Canadian Journal of Fisheries and Aquatic Sciences
Journal canadien des sciences halieutiques et aquatiques

Relationship between ontogenetic changes in foraging ecology and muscle lactate dehydrogenase activity in wild smallmouth bass (*Micropterus dolomieu*)

Journal:	<i>Canadian Journal of Fisheries and Aquatic Sciences</i>
Manuscript ID	cjfas-2015-0537.R1
Manuscript Type:	Article
Date Submitted by the Author:	16-Feb-2016
Complete List of Authors:	Laberge, Frederic; University of Guelph, Integrative Biology Edmunds, Nicholas; University of Guelph, Integrative Biology Yin-Liao, Irene; University of Guelph, Integrative Biology McCann, Kevin; University of Guelph,
Keyword:	FISHES < Organisms, FRESHWATER < Environment/Habitat, ECOLOGY < General, ENZYMES < General, FORAGING < General

SCHOLARONE™
Manuscripts

Relationship between ontogenetic changes in foraging ecology and muscle lactate dehydrogenase activity in wild smallmouth bass (*Micropterus dolomieu*)

Frédéric Laberge*, Nicholas Edmunds, Irene Yin-Liao, and Kevin S. McCann

Department of Integrative Biology, University of Guelph, Guelph, ON, Canada

E-mail addresses: N.E. (nick.b.edmunds@gmail.com), I. Y.-L. (iyinliao@mail.uoguelph.ca), K.S.M. (ksmccann@uoguelph.ca)

*Correspondence to: F. Laberge, Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada N1G 2W1. Tel.: 519-824-4120 ext. 56238, FAX: 519-767-1656. E-mail: flaberge@uoguelph.ca

Abstract

The activity of muscle glycolytic enzymes scales positively with body size in active fish; a phenomenon thought to counter the increased costs of burst swimming faced by larger individuals. Recent work argued that changes in these enzymes during ontogeny additionally reflect changes in foraging ecology. Here, we evaluated the relationship between muscle anaerobic metabolism and foraging ecology in a population of wild smallmouth bass by relating activity of muscle lactate dehydrogenase (LDH) to estimates of trophic position and habitat use obtained from stable isotope signatures. As expected, LDH activity increased with body size. However, further analysis showed associations between foraging ecology and LDH activity. Specifically, a shift to higher trophic position, indicating a change in diet, was paralleled by a shift to increased LDH activity. However, steady mass-specific decrease in LDH activity was observed as the fish grew above the size associated with this diet shift. Further, lower LDH activity was associated with increasing use of littoral carbon sources. These findings contribute to our understanding of how plasticity in muscle anaerobic potential is associated with fish foraging ecology.

Keywords: Fish, anaerobic metabolism, glycolysis, foraging ecology, ontogeny.

Rapport entre ontogénèse de l'alimentation et activité de la déshydrogénase lactique musculaire chez l'achigan à petite bouche (*Micropterus dolomieu*)

Resumé

L'activité des enzymes glycolytiques musculaires augmente avec la taille corporelle chez les poissons actifs; un phénomène qu'on croit lié au maintien de la capacité d'effort de pointe face à l'augmentation des coûts de la nage avec la croissance. Il a récemment été proposé que les changements d'activité de ces enzymes durant l'ontogénèse puissent aussi refléter des changements d'alimentation. Nous avons évalué le rapport entre le métabolisme musculaire anaérobie et l'écologie alimentaire chez une population sauvage d'achigan à petite bouche en associant l'activité de la déshydrogénase lactique (LDH) à des estimés de niveau trophique et d'utilisation de l'habitat obtenus par analyse d'isotopes stables. Comme anticipé, l'activité de la LDH augmente avec la taille. Des associations entre l'écologie alimentaire et l'activité de cette enzyme existent aussi. Plus spécifiquement, une augmentation de niveau trophique indiquant un changement d'alimentation se produit en parallèle d'une augmentation de l'activité de la LDH. Cependant, une baisse graduelle de l'activité de la LDH en fonction de la masse musculaire est observée au dessus de la taille associée avec ce changement d'alimentation. De plus, une baisse de l'activité de la LDH est associée avec l'utilisation accrue du carbone de sources littorales. Ces résultats aident à comprendre comment la plasticité du potentiel anaérobie musculaire est associée à l'écologie alimentaire des poissons.

Mots clés: Poissons, métabolisme anaérobie, glycolyse, écologie alimentaire, ontogénèse.

Introduction

The activity of muscle enzymes has been used to study how changes in metabolism accompany changes in body size in fish (Somero and Childress 1980; Sullivan and Somero 1983; Goolish 1991; Dickson et al. 1993; Martinez et al. 2000). While a mass-specific decrease in aerobic respiration rate is typically observed in muscle tissue as body size increases, the opposite scaling pattern is seen in the activity of anaerobic muscle enzymes. This increased anaerobic capacity in larger fish appears needed to maintain constant relative burst swimming speed (i.e. in body lengths per unit time) in the face of greatly increased costs of locomotion (i.e. power needed to overcome increased drag).

In addition to the general scaling relationship just mentioned, some interspecific differences in anaerobic metabolism have been related to differences in fish ecology (Childress and Somero 1990; Somero and Childress 1990; Sherwood et al. 2002a). For example, muscle lactate dehydrogenase (LDH) activity, a common indicator of anaerobic metabolism, displays higher scaling coefficients in active pelagic fish compared to less active benthic fish (Childress and Somero 1990); a pattern proposed to reflect selection for different burst swimming abilities across species. More recently, within-population variation in LDH activity during fish ontogeny has been used to propose the ‘diet-shift hypothesis’, whereby fish burst swimming activity and its related muscle anaerobic potential are reset to lower values whenever fish are able to switch diet to larger prey while growing (Sherwood et al. 2002a; 2002b). Such shifts would allow fish to maximise energy gains while minimizing foraging costs and could provide useful ecological information about energy budget at different life stages.

The muscle LDH activity referred to in the above studies is the maximal catalytic activity of the enzyme ($V_{\max}$) in the oxidation of NADH which accompanies the production of lactate from pyruvate. This reaction is involved in the maintenance of redox balance (regeneration of NAD^+) to extend anaerobic glycolysis, and thus can be conceived as an index of anaerobic metabolism capacity. The enzyme can also work in the opposite direction to produce pyruvate from lactate during recovery from exercise (Place and Powers 1984; Moyes et al. 1992). A link between LDH lactate production and burst swimming performance is commonly assumed, but it has been questioned in light of the fact that escape swimming speed usually does not correlate with LDH activity when body size is taken into account (Garenc et al. 1999; Gibb and Dickson 2002; Odell et al. 2003). On the other hand, LDH activity is strongly associated with endurance swimming in the cod (Martinez et al. 2003). It appears to enhance the capacity to produce burst-coast movements before reaching exhaustion. This suggests that LDH activity could be a good indicator of the swimming abilities important for repeated swimming bursts involved in active foraging (Sherwood et al. 2002a).

Support for the diet-shift hypothesis comes from evidence of an association between patterns of diet change and LDH activity during ontogeny and across fish populations exposed to different prey assemblages (Sherwood et al. 2002a; 2002b; Kaufman et al. 2006). However, it is not clear to what extent changes in body size could underlie the observed patterns in LDH activity associated with diet shifts. The diet-shift hypothesis would predict shifts in LDH activity concurrent with changes in diet even after correction for body size. We set out to assess the generality of this hypothesis by measuring trophic position (diet indicator) and white muscle LDH activity in a population of smallmouth bass spanning a wide range of body sizes. Additionally, we assessed LDH activity against an estimate of littoral and pelagic habitat use

under a hypothesized negative relationship between pelagic habitat foraging and anaerobic swimming capacity. Namely, we posited that fish obtaining food from pelagic sources would favour aerobic over anaerobic swimming capacity to support their ability to swim in open waters characteristic of this habitat.

Methods

Sampling

Fish were collected from Big Sound Bay in Lake Huron near Parry Sound, Ontario, Canada during August 17-24, 2013 and August 23-29, 2014 using a variety of techniques (angling, minnow traps, seine nets, and gill nets). A total of 62 smallmouth bass (*Micropterus dolomieu*) were used for stable isotope analyses, while only the 36 fish caught in 2013 were used for the LDH activity assay. Sampling and procedures were approved by the Ontario Ministry of Natural Resources (permits # UGLMU2013-06a, UGLMU2014-07) and the University of Guelph animal care committee.

Captured fish were weighed and measured (total length to the nearest 0.5 cm) daily. Large fish were weighed to the nearest 10 g with a Rapala Pro Select Digital Scale and small fish were weighed to the nearest 0.01 g with a laboratory balance (Mettler Toledo PB1502-S). Muscle samples were taken from the dorsal caudal musculature (skin was cut out) of each fish for stable isotope and LDH analyses. In very small fish, a whole muscle fillet was taken from one side to obtain enough tissue for analysis. The muscle samples were immediately frozen at -20°C and remained frozen until analysis. In addition to fish, bivalve mussels and littoral snails

were collected and used in this analysis as isotopic baselines for pelagic and littoral habitats respectively.

Stable isotope analyses

The trophic ecology of fish can be studied by gut content analysis or indirect methods such as stable isotope signatures (Vander Zanden et al. 1998; 2000). Here, stable isotopes fractions of ^{15}N and ^{13}C were used to acquire measures of trophic position and habitat use, respectively. Frozen muscle samples from individual fish and baseline organisms were dried at 70°C for 2 days, ground into powder and sent to the University of Windsor GLIER laboratories for isotopic analysis (Windsor, ON, Canada). $\delta^{13}\text{C}$ isotopic values were corrected for fat content using the equation $\delta^{13}\text{C}_{(corr)} = \delta^{13}\text{C} + (-3.32 + 0.99 * \text{C:N})$ (Post et al. 2007). Random muscle samples analyzed in triplicate showed average coefficients of variation of 0.9% for nitrogen and 1.7% for carbon.

The nitrogen ratios of consumers and baselines were used to estimate fish trophic position with the equation:

$$\text{Trophic Position} = [(\delta^{15}\text{N}_{\text{Fish}} - \delta^{15}\text{N}_{\text{mussel}})/3.4] + 2.$$

N_{Fish} and N_{mussel} are the nitrogen signatures of fish and mussels, respectively (Post 2002). The value of 3.4 is the assumed increase in $\delta^{15}\text{N}$ per trophic level and a value of 2 is added because primary consumers were used instead of primary producers (Vander Zanden et al. 2000).

Carbon ratios of consumers and baselines were calculated to measure the dietary littoral carbon use of fish, which was used to represent habitat use with the equation:

$$\text{Percent Littoral Carbon usage} = (\delta^{13}\text{C}_{\text{Fish}} - \delta^{13}\text{C}_{\text{mussel}}) / (\delta^{13}\text{C}_{\text{snail}} - \delta^{13}\text{C}_{\text{mussel}})$$

where C_{Fish} , C_{mussel} and C_{snail} are the carbon signatures of consumers, mussels and snails respectively (Tunney et al. 2012). The scale of this equation ranges from 0 – 1, where values closer to 0 indicate greater use of pelagic carbon sources and values approaching 1 indicate greater use of littoral carbon sources, assuming that there is no or little contribution from profundal carbon sources to the smallmouth bass diet.

Lactate dehydrogenase assay

LDH activity was assayed based on methods described in Couture et al. (1998). A subsample of 0.3g of muscle tissue was homogenized in 3 ml of 100mM phosphate buffer (pH 7.0) to release LDH. The homogenate was then centrifuged for five minutes at 15000 rpm and stored on ice until use. All samples were used within six hours of homogenization. The homogenate was further diluted to obtain a dilution factor of 150 before use. Each assay began by adding 0.1mL of the clear fraction of the homogenate solution to 2.8mL 0.15mM NADH and 0.1mL 0.8mM pyruvate. Assays were conducted in a temperature-controlled spectrophotometer (Agilent 8453 UV-Vis) at 11°C for four minutes and involved monitoring at a wavelength of 340nm to follow the oxidation of NADH to NAD^+ . Samples were run in triplicate and the average coefficient of variation for all LDH assays was 12.6%. Results are expressed in international units (micromoles of substrate converted to product per min) per gram of wet muscle tissue.

Data analysis

Curve fitting procedures were conducted in GraphPad Prism version 5.04 to explore the relationship between body size (total length and $\log_{10}$ body mass), enzyme activity and isotopic

signatures. Curves of best fit were determined using Akaike's information criterion (AIC). The different models chosen for comparison were straight line, quadratic, segmented regression, and Gompertz (MIC or NIC determination). For the segmented regression model, the inflexion point breaking the two lines was determined by choosing the best fit over a range of inflexion point values. Correction for body size was accomplished by obtaining residuals from the best-fit relationships with body size. The pattern of results did not differ when using length or mass as a body size variable; therefore, only the results using body length are presented in the following for clarity. Raw values of trophic position and percent littoral carbon could not be normalized, so they were used unmodified. All residuals and LDH activity values were normally distributed.

Results

Ontogenetic patterns in diet

Previous studies have shown marked diet shifts during ontogeny of larval and juvenile young-of-the-year smallmouth bass (Easton et al. 1996; Vander Zanden et al. 1998; Dauwalter and Fisher 2008). Data on larger smallmouth bass is limited to the observation that juveniles living in a reservoir consume insects and fish, while adults consume mostly crayfish and insects (Long and Fisher, 2000). Our data provides evidence of a diet shift in this species despite the absence of larval or early juvenile fish in our sample due to the late summer sampling dates (this species breeds in the spring). Figure 1A shows that a shift in trophic position estimated from nitrogen isotopic signatures occurs around a value of 3.5. Interestingly, this shift corresponds to a size gap in our sample, which likely corresponds to different year classes in this system (year-0 juveniles <10cm and older fish >15cm). The best-fit Gompertz relationship ($R^2 = 0.76$, $df = 58$, $\Delta AIC = 1.5$

over segmented regression) illustrates that once a trophic position above 4 has been reached it plateaus at this level and remains there even in the largest fish.

The data for habitat use shown in Figure 1B also supports a diet shift in this population, as littoral habitat use values estimated from carbon isotopic signatures are generally lower in the smallest fish and then increase substantially in fish of intermediate sizes. This increase likely represents a switch to a more specialist diet of littoral benthic invertebrates as we observed, but did not quantify, plenty of crayfish in the stomachs of intermediate size smallmouth bass. Interestingly, the best-fit quadratic relationship ($R^2 = 0.47$, $df = 59$, $\Delta AIC = 26$ over straight line) shows a hump shape pattern highlighting the effect of low littoral carbon values in the very largest fish sampled ($>40\text{cm}$). It is possible that such low values in very large smallmouth bass represent another diet shift whereby the increasingly mobile large fish become more piscivorous than intermediate sizes preying on crayfish. However, this relationship is driven by only 3 or 4 fish and no equivalent change in trophic position (Fig. 1A) accompanies these low littoral carbon values. Nevertheless, it is possible that trophic values of large crayfish and fish prey cannot be discriminated based solely on nitrogen. In fact, pooled muscle samples of medium- and large-sized crayfish had trophic position values around 3.8, which is only slightly higher than average trophic position values for forage fish that bass could consume at this site (e.g. bluntnose minnow: 3.5, round goby: 3.2, yellow perch: 3.4; from Edmunds, 2015).

Ontogeny of LDH activity

Figure 1C shows the relationship between body length and white muscle LDH activity. The best-fit quadratic relationship ($R^2 = 0.59$, $df = 105$, $\Delta AIC = 22$ over straight line) shows an almost linear

increase in LDH activity up to the larger fish sampled, whereupon LDH activity appears to plateau. No obvious downward shift in LDH activity is seen.

Relationship between trophic position and LDH activity

Figure 2A shows the relationship between raw values of trophic position and LDH activity ($R^2=0.57$, $df=104$, Gompertz MIC best-fit with ΔAIC 12 over straight line). An upward shift around trophic level 3.5 is seen, which is reminiscent of the ontogenetic shift in trophic position shown in Figure 1A. Since body size is known to influence LDH activity in active fish, the relationship between ecological variables and enzyme activity was also analyzed after correction for body size. First, the relationship between raw trophic position and size-corrected LDH activity was analyzed to allow for the observation of potential shifts in LDH activity along a trophic position gradient (Fig. 2B). The best-fit relationship obtained with this data is a segmented regression with an upward line up to trophic position 3.5 followed by a downward line at higher trophic positions ($R^2=0.15$, $df=105$, ΔAIC 15 over straight line). This pattern suggests a mass-specific decrease in muscle LDH activity once fish have attained a trophic position around 3.5.

Subsequently, based on previous studies that investigated enzyme activity and burst swimming performance (Garenc et al. 1999; Gibb and Dickson 2002), we investigated the relationship between size-corrected trophic position and size-corrected LDH activity to see if fish with high relative trophic positions also have high relative LDH activity. No significant relationship was seen when size correction was applied to both variables (Fig. 2C).

Relationship between habitat use and LDH activity

Figure 3 shows that LDH activity decreases slightly as smallmouth bass become increasingly reliant on carbon from littoral sources. The apparently higher LDH activity values in littoral specialists (Fig. 3A, segmented regression with inflexion point at 0.65 is best fit, $R^2 = 0.25$, $df = 105$, with ΔAIC 3.3 over quadratic) do not hold after correction of LDH activity for body size (Fig. 3B, quadratic and segmented regression both best fits with $\Delta AIC < 1$, quadratic fit shown, $R^2 = 0.04$, $df = 105$). Size correction applied to both variables reveals a U-shaped pattern possibly suggesting that individual fish displaying unusual foraging behavior for this population have in general relatively higher LDH activity (Fig. 3C, quadratic best fit with ΔAIC 1.8 over segmented regression, $R^2 = 0.11$, $df = 105$).

Discussion

Two possible diet shifts were identified during smallmouth bass ontogeny based on changes in muscle nitrogen and carbon isotopic signatures. One shift occurs between year-0 and older fish, while the other, more tentative, would occur when bass reach a very large size (>40 cm total length). Scaling of LDH activity with body size in smallmouth bass is similar to the pattern described for the barred sand bass by Yang and Somero (1996), with the exception that LDH activity appears to plateau in the largest fish in the present study. Quadratic nonlinear best fits with body length and body mass (not shown) support this plateauing effect. Beyond the effect of body size, an association between foraging ecology and LDH activity is also apparent. An upward shift in trophic position is paralleled by a similar shift to higher LDH activity, but size correction revealed a mass-specific decrease in LDH activity in larger bass following this shift. Finally, analysis of carbon source signatures shows that lower LDH activity is associated with increasing use of the littoral habitat.

249

250 *Diet-shift hypothesis*

251 The diet-shift hypothesis formulated by Sherwood and colleagues (2002a) is based on saw tooth
252 patterns of LDH activity during ontogeny, where enzyme activity is suggested to reset to lower
253 values when a fish can forage on new, more energetically rewarding prey as it grows. Such a
254 pattern was not observed in the smallmouth bass population and life stages studied here. Instead,
255 a relatively steady increase in LDH activity was seen with increasing body size. Nevertheless,
256 larger smallmouth bass show reduced mass-specific LDH activity as they grow beyond a certain
257 size, after a shift in diet that can be inferred from trophic position and habitat use data. Reduced
258 muscle LDH activity with increased bass size was also observed in largemouth bass feeding on
259 small fish prey (Selch and Chips 2007). This pattern of result could provide support for the diet-
260 shift hypothesis, but unlike a saw tooth pattern indicative of a reset to a lower value, mass-
261 specific LDH activity does not recover and declines steadily after the inferred diet shift in
262 smallmouth bass. This result supports the view that shifts in diet can influence LDH activity, but
263 not in a way that muscle anaerobic potential is reset to a lower value when prey size increases. It
264 might imply that foraging on some prey requires increasing anaerobic muscle potential with
265 increasing prey size, while other prey do not. In the case of smallmouth bass, a diet of littoral
266 benthic invertebrates in larger fish (Long and Fisher, 2000, present study) might not require
267 increased anaerobic muscle potential for foraging success. Thus, the reset in LDH activity
268 suggested by Sherwood and colleagues (2002a) is not likely to be a general feature of fish
269 growth. This does not exclude the possibility that it might apply to the foraging activities of
270 some fish species or populations.

271

272 *Habitat use and LDH activity*

273 We hypothesized a negative relationship between pelagic habitat use and anaerobic swimming
274 capacity because open pelagic waters would require more sustained aerobic swimming capacity.
275 However, this hypothesis was not supported by the data because lower littoral carbon signatures,
276 implying greater use of the pelagic habitat, were associated with higher, not lower, LDH activity.
277 This finding could instead provide support for a greater need for endurance swimming in the
278 pelagic habitat, as can be inferred from results in cod (Martinez et al. 2003). In this species, LDH
279 activity is associated with endurance swimming and the capacity to produce burst-coast
280 movements before reaching exhaustion. Such burst-coast movements might be a more prominent
281 feature of fish locomotion in the pelagic habitat, and depend on a high anaerobic muscle
282 potential for efficient swimming.

283 Ontogenetic diet shifts inferred from stable isotope signatures in smallmouth bass are
284 accompanied by change in mass-specific muscle LDH activity. This suggests that foraging on
285 different prey can involve adjustment of anaerobic muscle potential. Thus, in addition to
286 previously demonstrated evolutionary change in anaerobic metabolism in species differing in
287 lifestyle (Childress and Somero 1990; Somero and Childress 1990; Sherwood et al. 2002a),
288 anaerobic metabolism is plastic during the lifetime of an individual fish (Sherwood et al. 2002a;
289 present study). The specific functional role of this metabolic plasticity remains to be elucidated,
290 but it could relate to changes in endurance swimming abilities.

291

292 **Acknowledgements**

293 This research was supported by NSERC discovery grants to K.S.M and F.L as well as an
294 undergraduate summer research scholarship from the College of Biological Sciences of the
295 University of Guelph to I.Y.-L.

296

Draft

297

298 Figure 1: Ontogenetic patterns of foraging ecology estimated from stable isotope signatures and
 299 lactate dehydrogenase (LDH) activity in a population of smallmouth bass. The panels depict the
 300 relationships between total body length and A) trophic position (n=62), B) littoral carbon usage
 301 (n=62), and C) LDH activity (n=36). The dark curves and hatched lines represent best-fit
 302 relationships obtained by nonlinear regression and the 95% confidence intervals. LDH activity
 303 values (IU/g wet tissue weight) in panel C are means $\pm$ SEM of triplicate measurements.

Figure 2: Relationship between trophic positions (TP) estimated from nitrogen stable isotope signatures and lactate dehydrogenase (LDH) activity in a population of smallmouth bass. The panels depict the relationships between A) raw values of trophic position and LDH activity, and B) raw values of trophic position and size-corrected values of LDH activity obtained from the curve shown in figure 1C. The dark curves and hatched lines represent best-fit relationships obtained by nonlinear regression and the 95% confidence intervals. Panel C shows size-corrected values of both trophic position and LDH activity. No significant relationship was seen between these variables. Size-corrected values of trophic position were obtained from the curve shown in

313 figure 1A. LDH activity values (IU/g wet tissue weight) are means $\pm$ SEM of triplicate
314 measurements.

315

Draft

316

317

318

319

320

321

322

323

Figure 3: Relationship between littoral carbon (LC) usage estimated from stable isotope signatures and lactate dehydrogenase (LDH) activity in a population of smallmouth bass. The panels depict the relationships between A) raw values of littoral carbon use and LDH activity, B) raw values of littoral carbon use and size-corrected values of LDH activity (from figure 1C), and C) size-corrected values of both littoral carbon use (from figure 1B) and LDH activity. The dark curves and hatched lines represent best-fit relationships obtained by nonlinear regression and the

324 95% confidence intervals. LDH activity values (IU/g wet tissue weight) are means $\pm$ SEM of
325 triplicate measurements.

Draft

326 **References**

- 327 Childress, J.J., and Somero, G.N. 1990. Metabolic scaling: a new perspective based on scaling of
328 glycolytic enzyme activities. *Amer. Zool.* **30**: 161-173.
- 329 Couture, P., Dutil, J.-D., and Guderley, H. 1998. Biochemical correlates of growth and condition
330 in juvenile Atlantic cod (*Gadus morhua*) from Newfoundland. *Can. J. Fish. Aquat. Sci.*
331 **55**: 1591-1598.
- 332 Dauwalter, D.C., and Fisher, W.L. 2008. Ontogenetic and seasonal diet shifts of smallmouth bass
333 in an Ozark stream. *J. Freshwater Ecol.* **23**: 113-121.
- 334 Dickson, K.A., Gregorio, M.O., Gruber, S.J., Loeffler, K.L., Tran, M., and Terrell, C. 1993.
335 Biochemical indices of aerobic and anaerobic capacity in muscle tissues of California
336 elasmobranch fishes differing in typical activity level. *Mar. Biol.* **117**: 185-193.
- 337 Easton, R.S., Orth, D.J., and Voshell Jr., J.R. 1996. Spatial and annual variation in the diets of
338 juvenile smallmouth bass, *Micropterus dolomieu*. *Environ. Biol. Fish.* **46**: 383-392.
- 339 Edmunds, N. 2015. The effect of food web structure on teleost fish brain size and morphology in
340 an aquatic ecosystem. M.Sc. Thesis, Department of Integrative Biology, University of
341 Guelph, Guelph, ON. <http://hdl.handle.net/10214/9118>
- 342 Garenc, C., Couture, P., Laflamme, M.-A., and Guderley, H. 1999. Metabolic correlates of burst
343 swimming capacity of juvenile and adult threespine stickleback (*Gasterosteus aculeatus*).
344 *J. Comp. Physiol. B* **169**: 113-122.

- 345 Gibb, A.C., and Dickson, K.A. 2002. Functional morphology and biochemical indices of
346 performance: is there a correlation between metabolic enzyme activity and swimming
347 performance? *Integr. Comp. Biol.* **42**: 199-207.
- 348 Goolish, E.M. 1991. Aerobic and anaerobic scaling in fish. *Biol. Rev.* **66**: 33-56.
- 349 Kaufman, S.D., Gunn, J.M., Morgan, G.E., and Couture, P. 2006. Muscle enzymes reveal
350 walleye (*Sander vitreus*) are less active when larger prey (cisco, *Coregonus artedii*) are
351 present. *Can. J. Fish. Aquat. Sci.* **63**: 970-979.
- 352 Long, J.M., and Fisher, W.L. 2000. Inter-annual and size-related differences in the diets of three
353 sympatric black bass in an Oklahoma reservoir. *J. Freshwater Ecol.* **15**: 465-474.
- 354 Martinez, M., Dutil, J.-D., and Guderley, H. 2000. Longitudinal and allometric variation in
355 indicators of muscle metabolic capacities in Atlantic cod (*Gadus morhua*). *J. Exp. Zool.*
356 **287**: 38-45.
- 357 Martinez, M., Guderley, H., Dutil, J.-D., Winger, P.D., He, P., and Walsh, S.J. 2003. Condition,
358 prolonged swimming performance and muscle metabolic capacities of cod *Gadus*
359 *morhua*. *J. Exp. Biol.* **206**: 503-511.
- 360 Moyes, C.D., Shulte, P.M., and Hochachka, P.W. 1992. Recovery metabolism of trout white
361 muscle: role of mitochondria. *Am. J. Physiol.* **262**: R295-R304.
- 362 Odell, J.P., Chappell, M.A., and Dickson, K.A. 2003. Morphological and enzymatic correlates of
363 aerobic and burst performance in different populations of Trinidadian guppies *Poecilia*
364 *reticulata*. *J. Exp. Biol.* **206**: 3707-3718.

- 365 Place, A.R., and Powers, D.A. 1984. Kinetic characterization of the lactate dehydrogenase
366 (LDH-B₄) allozymes of *Fundulus heteroclitus*. J. Biol. Chem. **259**: 1309-1318.
- 367 Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and
368 assumptions. Ecology **83**: 703-718.
- 369 Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., and Montana, C.G.
370 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with
371 lipids in stable isotope analyses. Oecologia **152**: 179-189.
- 372 Selch, T.M, and Chips, S.R. 2007. The cost of capturing prey: measuring largemouth bass
373 (*Micropterus salmoides*) foraging activity using glycolytic enzymes (lactate
374 dehydrogenase). Can. J. Fish. Aquat. Sci. **64**: 1761-1769.
- 375 Sherwood, G.D., Pazzia, I., Moeser, A., Hontela, A., and Rasmussen, J.B. 2002a. Shifting gears:
376 enzymatic evidence for the energetic advantage of switching diet in wild-living fish. Can.
377 J. Fish. Aquat. Sci. **59**: 229-241.
- 378 Sherwood, G.D., Kovacs, J., Hontela, A., and Rasmussen, J.B. 2002b. Simplified food webs
379 lead to energetic bottlenecks in polluted lakes. Can. J. Fish. Aquat. Sci. **59**: 1-5.
- 380 Somero, G.N., and Childress, J.J. 1980. A violation of the metabolism-size scaling paradigm:
381 activities of glycolytic enzymes in muscle increase in larger-size fish. Physiol. Zool. **53**:
382 322-337.
- 383 Somero, G.N., and Childress, J.J. 1990. Scaling of ATP-supplying enzymes, myofibrillar
384 proteins and buffering capacity in fish muscle: relationship to locomotory habit. J. Exp.
385 Biol. **149**: 319-333.

- 386 Sullivan, K.M., and Somero, G.N. 1983. Size- and diet-related variations in enzymic activity and
387 tissue composition in the sablefish, *Anoplopoma fimbria*. Biol. Bull. **164**: 315-326.
- 388 Tunney, T.D., McCann, K.S., Lester, N.P., and Shuter, B.J. 2012. Food web expansion and
389 contraction in response to changing environmental conditions. Nat. Comm. **3**: 1105.
- 390 Vander Zanden, M.J., Hulshof, M., Ridgway, M.S., and Rasmussem, J.B. 1998. Application of
391 stable isotope techniques to trophic studies of age-0 smallmouth bass. Trans. Am. Fish.
392 Soc. **127**: 729-739.
- 393 Vander Zanden, M.J., Shuter, B.J., Lester, N.P., and Rasmussen, J.B. 2000. Within-and among-
394 population variation in the trophic position of a pelagic predator, lake trout (*Salvelinus*
395 *namaycush*). Can. J. Fish. Aquat. Sci. **57**: 725-731.
- 396 Yang, T.-H., and Somero, G.N. 1996. Activity of lactate dehydrogenase but not its concentration
397 of messenger RNA increases with body size in barred sand bass, *Paralabrax nebulifer*
398 (Teleostei). Biol. Bull. **191**: 155-158.
- 399

Figure 1
207x405mm (300 x 300 DPI)

Figure 2
207x424mm (300 x 300 DPI)

Figure 3
211x427mm (300 x 300 DPI)