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Abstract

Anaerobic activities such as those associated with spawning, foraging for prey, and predator avoidance are difficult to quantify in wild fish. This study experimentally evaluated if the muscle enzyme lactate dehydrogenase (LDH) can be used to index recent anaerobic activity in fish by testing the hypothesis that muscle LDH activity will be greater in exercised fish than in rested fish. We used burst swimming motions in a swim tunnel to elicit anaerobic metabolism in a 5 day anaerobic exercise treatment (n = 30) and a rested control group (n = 30). On average the exercised fish produced significantly more LDH in their muscle tissue (average = 19.5 IU/*u*g, SE = 1.8, $F = 12.88$, df = 2, 57, $P < 0.001$) due to the increased anaerobic activity than rested fish (average = 13.4 IU/*u*g, SE = 0.9). However, large individual variability in LDH activity within groups resulted in some overlap between treatment groups. Therefore we suggest limiting the use of LDH activity to infer relative comparisons of anaerobic activity among groups until the relationship is more clearly understood.

Keywords: lactate dehydrogenase, anaerobic activity, anaerobic metabolism, burst swimming

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Introduction

Anaerobic activities that rely on burst swimming motions are associated with activities such as spawning, migration, foraging for prey, and predator avoidance (Sherwood et. al 2002*b*, Kaufman et al. 2006). Lactate dehydrogenase (LDH; enzyme code 1.1.1.27; IUBMB 1992) is a glycolytic muscle enzyme which is largely retained within the axial musculature of fish during anaerobic activities (Childress and Somero 1990; Selch and Chipps 2007).

White muscle LDH activity may provide an index of recent anaerobic activity (within ~7 days of capture; Garenc et al. 1999, Sherwood et al. 2002*a*; Sherwood et al. 2002*b*; Martinez et al. 2003). For example, LDH activity has been used to hypothesize an energetic explanation for female-biased sexual size dimorphism as male yellow perch LDH activity was higher than in females suggesting greater energy costs associated with foraging, predator avoidance and spawning and therefore leaving less energy for somatic growth (Schoenebeck and Brown 2012). Other studies have also used LDH to index recent fish anaerobic activity (Audet and Couture 2003, Rennie et al. 2005, Kaufman et al. 2006, Selch and Chipps 2007). However, the relationship between LDH and fish activity has not been experimentally determined (Schoenebeck and Brown 2012). Indeed, white muscle LDH activity should not be used to index recent fish anaerobic activity if LDH activity does not differ between fish using anaerobic metabolism and fish that are not using anaerobic metabolism (H_o). Alternatively, we would hypothesize anaerobic metabolism will result in greater white muscle LDH activity than in rested fish who are not experiencing anaerobic metabolism suggesting LDH activity could be used to index recent anaerobic activity in fish (Ha). Therefore, our objective was to experimentally determine if an exercised treatment group would have higher LDH activity compared to a rested control group.

Methods

***Experimental fish*:** Lactate dehydrogenase can vary with water temperature, fish mass, sexual maturity, and prey source and therefore these variables were controlled during the experiment (Childress and Somero 1990, Rennie et al. 2005, Selch and Chipps 2007, Schoenebeck and Brown 2012). Immature bluegill sunfish (*Lepomis macrochirus)* of

similar size were used to control for variability associated with fish mass and reproductive maturation. Fish were collected from Cottonmill Lake, Kearney, Nebraska using boat electrofishing. Fish were housed in a divided 1900L holding tank maintained at a water temperature of 17.5°C and fed bloodworms (*ad libitum*) throughout the experiment.

***Swimming assessment*:** Fish were acclimated for 7 days prior to the start of the experiment and then randomly divided into two groups, exercised (n = 30) and rested (n = 30). Treatment fish were exercised using a 10-liter swim tunnel to stimulate burst swimming motions twice daily (9 AM and 5 PM) for five minutes or until fish reach exhaustion for a total of 10 exercise trails in five days (Prenosil et al. 2016). After fish were placed in the swim tunnel, water velocity within the swim tunnel was increased from a resting rate of 0 cm/s to 45 cm/s, which took approximately 15 seconds (Prenosil et al. 2016). Fish that did not swim against the current were prodded with a wire to elicit a burst swimming response. Rested fish were placed in the swim tunnel twice daily for five minutes with no current (0 cm/s) for a total of five days.

Tissue and Statistical Analysis: Fish were sacrificed (IACUC #021213) and the white axial muscle was filleted using a sterilized scalpel and stored at -80°C. Muscle fillets were ground into a homogenous powder using liquid nitrogen and the homogenized powder was resuspended in 2 mL of phosphate buffer 1XPBS (15 M Na2HPO4, 15 M NaHPO4, 20% glycerin, 5% beta mercaptoethanol, and 1X Sigma Aldrich Protease Inhibitor Cocktail; cat. # P2714). The resuspended samples were sonicated three times for 20 seconds. To further dilute the samples, one gram of each sample was resuspended in 500uL of 1XPBS and vortexed. Samples were centrifuged for 30 minutes at 13,000 rpm and the supernatant of each sample was removed and placed in new microcentrifuge tubes. Total protein concentrations (ug/L) were determined using 5 uL of each sample and the Pierce BCA (bicinchoninic acid; cat. #23225) microplate assay per manufacturer's instructions run in triplicate for each sample and standard. BIOO Scientific MaxDiscovery Lactate Dehydrogenase Enzymatic Assay Kits (3460-04) with a sensitivity of 10U/L were calibrated to confirm assay linearity and run in triplicate per manufacturer's instructions to estimate the activity of LDH. The LDH activity for each sample was divided by the corresponding average total protein concentration as determined by the BCA assay to normalize LDH activity to the total protein concentration (IU/ug).

The model LDH = length (continuous) + treatment group (exercised or rested) was used to account for potential differences in length and to test the null hypothesis that LDH activity of exercised fish was not significantly different from rested fish (grouping factor) at $\alpha = 0.05$. Mann-Whiney *U* tests were used to compare fish length and survival between exercised and rested fish and post-hoc to compare LDH activity and length of fish that did not complete all 10 trials (n =7) to those that did complete all 10 trials from the exercised group (n = 23).

Results

Experimental fish length was not significantly different between groups ($U = 412$, $P = 0.573$) and averaged 80.0 mm (SE = 0.8 mm) for the exercised group and averaged 80.8 mm (SE = 0.8 mm) for the rested group. Survival over the 5 day experiment was significantly lower ($U = 345$, $P = 0.005$) for the exercised group completing an average of 9.5 of the 10 trials (SE = 0.2) compared to zero mortality of the rested group in which all 30 fish completed 10 trials (SE = 0). The seven exercised mortalities were significantly smaller in length (average = 76.0 mm SE = 1.3, $U = 26.5$, $P = 0.008$) compared to the other 23 exercised fish that completed all 10 trials (average = 81.3 mm, SE = 0.8). There was no difference in LDH activity between the 7 exercised fish mortalities (average = 20.0 IU/*u*g, SE = 3.1) compared to the other 23 exercised fish that completed all 10 trails (average = 19.3 IU/*u*g, SE = 2.2, $U = 95$, $P = 0.477$). Of the seven exercised fish mortalities, one fish completed 6 of the 10 exercise trials, two fish completed 7 trials, two fish completed 8 trials, and two fish completed 9 trials.

Individual fish LDH varied within experimental groups ranging from 5.3 to 23.0 IU/*u*g for rested fish and 5.7 to 51.8 IU/*u*g for exercised fish. Lactate dehydrogenase activity was positively related to length (Figure 1) and while accounting for length exercised fish had significantly more lactate dehydrogenase activity in their muscle tissue (average = 19.5 IU/*u*g, SE = 1.8) than rested fish (average = 13.4 IU/*u*g, SE = 0.9, $F = 12.88$, df = 2, 57, $P < 0.001$) presumably due to increased anaerobic activity elicited by the swim trials.

Discussion

Exercise trials were successful in eliciting burst swimming motions and anaerobic metabolism and on average produced greater LDH activity within the muscle tissue.

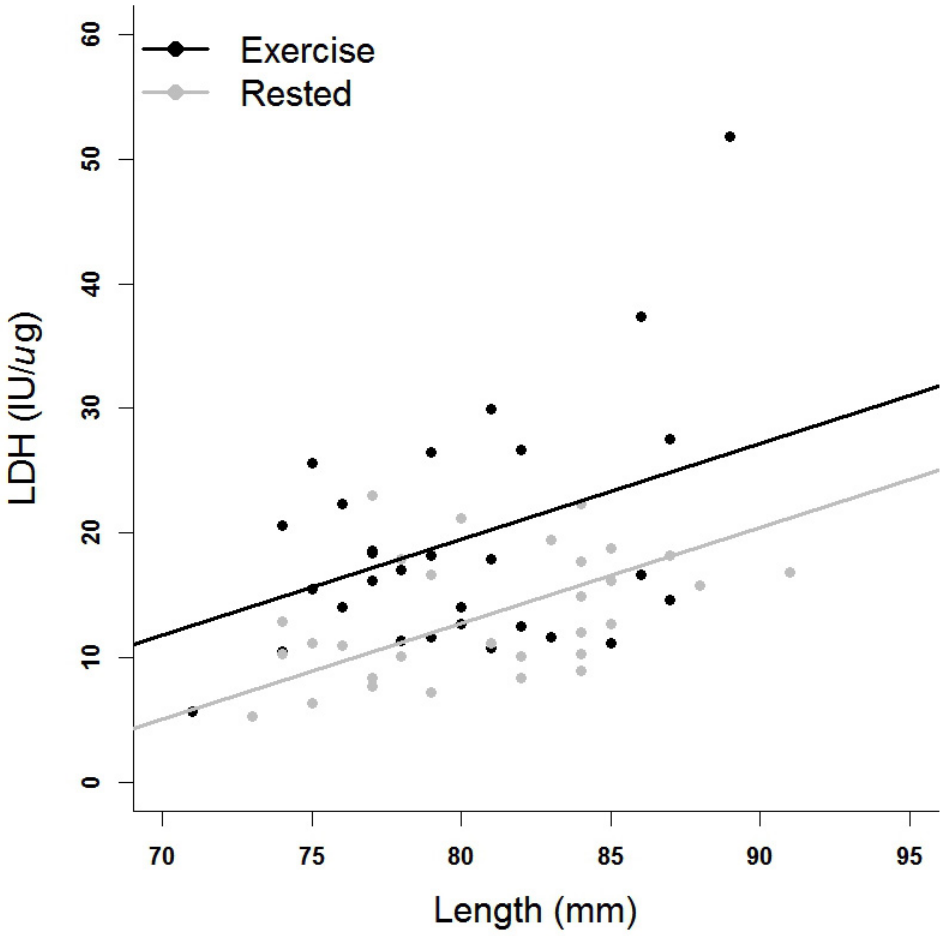

Figure 1. Lactate dehydrogenase activity (normalized to total protein concentration) and corresponding fish lengths in exercised (solid circles, n = 30) and rested (open circles, n = 30) groups shown with regression lines.

Therefore, we reject H_o suggesting muscle LDH activity could be used as an index of recent anaerobic activity in fish. This study provides a starting point to the experimental determination of using LDH as an index of anaerobic activity. Future studies should consider several things to further elucidate the shape of the relationship including the length of the experiment, the water velocity, length and number of trials per day, and length of the fish.

The length of the trials and the water velocity used in this study were appropriate in eliciting repeated burst swimming motions for the size of fish used. The water velocity was fast enough that exercised fish could not swim against the current without the use of burst swimming motions. In addition, the duration of the trials were long enough to stimulate many repeated burst swimming motions and therefore anaerobic metabolism. In addition to a similar exercised group used in this study (10 trials in 5 days), we suggest future studies incorporate a treatment group with 50% of the exercise trials or 5 trials in 5 days to further elucidate the shape of the relationship between anaerobic metabolism and LDH activity.

We caution against using more than 10 exercise trials within 5 days for similar length fish as 7 of 30 fish used in exercise trials died before completing all 10 swim trials. Anecdotally, exercised fish were able to complete the first 4 swim trials without showing signs of exhaustion but following trial 5 the exercised fish were beginning to reach exhaustion quicker and appeared weaker (i.e., decreased number and length of burst swimming motions and lack of balance following trials) and the first mortality occurred following trial 6.

Similar sized fish were used in an attempted to control for fish length as previous studies have documented LDH positively correlate with fish size (Childress and Somero 1990, Rennie et al. 2005, Schoenebeck and Brown 2012). Although similar sized fish were used in this experiment (the range of fish lengths used in each group was only 18 mm) this difference in size was large enough to result in a significant positive correlation between LDH activity and fish length. Larger fish within the exercised group were hardier during the experiment and better able to survive the exercise trails however LDH activity did not significantly differ between the larger 23 fish which completed all 10 trials and the smaller 7 exercised fish mortalities. Small differences in fish length explained changes in LDH activity highlighting the need to control (experiments) or account (wild fish) for fish size when using LDH activity.

On average, fish in the exercised group had significantly greater LDH activity than fish in the rested group but large individual variability in LDH activity within groups resulted in overlap between the two experimental groups. We suggest future studies concentrate on identifying mechanisms underlying within group variability in LDH activity. Given our study results (i.e., large within group variability), the use of LDH as an index may be limited to relative questions directed at a group of fish instead of at the individual level until the shape of the relationship between anaerobic activity and individual LDH activity can be further elucidated. Furthermore, we caution the application of LDH as an index of activity for wild fish when confounding variables that can impact LDH activity are either not controlled or are simply unknown.

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