH activity was moderately high in muscle tissue and quite low in hemolymph and the hepatopancreas (Figure 6). LDH activities were similar between the two species in these tissues. In *Pandalus* LDH was also low in the eye, but in *Hemisquilla* by far the highest LDH activities of any tissue were found in the eye.

Discussion

Lactate accumulation in *Hemisquilla* during anaerobiosis was substantial and similar to that of *Pandalus*. It is clear that, as reported for other crustaceans (Zebe, 1991) and as seen in *Pandalus*, lactate is a major end product of anaerobic metabolism in *Hemisquilla*. Concomitant with lactate production we observed a decrease in pH in the tissues. The mechanisms for extended anaerobiosis in *Hemisquilla* as compared to that in *Pandalus* are not obvious. Both have similar LDH activity in most of the body tissues, and the buffering capacity of *Hemisquilla* tissue and blood are no better than that of *Pandalus*. However, all *Pandalus* individuals tested in anoxia died after a buildup of only an average of 0.017 moles lactate/g wet weight in their tissues and a drop in pH of only 0.27. *Hemisquilla*, on the other hand, withstood a buildup of an average of 0.038 moles lactate/g wet weight and a pH drop of 0.48 in their tissues, and this was associated with the death of only some individuals. *Hemisquilla's* ability to withstand extended anaerobiosis thus appears to be linked not to avoidance of lactate buildup in the tissues or to increased buffering capacity but simply to greater metabolic tolerance to lactate buildup and resulting acidification.

The eyes of these crustaceans, chosen as a representative of neural tissues, sharply differed from other tissue in several characteristics. The pH of the eyes was 1.5 units higher than that of the other tissues, and the buffering capacity was approximately triple. This suggests that the maintenance of alkaline conditions may be especially critical in the eye. Lactate seemed to build up less in the eye than in other tissues (though the difference was not significant), suggesting that this neural tissue may be partly protected from buildup of harmful metabolites such as lactate even under fully anoxic conditions. In this regard the extremely high activities of LDH in the eyes of *Hemisquilla* come as quite a surprise, especially as they are not associated with an increased buildup of lactate there. Perhaps the LDH in the eyes of *Hemisquilla* is specialized for the local detoxification of lactate by converting it back to pyruvate.

Summary



- **_Hemisquilla californiensis_ is a burrow-dwelling crustacean which routinely experiences environmental anoxia.**
- **_Hemisquilla_ utilizes lactate fermentation to provide energy during anaerobiosis.**
- **There was no difference in buffering capacity between _Hemisquilla_ and the control, _Pandalus platyceros._**
- **Tissue pH trended downward with extended anaerobiosis, but not significantly.**
- **_Hemisquilla_ has a similar pattern of LDH activity as does _Pandalus_ in most tissues, but LDH activity in _Hemisquilla's_ eyes is very high.**
- **Eyes of both species tend to be buffered very well and they both had a high pH level.**

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