

# The cost of capturing prey: measuring largemouth bass (*Micropterus salmoides*) foraging activity using glycolytic enzymes (lactate dehydrogenase)

Trevor M. Selch and Steven R. Chipps

**Abstract:** We used muscle-derived lactate dehydrogenase (LDH) to measure effects of prey size and vegetation density on anaerobic foraging activity by largemouth bass (*Micropterus salmoides*). Largemouth bass (240–303 mm total length, TL) were fed bluegill (*Lepomis macrochirus*) prey (range 33–83 mm TL) in laboratory feeding trials. Prey selectivity experiments showed that small bluegills (<50 mm) were strongly preferred (>88%) over larger (>65 mm) individuals. Largemouth bass activity, as indexed by LDH, increased with increasing prey size and was 20% higher in fish feeding on large (mean size = 80 mm) versus small (mean size = 35 mm) bluegill. Bioenergetics modeling revealed that food consumption was appreciably underestimated (29%–34%) for largemouth bass foraging on large bluegills (65 and 80 mm), implying that activity costs vary with prey size, consistent with LDH measurements. In contrast to prey size, vegetation density had little effect on anaerobic energy expenditure of largemouth bass. For two size groups of largemouth bass (mean = 244 and 316 mm) foraging on 50 mm bluegill, mean LDH activity was similar across simulated vegetation densities ranging from 70 to 350 stems·m<sup>-2</sup>. These findings highlight the importance of prey size on foraging costs by piscivores and the difficulties of accounting for activity level in bioenergetics models.

**Résumé :** Nous utilisons de la lactate déshydrogénase (LDH) extraite des muscles de l'achigan à grande bouche (*Micropterus salmoides*) afin de mesurer les effets de la taille des proies et de la densité de la végétation sur la recherche anaérobie de nourriture. Nous avons nourri des achigans à grande bouche (240–303 mm de longueur totale, TL) de proies consistant en des crapets arlequins (*Lepomis macrochirus*) (étendue de TL 33–83 mm) dans des essais alimentaires en laboratoire. Des tests de sélectivité des proies indiquent que les petits crapets (<50 mm) sont nettement préférés (>88 %) aux individus plus grands (>65 mm). L'activité des achigans à grande bouche, telle que mesurée par la LDH, augmente en fonction de la taille des proies et elle est 20 % plus élevée chez des pêches qui se nourrissent de grands (taille moyenne = 80 mm) plutôt que de petits (taille moyenne = 35 mm) crapets. Une modélisation bioénergétique montre que la consommation de nourriture est clairement sous-estimée (29–34 %) chez les achigans à grande bouche qui se nourrissent de grand crapets (65 et 80 mm), ce qui veut dire que les coûts de l'activité varient en fonction de la taille des proies, ce qui est en accord avec les mesures de LDH. Contrairement à la taille des proies, la densité de la végétation a peu d'effet sur la dépense anaérobie d'énergie chez l'achigan à grande bouche. Chez des groupes d'achigans à grande bouche de deux tailles différentes (moyennes de 244 mm et de 316 mm) qui se nourrissent de crapets de 50 mm, l'activité moyenne de la LDH ne varie pas le long d'un gradient simulé de densités de la végétation, allant de 70 à 350 tiges·m<sup>-2</sup>. Ces résultats mettent en évidence l'importance de la taille des proies sur le coût de la recherche alimentaire chez les piscivores et la difficulté qu'il y a à tenir compte des niveaux d'activité dans les modèles bioénergétiques.

[Traduit par la Rédaction]

## Introduction

Prey size has an important influence on capture success by piscivores (Wahl and Stein 1989; Einfalt and Wahl 1997). Because prey evasiveness and maneuverability generally increase with body size (Howick and O'Brien 1983), it follows that additional effort is needed by predators to capture larger

prey. As a result, swimming demands required to capture large prey may have considerable metabolic costs for piscivores and important implications for energy budgets (Boisclair and Leggett 1989; Krohn and Boisclair 1994; Sherwood et al. 2002a).

Attempts to incorporate prey size into optimal foraging models have been met with mixed success (Juanes 1994).

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**T.M. Selch<sup>1</sup> and S.R. Chipps.** US Geological Survey South Dakota Cooperative Fish and Wildlife Research Unit,<sup>2</sup> SNP 2140B, Department of Wildlife and Fisheries Sciences, South Dakota State University, Brookings, SD 57007, USA.

<sup>1</sup>Corresponding author (e-mail: [trevor.selch@sdstate.edu](mailto:trevor.selch@sdstate.edu)).

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Often times, foraging activity is related to behaviors such as time spent searching, following, pursuing, or handling prey (Savino and Stein 1982, 1989b; Einfeldt and Wahl 1997). Associating behaviors with actual energy costs has been difficult to determine experimentally (Niimi and Beamish 1974). Indeed, prey sizes that minimize handling time per unit energy gained often do not match those determined from preference experiments (Einfeldt and Wahl 1997), suggesting that handling time is an inaccurate method of determining size selectivity by piscivores.

Vegetation density can also play an important role in the foraging dynamics of fishes. In dense vegetation, piscivorous fishes often spend more time searching for prey and have lower attack and capture rates than in sparsely vegetated environments (Werner and Mittelbach 1981; Savino and Stein 1982, 1989b). As a result, predators such as largemouth bass (*Micropterus salmoides*) generally capture fewer prey and exhibit lower growth rates in dense versus sparse vegetation (Anderson 1984; Olson et al. 1998). Because capture efficiency (i.e., number of captures per number of strikes) varies little with vegetation density (Savino and Stein 1982; Valley and Bremigan 2002; Chipps et al. 2004), encounter rate likely limits foraging success for largemouth bass in dense vegetation (Savino and Stein 1982, 1989a). In dense vegetation, the ambush-type behavior exhibited by largemouth bass may act to conserve energy by reducing costly chasing (e.g., pursuit) behaviors (Savino and Stein 1982).

Although foraging activity can be related to the time spent in certain behaviors (e.g., search, orientation, follow, pursuit, strike, and capture; Savino and Stein 1982), the effect of these behaviors on energetic costs are difficult to quantify. Moreover, aerobic swimming activity is relatively cheap compared with anaerobic activity expenditures in piscivores (Kaufman et al. 2006). Pursuit behavior, in particular, involves burst swimming characterized by anaerobic metabolism and may be the most costly activity leading up to a successful capture, despite the relatively small amount of time spent in this activity. As a by-product of anaerobic metabolism, lactate dehydrogenase (LDH) measured from white muscle tissue has been linked with foraging activity in fishes, providing a useful index for quantifying relative foraging costs (Sherwood et al. 2002a).

The production of glycolytic enzymes (i.e., LDH) occurs in the axial musculature of fish (Sullivan and Somero 1980; Childress and Somero 1990). When speed bursts are used to catch prey, the lactate pathway is needed to create energy at faster rates than possible by aerobic processes (Goolish 1995). White muscle powers anaerobic burst swimming in fish. During activity bouts, fish release a small portion of lactate into the bloodstream; however, the anaerobic white muscle tissue retains most of the lactate (Jobling 1994), providing a useful measure of anaerobic activity (Sherwood et al. 2002b).

Here, we explore the influence of prey size and vegetation density on largemouth bass foraging activity. We postulate that foraging activity, as indexed by LDH, is positively scaled to prey size, corresponding with heightened anaerobic activity costs of capturing larger prey. Using bioenergetics modeling, we then evaluate the influence of prey size on accuracy of model predictions. In separate experiments, we

examined the influence of vegetation density on anaerobic activity cost of largemouth bass foraging on bluegill (*Lepomis macrochirus*) prey. Because capture efficiency varies little across vegetation density, we postulate that largemouth bass conserve energy when foraging in dense vegetation by adopting an ambush foraging strategy (cf. Savino and Stein 1982). If true, then anaerobic activity should vary little across vegetation density.

## Materials and methods

### Size selectivity

Size selectivity trials were conducted in laboratory tanks to determine the size of prey most preferred by largemouth bass. Largemouth bass (240–303 mm total length, TL) and bluegill (33–82 mm TL) were collected from local ponds and lakes in Deuel, Brookings, and Day counties, South Dakota, USA. Bluegill used in the experiments were measured for total length and assigned to one of four size groups: 35, 50, 65, or 80 mm TL ( $\pm 2$  mm). The largest prey used (i.e., 80 mm TL) were smaller than the maximum size prey that the largemouth bass were able to consume based on a maximum prey-to-predator length ratio (P:PR) of 0.40 (Timmons et al. 1980); P:PR ratios in our experiments ranged from 0.13 to 0.30.

Prior to starting a feeding trial, largemouth bass ( $n = 130$ ) were held in 1500 L rectangular, flow-through tanks (2.8 m length  $\times$  0.75 m width  $\times$  0.75 m depth) with a constant flow of 1.6 L $\cdot$ min $^{-1}$  and a 12-h dark – 12-h light photoperiod. Largemouth bass were maintained on a diet of fathead minnows (*Pimephales promelas*) for 1–2 weeks and were then starved for 24 h before starting prey selectivity trials. Bluegills were held in a separate 700 L recirculating tank (1.3 m diameter  $\times$  0.7 m depth) for 2–4 days before being used in trials and were maintained on a diet of zooplankton. Feeding trials ( $n = 130$ ) were conducted in 760 L circular tanks (1.4 m diameter  $\times$  0.76 m depth) by placing a single largemouth bass in a holding chamber and introducing four bluegill, one from each of the four size categories of 35, 50, 65, and 80 mm TL. Individual bluegill were measured before being added to tanks and never varied by more than  $\pm 2$  mm from selected length categories. The tanks and fish used in our trials were similar in size to those used in other experiments examining foraging behavior by largemouth bass (Savino and Stein 1982; Schramm and Zale 1985; Hoyle and Keast 1987). Plastic cylindrical cages (35 cm diameter  $\times$  100 cm height) constructed of 10 mm mesh were used as holding chambers for largemouth bass. After a 20 min acclimation period, the largemouth bass was released from the chamber and allowed to forage until a capture was made. Once a capture was made, the trial was ended by isolating the bass in the holding chamber; all remaining bluegill were netted and the length of the bluegill eaten was recorded. Capture data were summarized as the mean proportion and 95% confidence intervals (CI) (Johnson 1999) for each prey size category. For a given prey size, we considered size selectivity “neutral” if the 95% CI for proportion eaten included 0.25 (corresponding to random selection of 1 out of 4 prey). Positive selection was inferred if the 95% CI was above the random feeding value ( $>0.25$ ), and negative selec-

tion was inferred if the 95% CI fell below the random feeding value ( $<0.25$ ; Graeb et al. 2004, 2005).

### Experiment 1: effects of prey size on largemouth bass foraging activity

The effect of prey size on anaerobic activity in largemouth bass was measured in feeding trials conducted in the same tanks used for prey selectivity trials. Largemouth bass (240–303 mm TL) used in experiments ( $n = 36$ ) were collected by hook and line from a local pond in Brookings County, South Dakota, USA. Bluegills used in the experiments were collected by seining from Enemy Swim Lake, South Dakota, and were maintained on a diet of zooplankton in separate 700 L circular holding tanks (1.3 m diameter  $\times$  0.7 m depth).

Largemouth bass were held in 1500 L aerated rectangular tanks with a constant flow of  $1.6 \text{ L}\cdot\text{min}^{-1}$  and a 12-h dark – 12-h light photoperiod. Fish were acclimated in rectangular tanks for a minimum of 2 weeks and maintained on a diet of fathead minnows prior to the start of a feeding trial. Each bass was then randomly assigned to one of 12 circular tanks (760 L) and starved for 2 days prior to starting a trial. Water temperature in the tanks was maintained between 21 and 24 °C and never varied by more than 1 °C for any given trial. To provide structure in the tanks, we added a 1 m section of simulated vegetation consisting of a linear array of buoyant synthetic strips (approximately 70 strips total, with each strip measuring 2 cm  $\times$  0.5 cm  $\times$  35 cm; Aquamat® Inc., Calverton, Maryland) that were anchored on the bottom of the tanks and suspended vertically.

We fed largemouth bass one of four sizes of bluegill (35, 50, 65, or 80 mm). The size range of bluegills used in activity trials matched those used in size selectivity experiments. Because LDH provides a snapshot of recent energy expenditure, it generally accumulates in fish tissue within, but not earlier than, 7 days (Schulte et al. 2000; Sherwood et al. 2002a). Thus, largemouth bass were allowed to forage on bluegill for 10 consecutive days. To keep encounter rates constant, three bluegills were maintained in each tank and replaced daily depending on the number of bluegill consumed by each bass. At the start of each trial, and before new prey were added each day, largemouth bass were isolated for 15 min by slowly maneuvering them into the holding chamber. The number of bluegill consumed was recorded daily for each largemouth bass and any consumed prey were replaced with similar-sized bluegill ( $\pm 2$  mm). We measured length (mm, TL) and weight (g) of each largemouth bass before and after each trial. Three separate trials were conducted using a total of 36 largemouth bass (i.e., nine replicates per prey size category).

After 10 days of foraging on bluegill, largemouth bass were removed from the tanks, anesthetized in  $60 \text{ mg}\cdot\text{L}^{-1}$  clove oil (Peake 1998), weighed (g), measured for TL (mm), and sacrificed by cervical dislocation in accordance with Animal Use and Care Procedures (South Dakota State University) and Animal Welfare Considerations (Nickum et al. 2004). A sample (1–2 g) of white muscle tissue was then excised anterior to the caudal peduncle on the right side of the fish and frozen at  $-20$  °C until analysis.

Lactate dehydrogenase assays were performed using kit specifications (Lactate Dehydrogenase Assay, Diagnostic Chemicals Limited, Oxford, Connecticut) and sample prepa-

ration procedures outlined in Sherwood et al. (2002a). LDH activity, measured at 20 °C, was expressed as arbitrary absorbance units (at 340 nm) per gram of tissue wet weight. Because LDH scales positively with fish size (Sullivan and Somero 1980; Childress and Somero 1990), we tested for significant differences in LDH activity and mean daily consumption using an analysis of covariance (ANCOVA) with largemouth bass weight as the covariate and prey size as a grouping factor. Pairwise comparisons were made using Tukey's multiple-comparison test ( $P < 0.05$ ).

### Experiment 2: effects of vegetation density on largemouth bass foraging activity

We evaluated effects of vegetation density on largemouth bass foraging activity using two size ranges of largemouth bass (219–276 and 304–330 mm TL) and three vegetation densities (70, 210, and 350 stems $\cdot\text{m}^{-2}$ ). The small size group of largemouth bass (219–276 mm TL) was collected from Lake Cochrane (Deuel County, South Dakota, USA); the larger fish (304–330 mm TL) were collected from Enemy Swim Lake (Day County, South Dakota, USA). To simulate vegetation density, we added 1 m sections of Aquamat® ( $n = 1$  to 5 sections) to each tank. Low, moderate, and high vegetation consisted of 70, 210, and 350 stems $\cdot\text{m}^{-2}$ , respectively. Two feeding trials were conducted to evaluate effects of vegetation density on largemouth bass foraging activity. In the first trial, individual largemouth bass ( $n = 12$ ), ranging in size from 219 to 276 mm TL, were fed bluegill prey (50 mm TL) at one of three vegetation densities (low,  $n = 4$ ; moderate,  $n = 4$ ; or high,  $n = 4$ ). In the second trial, we used the larger bass (304–330 mm TL,  $n = 12$ ) fed similar-sized bluegill prey (50 mm TL) at each of the three vegetation densities. Largemouth bass were acclimated to experimental conditions as described in experiment 1. To begin a trial, three bluegill were added to each tank while bass were isolated in the holding chambers. Largemouth bass were fed 50 mm bluegill for 10 consecutive days; the number eaten by each bass was recorded and replaced daily so that three bluegill were maintained in each tank. After 10 days of foraging, largemouth bass were removed from the tanks, anesthetized, weighed, measured, and sacrificed as described in experiment 1. A sample (1–2 g) of white muscle tissue was then excised anterior to the caudal peduncle, frozen ( $-20$  °C), and later analyzed for LDH activity. The effects of vegetation density on mean daily consumption, growth, and LDH activity were analyzed using ANCOVA with largemouth bass weight as the covariate. Pairwise comparisons were made using Tukey's multiple-comparison test ( $P < 0.05$ ).

### Bioenergetics simulations

We used observed measures of food consumption and growth to explore the influence of prey size and vegetation density on accuracy of bioenergetics predictions. Growth data from experiments were used as input in a largemouth bass bioenergetics model (Hanson et al. 1997) to estimate mean food consumption for bass foraging on bluegill prey (35, 50, 65, or 80 mm TL) and under different vegetation densities (low, moderate, or high). Other input data required in the model included daily water temperature (°C), predator energy density ( $\text{J}\cdot\text{g wet weight}^{-1}$ ), and energy density of bluegill prey. Caloric density of largemouth bass and bluegill

**Table 1.** Feeding, growth, and anaerobic activity level (lactate dehydrogenase, LDH) for largemouth bass foraging on four size categories of bluegill prey (experiment 1).

Bluegill size category (mm)	<i>N</i>	Mean largemouth bass initial weight (g)	Mean largemouth bass final weight (g)	Mean no. of bluegill consumed·day <sup>-1</sup>	Mean daily growth (g·day <sup>-1</sup> )	LDH (U·g <sup>-1</sup> )
35	9	253.2 (20.5)	251.0 (18.2)	3.00 (0.00)a	-0.08 (0.10)	208 (8.3)a
50	9	277.0 (22.9)	287.6 (21.2)	2.99 (0.11)a	1.06 (0.08)	236 (15.9)ab
65	9	275.0 (16.4)	305.8 (15.3)	2.88 (0.04)a	3.08 (0.06)	237 (16.4)ab
80	9	271.1 (20.4)	310.0 (21.7)	1.82 (0.09)b	3.89 (0.11)	250 (12.7)b

**Note:** Initial and final weights of largemouth bass over the 10-day feeding trial were used as input in bioenergetics modeling simulations. Mean number of bluegill consumed and LDH values (arbitrary absorbance units) with the same letter are not significantly different (Tukey's multiple-comparison test,  $P > 0.05$ ). Values in parentheses represent 1 standard error.

was quantified using a Parr 1108 oxygen bomb calorimeter and used as input in the model.

Sources of error in model predictions were evaluated by decomposition of mean square error (MSE), obtained from least-squares regression of observed on predicted values. The MSE represents the variance around the 1:1 line, where sources of error from the mean component ( $Z$ ) are due to differences in predicted and observed values, the slope component ( $S$ ) represents error due to deviation of the slope from unity, and the residual component ( $R$ ) represents random error (Rice and Cochran 1984; Wahl and Stein 1991; Chipps and Wahl 2004). To evaluate systematic errors ( $Z$  and  $S$ ), we regressed observed values on predicted values and used Bonferroni joint confidence intervals to test the joint hypothesis that regression parameters had an intercept of 0 and a slope of 1 ( $P < 0.05$ ; Neter et al. 1985). If the joint hypothesis was rejected, we tested for differences between means and slope separately (Rice and Cochran 1984; Wahl and Stein 1991). Statistical analyses for all experiments were performed using SAS 9.1 (SAS Institute Inc., Cary, North Carolina).

## Results

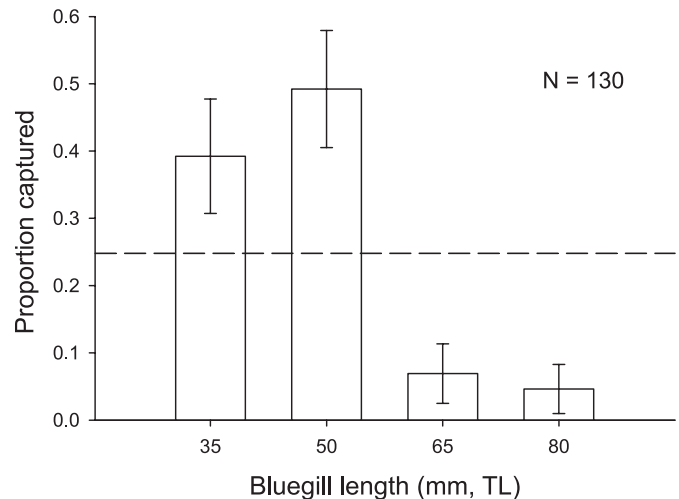
### Size selectivity

Prey selectivity decreased appreciably as bluegill size exceeded 50 mm (Fig. 1). Smaller bluegills (35 and 50 mm) represented over 88% of all fish consumed in prey selectivity trials. In contrast, 65 and 80 mm bluegills were chosen <12% of the time. The largest bluegill offered (80 mm) represented only 5% of total bluegills consumed by bass. When offered four sizes of bluegill prey, largemouth bass positively selected smaller bluegill (35 and 50 mm) and negatively selected larger bluegill (65 and 80 mm; Fig. 1).

### Experiment 1: effects of prey size on largemouth bass foraging activity

Mean daily consumption by largemouth bass ranged from 1.8 to 3 bluegills·day<sup>-1</sup> and varied significantly with prey size (ANCOVA,  $F_{[3,31]} = 50.53$ ,  $P < 0.001$ ). Daily consumption was similar for 35, 50, or 65 mm bluegills (Tukey's multiple-comparison test,  $P > 0.05$ ; Table 1); in these trials, largemouth bass consumed over 96% of total prey offered. When offered 80 mm bluegill, largemouth bass captured significantly fewer prey (1.8 bluegills·day<sup>-1</sup>,  $P < 0.05$ ) and consumed only 61% of the prey that were offered.

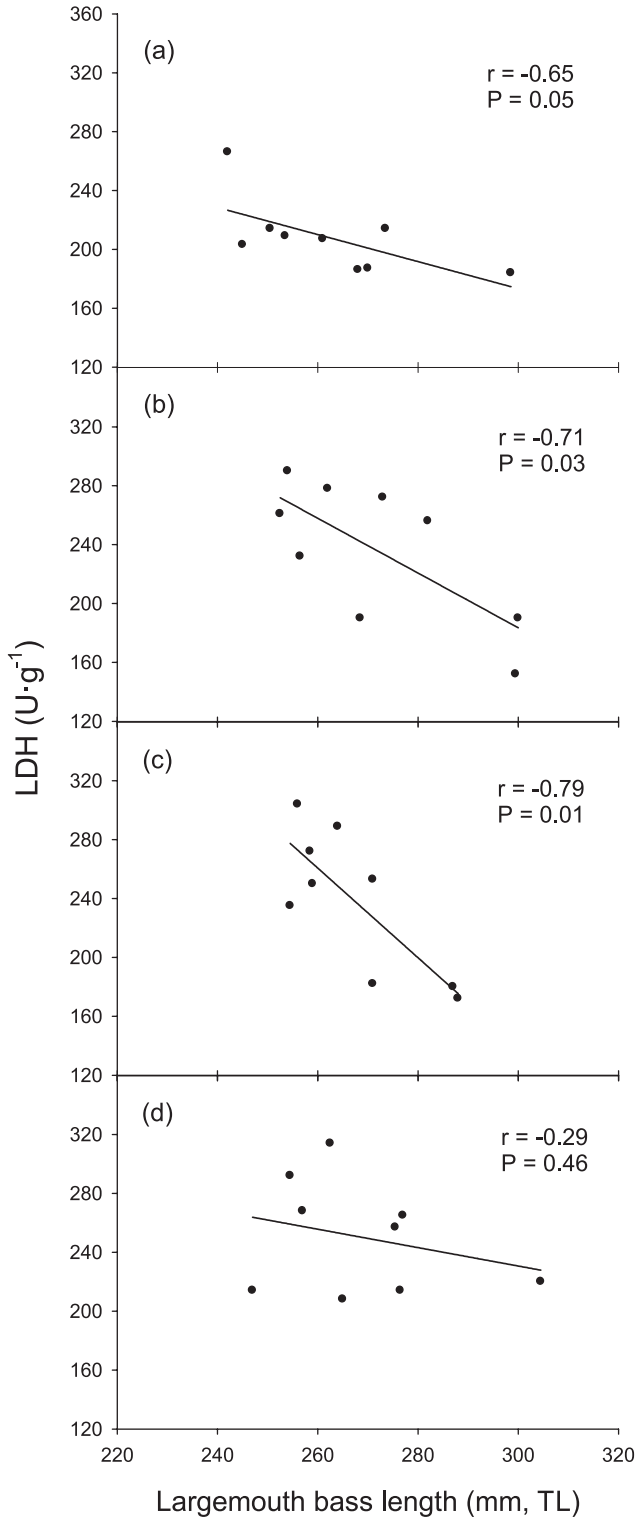
**Fig. 1.** Mean proportion of different-sized bluegills (*Lepomis macrochirus*) captured by largemouth bass (*Micropterus salmoides*) in prey selectivity trials. Error bars are 95% confidence intervals (CI). The random feeding value is represented by the horizontal, broken line at 0.25 (e.g., 1 out of 4 prey offered). When the 95% CI lies above the random feeding line, there is positive selection; when it overlaps the line, there is neutral selection; and when it lies below the line, there is negative selection.



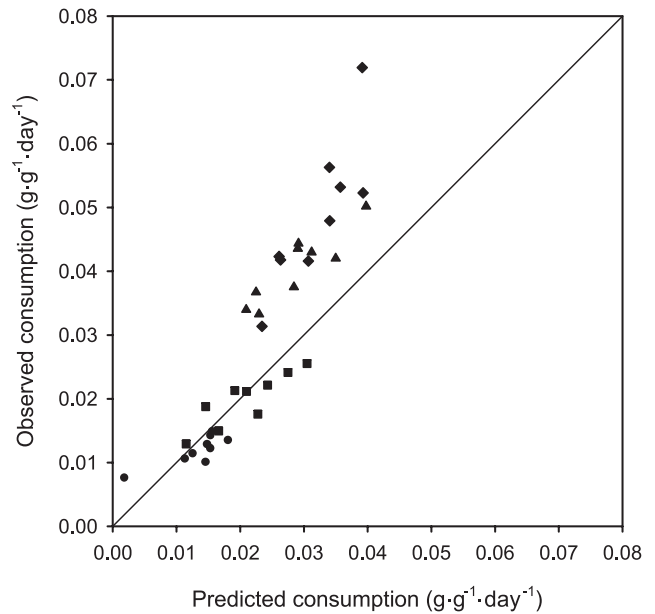
We found no interaction between prey size and largemouth bass weight on LDH activity measured from largemouth bass (ANCOVA,  $F_{[3,28]} = 1.15$ ,  $P = 0.35$ ). However, LDH activity varied significantly with mean weight of largemouth bass ( $F_{[1,31]} = 12.59$ ,  $P = 0.001$ ). Across the range of fish sizes we examined, LDH generally decreased as largemouth bass size increased (Fig. 2), implying that relative prey size influenced anaerobic activity expenditure. Similarly, mean prey size had a significant influence on LDH activity in largemouth bass ( $F_{[3,31]} = 4.88$ ,  $P = 0.003$ ; Table 1). On the average, LDH activity increased with prey size and was significantly lower for bass foraging on small (35 mm) versus large bluegills (80 mm; Table 1).

Consumption rate over the 10-day feeding trial ranged from 0.008 to 0.072 g·g<sup>-1</sup>·day<sup>-1</sup> for bass feeding on 35 to 80 mm bluegills (Fig. 3). Energy density for largemouth bass averaged 4649 J·g wet weight<sup>-1</sup> ( $n = 36$ , standard error (SE) = 46). Energy density for bluegill varied with body size and averaged 3846, 3870, 4406, and 4464 J·g wet weight<sup>-1</sup> for 35, 50, 65, and 80 mm bluegills, respectively.

**Fig. 2.** Muscle lactate dehydrogenase (LDH) activity (units per gram wet weight of muscle tissue) versus mean total length (mm) for largemouth bass (*Micropterus salmoides*) foraging on (a) 35, (b) 50, (c) 65, or (d) 80 mm bluegill (*Lepomis macrochirus*). Pearson's correlation coefficients and significance values are given.



**Fig. 3.** Relationship between observed consumption rate ( $\text{g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$ ) and predicted consumption rate ( $\text{g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$ ) calculated using the bioenergetics model for largemouth bass (*Micropterus salmoides*) feeding on different-sized bluegills (*Lepomis macrochirus*): 35 (●), 50 (■), 65 (▲), and 80 (◆) mm total length. Tests of the joint hypothesis that regression parameters had an intercept of 0 and slope of 1 were rejected for the 65 and 80 mm prey groups, indicating poor model fit to observed data. The 1:1 relationship is shown by the diagonal line.



Bioenergetic estimates of food consumption agreed well with observed values for largemouth bass foraging on 35 and 50 mm bluegills (Fig. 3). Decomposition of MSE indicated that most of the variability between observed and predicted values for bass foraging on 35 and 50 mm bluegill was attributable to random error (Table 2). Regression of observed on predicted values revealed that the 95% joint confidence region included an intercept of 0 and a slope of 1.0 (Table 2). In contrast, as prey size increased (65 and 80 mm), model predictions underestimated observed food consumption (Fig. 3). Decomposition of MSE indicated that most of the variability between observed and predicted values was attributable to systematic error (mean and slope; Table 2). Regression of observed on predicted values revealed that the 95% joint confidence region did not include an intercept of 0 for bass foraging on 65 mm bluegill (Table 2). For bass foraging on 80 mm bluegill, the 95% joint confidence region did not include an intercept of 0 or a slope of 1.0 (Table 2). Separate tests of the slope and intercept revealed that the intercept was significantly different than 0 ( $P = 0.05$ ) and the slope was different than 1.0 ( $P < 0.001$ ).

To calibrate the model for largemouth bass foraging on larger prey (65 and 80 mm bluegill), we adjusted the activity multiplier in the bioenergetics model until predicted consumption values matched those measured in the experiments. The standard version of the model uses an activity multiplier of one ( $ACT = 1$ ; Hanson et al. 1997). To approximate observed consumption (i.e., within 5% of observed values),  $ACT$

**Table 2.** Proportion of mean square error due to systematic (mean and slope) and random (residual) components for the relationship between observed and predicted food consumption using the bioenergetics model for largemouth bass.

Treatment	Sources of error			$\beta_0 \pm 95\% \text{ CI}$	$\beta_1 \pm 95\% \text{ CI}$
	Mean ( <i>Z</i> )	Slope ( <i>S</i> )	Residual ( <i>R</i> )		
<b>Experiment 1</b>					
35 mm	0.128	0.411	0.461	-0.007±0.014	1.715±1.153
50 mm	0.031	0.167	0.802	-0.005±0.007	1.282±0.330
65 mm	0.709	0.007	0.284	-0.011±0.004	0.991±0.091
80 mm	0.527	0.032	0.441	0.011±0.004	0.429±0.076
<b>Experiment 2</b>					
Lake Cochrane	0.622	0.102	0.276	-0.018±0.006	1.315±0.236
Enemy Swim Lake	0.001	0.191	0.808	0.001±0.009	0.956±0.681

**Note:** Values close to 0 for mean (*Z*) and slope (*S*) and close to 1 for residual values (*R*) indicate that errors are not systematic. Bonferroni joint confidence intervals (CI) for an intercept ( $\beta_0$ ) of 0 and a slope ( $\beta_1$ ) of 1 are presented for each treatment.

**Table 3.** Feeding, growth, and anaerobic activity level (lactate dehydrogenase, LDH) for largemouth bass foraging on 50 mm bluegill in different vegetation densities (experiment 2).

Vegetation density (stems·m <sup>-2</sup> )	<i>N</i>	Mean largemouth bass initial weight (g)	Mean largemouth bass final weight (g)	Mean no. bluegill consumed·day <sup>-1</sup>	Mean daily growth (g·day <sup>-1</sup> )	LDH (U·g <sup>-1</sup> )
<b>Lake Cochrane</b>						
70	3	189.7 (24.4)	195.5 (19.3)	3.00 (0.00)	0.58 (0.55)	226.8 (19.8)
210	4	211.7 (21.4)	216.5 (17.0)	2.98 (0.03)	0.48 (0.48)	219.9 (17.5)
350	4	189.6 (17.9)	190.7 (17.5)	2.75 (0.08)	0.11 (0.10)	238.3 (17.2)
<b>Enemy Swim Lake</b>						
70	4	419.8 (21.7)	429.2 (22.2)	2.97 (0.03)	1.05 (0.32)	303.9 (13.7)
210	4	464.5 (22.3)	472.3 (19.8)	2.69 (0.09)	0.86 (0.45)	326.4 (14.0)
350	4	432.6 (29.9)	439.3 (28.3)	2.58 (0.10)	0.75 (0.34)	301.6 (13.3)

**Note:** Results are summarized for both the small (Lake Cochrane) and large (Enemy Swim Lake) size groups of largemouth bass fed for 10 days. Mean LDH values (arbitrary absorbance units) were not significantly different among vegetation treatments (Tukey's multiple-comparison test,  $P > 0.05$ ) for either group of largemouth bass. Values in parentheses represent 1 standard error.

values had to be increased to 2 for largemouth bass foraging on 65 mm bluegill and to 2.5 for fish foraging on 80 mm bluegills; these values are within the range of activity multipliers used in other models (1 to 11.7; Hanson et al. 1997).

#### Experiment 2: influence of vegetation density

Vegetation density had little influence on the number of bluegills consumed by largemouth bass from Lake Cochrane (ANCOVA,  $F_{[2,7]} = 0.95$ ,  $P = 0.47$ ) or Enemy Swim Lake ( $F_{[2,8]} = 1.45$ ,  $P = 0.30$ ; Table 3). Similarly, mean daily growth did not differ across vegetation treatments for largemouth bass from Lake Cochrane (ANCOVA,  $F_{[2,7]} = 3.84$ ,  $P = 0.06$ ) or Enemy Swim Lake ( $F_{[2,8]} = 0.53$ ,  $P = 0.67$ ; Table 3). We found no evidence that LDH activity varied significantly in largemouth bass foraging in low, moderate, or high vegetation density from Lake Cochrane (ANCOVA,  $F_{[2,7]} = 0.30$ ,  $P = 0.82$ ) or Enemy Swim Lake (ANCOVA,  $F_{[2,8]} = 1.06$ ,  $P = 0.42$ ).

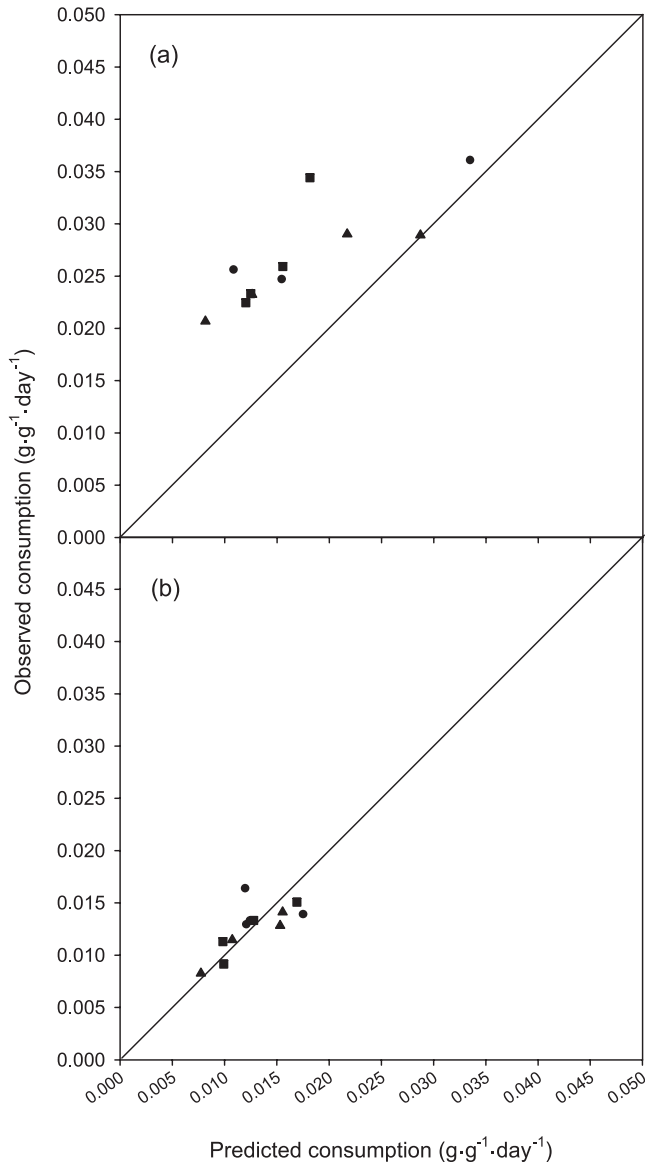
For larger bass (Enemy Swim Lake) feeding on 50 mm bluegill, bioenergetic estimates of food consumption agreed well with observed values (Fig. 4). Decomposition of MSE indicated that most of the variability between observed and predicted values was attributable to random error (81%; Table 2). Regression of observed on predicted values revealed that the 95% joint confidence region included an intercept of

0 and a slope of 1.0 (Table 2). However, for smaller bass (Lake Cochrane) feeding on 50 mm bluegill, the model underestimated observed consumption (Fig. 4). Decomposition of MSE indicated that most of the variability between observed and predicted values for Lake Cochrane fish was attributable to systematic (mean and slope) components (72%; Table 2). Regression of observed on predicted values revealed that the 95% joint confidence interval did not include an intercept of 0 or a slope of 1.0 (Table 2). Separate tests of the slope and intercept showed that the intercept value was significantly different than 0 ( $P = 0.05$ ), but the slope was not significantly different than 1.0 ( $P = 0.32$ ).

#### Discussion

Largemouth bass selected relatively small bluegills (35 and 50 mm) when offered a range of prey sizes within their gape limitation ( $P:PR < 0.40$ ; Timmons et al. 1980). Because the evasive ability of bluegills increases with body size (Howick and O'Brien 1983), it follows that more effort is needed to capture larger prey. LDH in largemouth bass scaled positively with prey size, demonstrating that anaerobic activity cost increased with prey size. Nonetheless, anaerobic activity cost alone did not explain observed patterns of prey selection; consumption of 50 mm bluegill was no-

**Fig. 4.** Relationship between observed consumption rate ( $\text{g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$ ) and predicted consumption rate ( $\text{g}\cdot\text{g}^{-1}\cdot\text{day}^{-1}$ ) calculated using the bioenergetics model for largemouth bass (*Micropterus salmoides*) from (a) Lake Cochrane and (b) Enemy Swim Lake for three vegetation treatments: 70 (●), 210 (▲), and 350 (■) stems·m<sup>-2</sup>. Bluegills (*Lepomis macrochirus*) used in trials were 50 mm total length. The 1:1 relationship is shown by a diagonal line.



ticeably higher (49%) than that for the smallest bluegills (35 mm) offered (39%). This may be due to differences in prey vulnerability as influenced by both encounter rate and capture efficiency. Because encounter rate and capture efficiency are differentially related to prey size, the size of preferred prey often represents an optimal combination of both (Juanes 1994), which may explain why the smallest bluegills were not the most frequently consumed.

In general, we found negative correlations between LDH activity and largemouth bass size. Although LDH is known to scale positively with fish size (Somero and Childress 1980; Childress and Somero 1990), the size range of largemouth bass that we used was small compared with that typi-

cally used to show allometric scaling of LDH. For a given prey size, smaller bass had to forage on relatively larger prey. We interpret this within-treatment variability in LDH as consistent with the across-treatment variation in LDH that we observed. That is, LDH increased as the ratio of prey-to-predator size increased; smaller bass foraging on relatively larger prey exhibited higher LDH levels.

Fish activity is regarded as a variable component of bioenergetics models yet is often modeled as a fixed multiplier of standard metabolism (Boisclair and Sirois 1993; Hansen et al. 1993; Krohn and Boisclair 1994). Recent studies have shown that LDH is linked with independent measures of foraging activity in fishes (Sherwood et al. 2002b; Rennie et al. 2005). In a study of fast- and slow-growing yellow perch populations, fish from the faster-growing population consumed less food than perch from the slower-growing population (Rennie et al. 2005). Higher LDH and slower growth of perch were attributed to increased foraging activity resulting from low prey density. Similarly, prey size and (or) morphology are known to affect capture success (Wahl and Stein 1989; Einfalt and Wahl 1997) and can influence activity level in fish predators (Sherwood et al. 2002b).

Our results showed that increased anaerobic activity associated with capturing larger prey was not a trivial component of energy allocation. Using a corroborated bioenergetics model for largemouth bass (Rice and Cochran 1984; Whitledge and Hayward 1997), we found that food consumption was appreciably underestimated (29%–34%) for bass foraging on large (65 and 80 mm) bluegill. Because feeding and growth rates varied for largemouth bass fed different prey sizes, systematic error in model parameters may account for observed differences between actual and predicted consumption (Madenjian and O'Conner 1999; Chipps et al. 2000; Bajer et al. 2004). Consumption-dependent parameters that include waste losses (egestion and excretion) and specific dynamic action are believed to vary with feeding rate and contribute to errors in model estimates (Bajer et al. 2004). However, our results suggest that activity level, and not consumption-dependent error, was responsible for differences between observed and predicted food consumption. To accurately balance the energy budget for largemouth bass foraging on 80 mm bluegill, all consumption-dependent parameters (egestion, excretion, and specific dynamic action) in the model would have to be increased by over 100%. It is unlikely that this amount of error exists in these parameters. Rather, activity patterns, as shown by the LDH analysis, better explain discrepancies between observed and predicted consumption.

Anaerobic activity, as indexed by LDH, is not readily incorporated as a measure of fish activity in bioenergetics models. Rather, activity is usually represented as a fixed multiplier of standard metabolism (Hanson et al. 1997). Because oxygen is needed to recover from a lactic acid oxygen debt (Jobling 1994), scaling aerobic respiration to account for increased anaerobic metabolism may provide a reasonable approximation of relative activity. For largemouth bass foraging on 80 mm bluegill, increasing the activity multiplier for metabolism from 1.0 to 2.5 resulted in bioenergetics estimates that agreed well with observed values. As an integrated measure of recent feeding history, LDH may provide a reasonable way to account for variable foraging activity,

although additional work is needed to quantify relationships between LDH and activity rate multipliers.

The influence of prey size on LDH activity in walleyes was recently studied by Kaufman et al. (2006). In their study, LDH activity was significantly lower for walleyes feeding on cisco (*Coregonus artedii*) than for walleyes foraging on smaller prey in lakes without cisco (Kaufman et al. 2006). The authors concluded that walleyes experience an energetic advantage by foraging on larger prey. However, the influence of prey size can be confounded by morphological and (or) behavioral differences between prey types. Although larger prey taxa may confer an energetic advantage to large piscivores, it is important to recognize that foraging success varies with prey morphology and behavior, making it difficult to isolate effects due solely to prey size. For a single forage species, our findings demonstrate that larger individuals can require more activity to capture than smaller conspecifics.

Effects of vegetation density on foraging success of piscivores have been well documented (Savino and Stein 1982, 1989a, 1989b). Dense vegetation reduces encounters between predators and their prey thereby reducing foraging success (Persson and Crowder 1998). Studies with largemouth bass have shown that dense vegetation results in increased search time and reduced attack rates (Savino and Stein 1982, 1989a). This implies that encounter rate, rather than capture efficiency, has the greatest effect on foraging success by largemouth bass in dense vegetation (Valley and Bremigan 2002). As we have shown, anaerobic activity in largemouth bass varied little with vegetation density. In dense vegetation, the ambush-type foraging tactic used by bass (Savino and Stein 1982) appears to conserve energy by reducing costly pursuit behaviors that require burst swimming (e.g., anaerobic metabolism).

Variation in largemouth bass LDH activity between experiments 1 and 2 may be attributable to several factors. Largemouth bass collected from Enemy Swim Lake (experiment 2) were more than twice as large as fish used in other feeding trials (both experiments 1 and 2). Although LDH did not vary with vegetation density, it was noticeably higher (about 30%) than that observed in other treatments (experiments 1 and 2). In contrast, largemouth bass from Lake Cochrane (experiment 2) were among the smallest fish used and exhibited LDH values that were lower than those observed in Enemy Swim Lake fish. These differences may be due to the allometric scaling of LDH when considering the broader size range of fish used in experiment 2 (Somero and Childress 1980; Childress and Somero 1990). For this reason, it is important to consider predator size when comparing the influence of prey size – morphology on anaerobic activity. Alternatively, intrinsic differences may exist between these two source populations that we were unable to determine in lieu of any baseline data from similar-sized fish from each lake. Unlike experiment 1, the broad range in largemouth bass size combined with potential population differences make it difficult to interpret the effects of relative prey size on anaerobic activity levels observed in experiment 2. We did find, however, that the bioenergetics model significantly underestimated total food consumption for small largemouth bass (Lake Cochrane) foraging on relatively large prey.

Activity level can be a large and variable component of fish energy budgets (Boisclair and Leggett 1989; Hanson et al. 1997). Identifying physical and biological factors that influence activity patterns can be important for understanding effects on model accuracy. As an indicator of fish activity, LDH integrates anaerobic energy costs associated with short-term, albeit costly, activity (i.e., burst swimming). As such, LDH may prove useful as a means for quantifying relative activity of free-ranging fishes, ultimately improving our ability to model activity patterns in bioenergetics models.

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