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Functional Morphology and Biochemical Indices of Performance: Is there a Correlation Between Metabolic Enzyme Activity and Swimming Performance?[1]

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SYNOPSIS. Comparative physiologists and ecologists have searched for a specific morphological, physiological or biochemical parameter that could be easily measured in a captive, frozen, or preserved animal, and that would accurately predict the routine behavior or performance of that species in the wild. Many investigators have measured the activity of specific enzymes in the locomotor musculature of marine fishes, generally assuming that high specific activities of enzymes involved in aerobic metabolism are indicators of high levels of sustained swimming performance and that high activities of anaerobic metabolic enzymes indicate high levels of burst swimming performance. We review the data that support this hypothesis and describe two recent studies we have conducted that specifically test the hypothesis that biochemical indices of anaerobic or aerobic capacity in fish myotomal muscle correlate with direct measures of swimming performance. First, we determined that the maximum speed during escapes (C-starts) for individual larval and juvenile California halibut did not correlate with the activity of the enzyme lactate dehydrogenase, an index of anaerobic capacity, in the myotomal muscle, when the effects of fish size are factored out using residuals analysis. Second, we found that none of three aerobic capacity indices (citrate synthase activity, 3-hydroxy-o-acylCoA dehydrogenase activity, and myoglobin concentration) measured in the slow, oxidative muscle of juvenile scombrid fishes correlated significantly with maximum sustained speed. Thus, there was little correspondence between specific biochemical characteristics of the locomotor muscle of individual fish and whole animal swimming performance. However, it may be possible to identify biochemical indices that are accurate predictors of animal performance in phylogenetically based studies designed to separate out the effects of body size, temperature, and ontogenetic stage.

INTRODUCTION

One aspect of the discipline of functional morphology addresses the following question: What factors determine or limit animal performance? In this paper, we use the locomotor performance of teleost (*i.e.*, derived bony) fishes as a model system in which to investigate the relationship between biochemical determinants of organism function and whole-animal performance. Teleost fishes represent an excellent model system for several reasons. First, teleosts are the most speciose and abundant vertebrates; they use diverse locomotor modes in diverse habitats and demonstrate diverse morphologies. Second, most of the body of a fish is composed of muscle used for locomotion (the segmentally arranged myotomes), and different subtypes of locomotor muscle fibers are found in discrete regions of the body. Third, the partitioning of different fiber types within the myotome allows homogenous samples of each fiber type to be easily removed and tested *in vitro.*

Partitioning of locomotor muscle and tasks

Within the body of a teleost fish, the ''red'' muscle (composed of slow oxidative, or Type I, fibers) is a relatively small proportion of the myotomal muscle, comprising approximately 0.5–13% of body mass (Goolish, 1989; reviewed by Dickson, 1995), and is typically positioned along the midline of the posterior two-thirds of the body (Fig. 1). Numerous electromyographic studies have demonstrated that this muscle powers sustainable, aerobic swimming in most teleost fishes (for example, Johnston, 1981; Rome *et al.*, 1988; Gillis, 1998). In contrast, ''white'' muscle (composed of fast glycolytic, or Type IIb, fibers) comprises the bulk of the axial musculature (Fig. 1). In most teleost fishes, this muscle provides the power for accelerations and short-duration sprint swimming supported by anaerobic pathways of ATP production (Johnston, 1981; Rome *et al.*, 1988). Relatively little of the myotome is composed of muscle of intermediate fiber type (fast oxidative, or Type IIa, fibers) in most teleost fishes, although there are some exceptions (Johnston, 1981; Rome *et al.*, 1988).

These distinct regions of the myotomal muscle provide the power for particular behaviors. White muscle contracts to produce the force required for rapid movements, particularly sprint swimming and escape responses, used to escape from predators, capture elusive prey, and pursue mates. In contrast, red muscle contracts to produce the force required for slower, sustainable movements, including migrations over long distances, routine movements, and foraging for non-elusive food.

It has been suggested that morphological specialization for a given type of locomotor performance or ecological role has required trade-offs, leading to reduced performance using other locomotor behaviors

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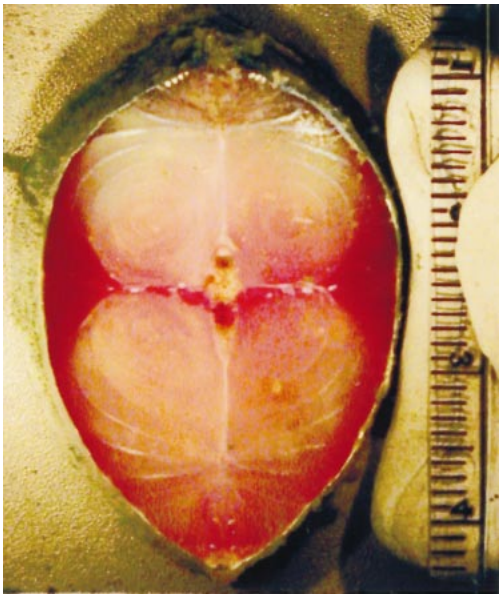

FIG. 1. A cross-section through the body of a 233 mm fork length juvenile chub mackerel, *Scomber japonicus*, at a position along the body equal to 60% of fork length, illustrating the regions of the axial musculature used for different swimming behaviors. The red muscle is positioned along the midline on either side of the fish, the white muscle comprises most of the rest of the section, and the vertebral column is approximately in the middle. A ruler to the right of the section gives the scale in centimeters. Photo by Jennifer Hoskinson.

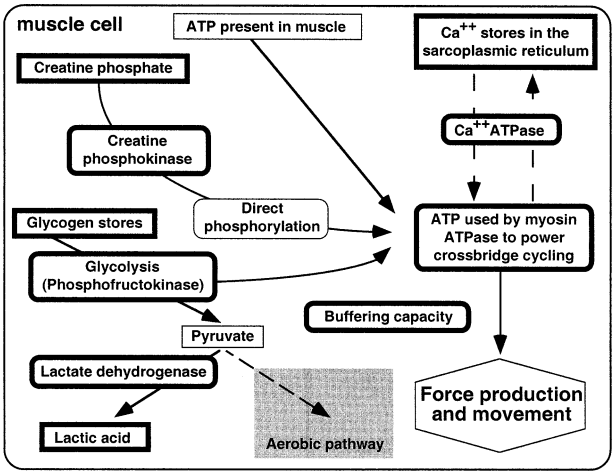

FIG. 2. Anaerobic ATP production in muscle fibers: a schematic representation of some of the important fuel stores and biochemical pathways involved in ATP production and force generation inside a muscle cell during anaerobic locomotion. A heavy line surrounding a variable indicates that it has been suggested as a possible predictor of anaerobic swimming performance.

(Webb, 1984). For example, while tunas are specialized for ''stiff-bodied'' locomotion with reduced drag, they are also less maneuverable (Blake *et al.*, 1995). Recent work on cod (Reidy *et al.*, 2000) showed a negative correlation between an individual's maximum aerobic swimming speed and its sprint swimming performance (*i.e.*, short bouts of acceleration above the maximum aerobic swimming speed). Yet, cod that attained high burst swimming speeds (*i.e.*, maximum speed achieved during the escape response) also reached high maximum sustainable swimming speeds (Reidy *et al.*, 2000). Domenici and Blake (1997) have argued that the morphological and physiological systems involved in cruising, maneuvering, and accelerating are decoupled from one another. Thus, in linking whole-animal swimming performance to biochemical indices of performance, it is important to consider the type of behavior involved, how it is measured, and the tissue and metabolic pathway responsible for powering that locomotor activity.

Methods to measure swimming performance

Certain experimental protocols have been used to assess a fish's swimming performance (reviewed by Hammer, 1995). Typically for anaerobic swimming performance, fish begin at a standstill or a low swimming speed and are given a negative stimulus to provoke a ''C-start'' and very rapid escape swimming (reviewed by Domenici and Blake, 1997). The maximum speed obtained during this trial is considered the maximum burst swimming speed, or U_{max}. Recently, another acceleration performance test in which fish are rapidly accelerated in a swimming tunnel against steadily increasing water speeds has been used to quantify maximum sprint swimming performance (Reidy *et al.*, 2000).

For aerobic swimming performance, fish are usually placed in a flow tunnel (following methods first described by Brett, 1964) and required to match the speed of water in the chamber for a predetermined length of time. After the fish has swum at this speed for the required time, the speed is increased by some increment, and the fish is required to match water speed for another time period. This procedure is repeated until the fish can no longer match the water speed in the flow chamber for the entire period and fatigues. The maximum sustainable swimming speed (or U_{crit}) is then calculated by a formula developed by Brett (1964) which takes into account the fraction of the period that the fish was able to swim at the final fatigue speed. Others have used the endurance of fish swimming steadily at a given speed in a flume or a gantry tank (*e.g.*, He and Wardle, 1988) to measure maximum sustainable swimming performance.

Metabolic pathways and biochemical indices

Sustainable, steady swimming and burst swimming rely on different metabolic pathways to provide the ATP used to produce muscle contractions. Burst swimming relies on anaerobic pathways of ATP production and uses fuel sources contained within the muscle (Fig. 2). The specific activity of key enzymes involved in these pathways, the level of anaerobic fuel stores, and other factors within the white myotomal muscle have been suggested as potential indicators of a fish's anaerobic performance. For example, creatine phosphate provides ATP during the first approximately 30 sec of

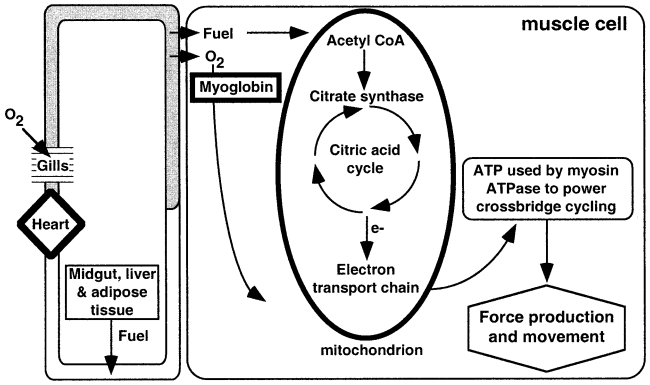

FIG. 3. Aerobic ATP production in muscle fibers: a schematic representation of some of the important stores and biochemical pathways involved in ATP production and force generation for a muscle cell during aerobic locomotion. Unlike in an anaerobic system, the cell's ability to produce ATP aerobically is potentially limited by the ability of organ systems outside of the muscle cell to deliver oxygen and fuel to the muscle cell, as well as the internal components of the muscle. A heavy line surrounding a variable indicates that it has been suggested as a possible predictor of aerobic swimming performance.

sprinting via direct phosphorylation of ADP (Dobson *et al.*, 1987). Thus, the concentration of creatine phosphate (a high-energy-phosphate store) and the specific activity of creatine phosphokinase (the enzyme that catalyzes the reaction creatine phosphate + ADP ↔ creatine + ATP) in the white muscle have been proposed as indicators of anaerobic capacity. Similarly, glycogen stores, the activity of enzymes of the glycolytic pathway and of lactate dehydrogenase (which catalyzes the reaction pyruvate + NADH ↔ lactate + NAD^+ to maintain redox balance during anaerobic glycolysis), and buffering capacity (which allows the muscle to defend intracellular pH during rapid anaerobic ATP turnover) of the white muscle have all been used to assess fish anaerobic capacity (Childress and Somero, 1979; Somero and Childress, 1980, 1990; Dickson and Somero, 1987; Dickson, 1995, 1996). Finally, the activity of myosin ATPase (located on the myosin heads within the sarcomere), Ca^{++}ATPase, or concentrations of parvalbumin may limit the rate of contraction and relaxation of the muscle fibers that produce the force required for movement (Rome, 1998; Thys *et al.*, 1998; Rome *et al.*, 1999; Swank and Rome, 2001), and thus could limit muscle contraction kinetics and maximum swimming speed.

Sustained swimming relies on the aerobic production of ATP within the muscle cell (Fig. 3), and the ability of the circulatory system to deliver oxygen and fuel to the cell (Weber and Haman, 1996). The ability to obtain and deliver oxygen is potentially limited by the respiratory surfaces, ventilation and perfusion rates, the oxygen carrying capacity of the blood, cardiac output, vascularization of the tissue, and the myoglobin content of the muscle. Fuels must be mobilized and delivered to the muscle cells in order to be catabolized aerobically for ATP production to sustain muscle contraction. The ability of a muscle cell to produce ATP during steady swimming is also potentially limited by the number of mitochondria in the cell and the activity of enzymes involved in the intra-mitochondrial aerobic pathways.

Supporting evidence

Evidence to support the idea that the biochemical parameters discussed above can be altered to improve organism performance comes from four areas of research: acclimation studies, scaling studies, comparative studies and training studies. There have been numerous such studies of various fish species in many different laboratories. Here, we summarize a few examples from these studies that lend credence to the theory that these parameters may be predictors of individual performance.

Red muscle power output is reduced at low temperatures, and the recruitment of the white myotomal muscle, the transition to anaerobically powered swimming (*i.e.*, maximum sustainable speed), occurs at lower speeds in cold *versus* warm temperatures (Rome, 1995). To increase sustainable swimming performance, fish that are acclimated or acclimatized to cold temperatures could adjust their muscle properties to compensate for the temperature effects. Acclimation studies consistently demonstrate that muscle mitochondrial density and mitochondrial metabolic enzyme activities increase when teleost fishes are cold-acclimated (for reviews see Moerland, 1995; Sidell, 1998), allowing the fish to elevate metabolic rate. However, other cold-acclimation studies have not yielded clear patterns. For example, some fish species show an elevation in white muscle myosin ATPase activity or in Ca^{++}ATPase activity in response to cold-acclimation, whereas others do not (Fleming *et al.*, 1990; Johnson and Bennett, 1995; Temple and Johnston, 1997). In addition, rates of activation and relaxation have also been shown to change in response to cold-acclimation, but the physiological underpinnings of these changes are not yet clear (Rome, 1995; Temple and Johnston, 1997; Swank and Rome, 2001).

Scaling studies have also suggested that there may be biochemical correlates of organism performance. For example, many active teleost fishes show an increase in lactate dehydrogenase (LDH) specific activity with an increase in body size (Somero and Childress, 1980, 1990; Childress and Somero, 1990). This increase in LDH activity has been invoked as a potential mechanism for maintaining size-independent maximum sprint-swimming speed. Generally, larger animals require proportionally more muscle power than do smaller animals, because the mass that must be moved increases faster than the cross-sectional area of the muscle that is used to produce the movement (Hill, 1950). Theoretically, the additional activity of LDH reflects the increased glycolytic capacity of the white myotomal muscle of the larger animals, which could allow the fish to produce more power per gram muscle during sprint swimming. Less active teleosts do not show this pattern; it has been proposed that those fish-

es do not need to maintain high anaerobic performance as they grow larger (Childress and Somero, 1990).

Comparative studies suggest that, in the axial muscle, more active teleost species have higher specific activities of key aerobic enzymes, such as citrate synthase (CS), which catalyzes the first reaction of the citric acid cycle and is an index of tissue mitochondrial density, than do less active fishes (Childress and Somero, 1979; Sullivan and Somero, 1980; reviewed by Dickson, 1995). This relationship has been found for both red and white myotomal muscle, but its interpretation is somewhat limited because fish activity levels have been based on morphology and natural history information rather than accurate measurements of swimming performance. In another interspecific study of marine teleosts, CS activity in the white muscle was shown to correlate with fish oxygen consumption rate (Torres and Somero, 1988). It is clear why an aerobic capacity indicator (CS activity) in the aerobic locomotor muscle would correlate with fish routine activity and oxygen consumption rate, but not as clear why CS in the anaerobic white muscle would show such a correlation. It has been suggested that fishes that produce more lactate in the white muscle need a higher aerobic capacity to restore intracellular glycogen stores within the white muscle, which is an aerobic process (Dickson, 1995). Alternatively, since white myotomal muscle is the tissue that comprises the greatest percentage of body mass of fishes, white muscle aerobic capacity may reflect overall organismic aerobic capacity and standard metabolic rate.

Perhaps the most compelling evidence for a correlation between tissue biochemical indices and organism performance should be provided by training studies. An increase in a given parameter in response to a particular training regime would seem to demonstrate a cause-and-effect relationship. However, although many endurance training studies have been conducted, there seems to be little consensus in the results (reviewed by Davison, 1997). Cod increase the myoglobin concentration in the red muscle (Love *et al.*, 1977), and blood hemoglobin concentration increases in some fish species, but not in others (reviewed by Davison, 1997). In many fish species, but not all, red muscle fiber diameter and capillarization increase in response to aerobic training. The literature on changes in metabolic enzyme activity in response to training has been equally ambiguous, with no consistent trends present among species (reviewed by Davison, 1997). Relatively little work has examined changes that occur as a result of a sprint training regime (reviewed by Davison, 1989, 1997).

Predictions and general hypotheses

Therefore, on the basis of basic physiological principles and the types of studies summarized above, it appears that certain parameters should be predictors of swimming performance (Table 1). For example, burst swimming performance could be enhanced by (1) increased amounts of anaerobic fuels; (2) increased activity of glycolytic enzymes and lactate dehydrogenase; (3) increased buffering capacity; or (4) increased myosin ATPase activity, Ca^{++}ATPase activity, or parvalbumin concentration—all in the white myotomal muscle. In contrast, sustained swimming performance might be improved by (1) increased oxygen delivery capacity to the red myotomal muscle; (2) increased stores of lipids in the red muscle and/or adipocytes; (3) increased metabolism of aerobic fuels by the red muscle; (4) increased specific activity within the red muscle of enzymes in pathways of aerobic ATP production; or (5) increased number of red muscle mitochondria.

TABLE 1. *Potential mechanisms to increase swimming performance in teleost fishes.*

Burst or sprint swimming performance	Sustainable swimming performance
Increased concentrations of anaerobic fuels in the muscles	Increased stores and metabolism of lipids
Increased activity of glycolytic enzymes and lactate dehydrogenase	Increased activity of enzymes in the aerobic pathway
Increased buffering capacity	Increased oxygen delivery capacity (*e.g.*, increased gill surface area, heart performance, blood hemoglobin content, number of capillaries, concentration of myoglobin, etc.)
Increased myosin ATPase activity, Ca++ ATPase activity, and parvalbumin concentration	Increased number of mitochondria

To test the general hypothesis that muscle biochemical parameters may be indicators of individual performance, we conducted two sets of experiments in which we compared individual swimming performance to specific biochemical indices. In the first study, we compared maximum swimming speed during a C-start in larval and juvenile California halibut, *Paralichthys californicus*, with the lactate dehydrogenase activity in the axial musculature. We used halibut larvae and juveniles because burst swimming is important to marine fish at these stages for the capture of individual prey items and to escape predation (for example, Webb, 1981), and a previous study (Kaupp and Somero, 1989) found changes in muscle enzyme activity during metamorphosis that may correlate with changes in swimming behavior. In the second study, we compared maximal sustained swimming speeds in chub mackerel, *Scomber japonicus*, and kawakawa tuna, *Euthynnus affinis*, with several potential indicators of aerobic capacity. These scombrid species were chosen because they are active, pelagic swimmers specialized for continuous swimming.

METHODS

California halibut, *Paralichthys californicus*, were obtained as larvae or juveniles from a local hatchery (Redondo Beach California Halibut Hatchery Program) and held at 18°C in 1-liter containers of filtered seawater. Individual larval halibut were transferred to

a small glass Petri dish, and a Peak Performance Technologies high-speed (120 Hz) video camera was mounted over the dish (with the lens perpendicular to the base of the dish). Individual juvenile halibut were transferred to small rectangular aquaria and videotaped from a lateral view (with the lens perpendicular to the side of the tank). Both videotaping arenas contained a calibrated grid in the field of view. Escape responses were triggered by gently prodding the fish with a blunt probe. Multiple escape responses were recorded for each individual, and maximum speed during the escape was determined as described below. After the escape responses were recorded, each individual was quick-frozen in seawater at −80°C in a small cryogenic tube.

To perform the enzyme assays, individuals were thawed and quickly measured (mass and length). The head and viscera were removed from the fish, and the post-cranial body was homogenized on ice in a ground-glass homogenizer in 50 mM imidazole buffer, 2 mM ethylenediamine tetra acetic acid (EDTA), pH 6.6 at 20°C. The homogenate was centrifuged to remove cellular debris and the supernatant was used for assays conducted at 18 ± 0.2°C to determine the specific activity of LDH under saturating substrate concentrations, following methods described in Dickson and Somero (1987), which were modified for a microplate reader for the smallest specimens. Enzyme activity was calculated in international units (I.U., or micromoles of substrate converted to product per minute) per gram wet weight of tissue. To test for possible variations in individual condition that would affect the enzyme activity measurements, the concentration of protein in each tissue supernatant (*i.e.*, soluble protein) was determined with a microplate reader using a BioRad protein assay kit with bovine plasma gamma globulin as a standard. Then, we tested for a significant correlation between enzyme activity (in I.U. per g tissue) and protein content (in mg protein per g tissue).

Sequential video images of each escape response were uploaded field-by-field to a Macintosh personal computer and saved as image files. These image files were transferred to an IBM-compatible PC running *Didge* Image Digitizing Software (Cullum, 1999), which was used to determine the coordinates of 11 points along the midline of the fish. These points were tracked over time, and the point closest to the center of mass (approximately 30% of total length; verified using preserved specimens) was used to assess the movement of the fish during the escape response. From these data, various kinematic variables (including maximum swimming speed, or U_{max}) were calculated (similar to methods described in Hale, 1999).

For the experiments on aerobic swimming performance in scombrid fishes, juvenile kawakawa tuna (*Euthynnus affinis*, 110 to 250 mm fork length or FL) and chub mackerel (*Scomber japonicus*, 133 to 250 mm FL) were captured by hook and line from the wild, and placed in a large holding tank with filtered seawater maintained at 24 ± 1°C. After acclimating to captivity for at least two weeks, individual fish were placed in a temperature-controlled, variable-speed respirometer held at 24 ± 0.2°C. The respirometer was then sealed, and a black cloth was placed over the central chamber (which contained the fish) to reduce disturbance. After a four-hour adjustment period, during which aerated or oxygenated water was circulated through the respirometer at a slow speed, the maximum sustainable speed was measured using a modification of the Brett (1964) procedure, as described in Sepulveda and Dickson (2000). Individual fish swam for 30 min at each speed until it could no longer maintain position using steady, continuous tail beats and shifted to a ''burst-and-glide'' swimming mode 3 times within 30 sec. The maximum swimming speed that the fish was able to sustain for a complete 30-min period while swimming using a gait characterized by steady, continuous tail beats (U_{sust}), assumed to be powered by the slow-twitch, oxidative myotomal muscle (Rome, 1995; Webb, 1998), was used as a measure of aerobic swimming performance. At the end of the experiment, the fish was sacrificed and quick-frozen at −70°C or −80°C.

To run assays on the muscle tissue of the scombrids, a small sample of each tissue type (red and white myotomal muscle and heart) was removed from each frozen fish. Tissue homogenates were prepared as described above for the halibut, and the supernatants were used for both spectrophotometric enzyme assays and high performance liquid chromatography assays for myoglobin. Two enzyme assays were run at 24 ± 0.2°C on the tissues. First, citrate synthase assays were conducted on white, red, and heart muscle samples. Second, an assay for 3-hydroxy-o-acylCoA dehydrogenase (HOAD), which catalyzes a key reaction in the β-oxidation of fatty acids, was performed on the red muscle and heart samples. All assays were run under conditions in which the enzyme was saturated with substrate, as determined by preliminary analyses. For CS, the assay mixture contained 0.5 mM oxaloacetate, 0.1 mM acetyl CoenzymeA, 0.1 mM dithiobis-nitrobenzoic acid, 2.0 mM $MgCl_2$, 80 mM Tris buffer, pH 8.0 at 24°C. The HOAD assay was run in the physiological direction of β-ketoacyl-CoA formation, in a solution containing 0.5 mM β-L-hydroxybutyrl CoenzymeA, 0.15 mM NAD^+, 5 mM EDTA, 80 mM Tris buffer, pH 8.0 at 24°C. In all cases, control activity was measured in the absence of substrate.

The concentration of myoglobin was measured in the tissue homogenates using high-performance liquid chromatography (HPLC), following methods in Kryvi *et al.* (1981). Tissue homogenate samples were diluted, chemically reduced with dithionite, and filtered through a 45-μm syringe filter to remove particulates. A small (25 μl) sample was injected into a Shimadzu HPLC system and a size-exclusion, gel permeation column was used to separate myoglobin from other molecules on the basis of molecular size, retention time in the column, and spectral characteristics. The concentration of myoglobin was calculated from the

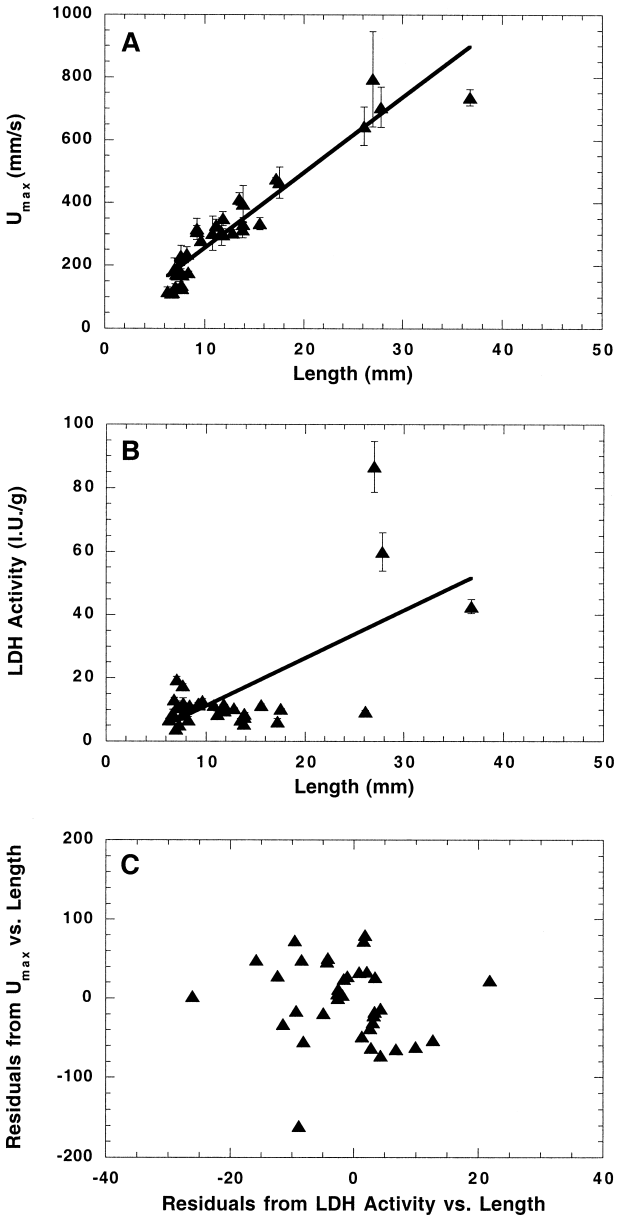

FIG. 4. Graphs illustrating the relationship between fish total length (mm), maximum swimming speed during an escape response (U_{max}), and lactate dehydrogenase (LDH) activity (International Units per gram; see explanation in text) in larval and juvenile California halibut, *Paralichthys californicus* (n = 34). Error bars represent one standard error of the mean. (A) Maximum speed (U_{max}) is positively correlated ($P \leq 0.05$) with length; this relationship is described by the linear equation $y = 14.4 + 24.0x$. (B) LDH activity also is positively correlated ($P \leq 0.05$) with length; this relationship is described by the linear equation $y = 4.4 + 1.5x$. (C) After size effects are removed with an analysis of residuals, there is no significant relationship between swimming speed and LDH activity.

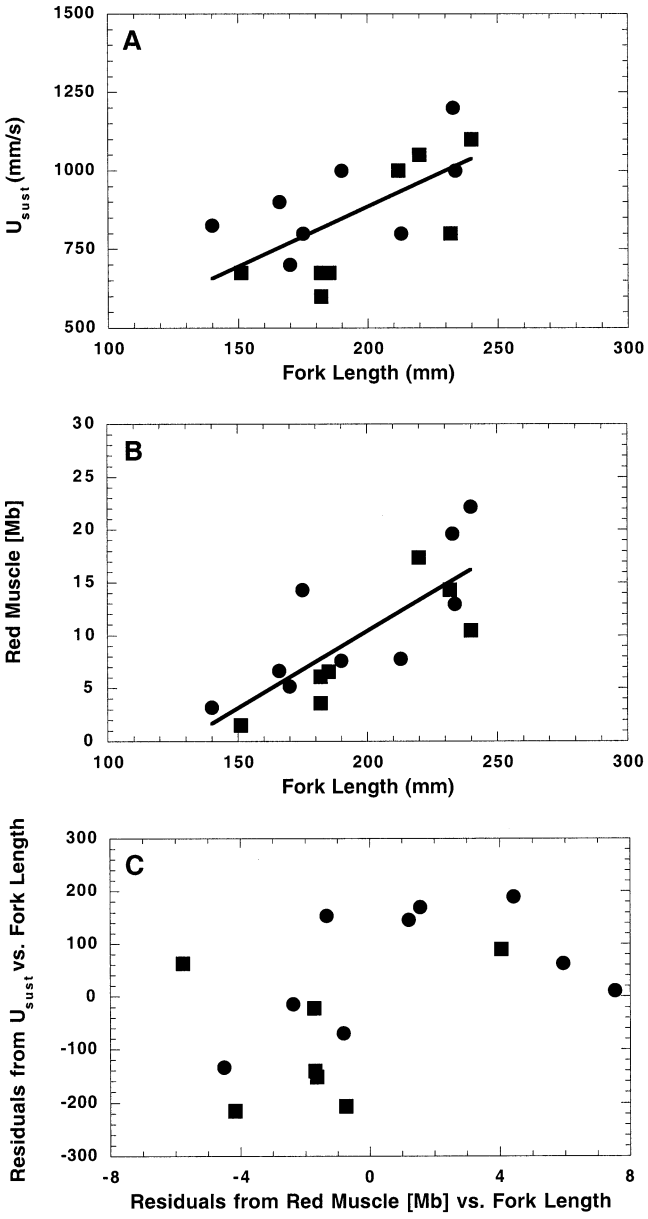

FIG. 5. Graphs illustrating the relationship between fish fork length (mm), maximum sustainable swimming speed powered by the red muscle (U_{sust}), and red muscle myoglobin concentration ([Mb]) in juvenile chub mackerel, *Scomber japonicus* (circles), and kawakawa tuna, *Euthynnus affinis* (squares). (A) U_{sust} is positively correlated ($P \leq 0.05$) with length; this relationship is described by the linear equation $y = 120 + 3.8x$. (B) Red muscle [Mb] is also is positively correlated ($P \leq 0.05$) with length; this relationship is described by the linear equation $y = -18.8 + 0.15x$. (C) After size effects are removed with an analysis of residuals, there is no significant relationship between U_{sust} and red muscle myoglobin concentration ($y = -4.3 + 17.3x$; $P = 0.06$). In addition, the correlation between residuals is not significant for either species when analyzed separately.

area of a peak observed at 413 nm, the wavelength at which the heme group is detected, in comparison to a standard curve. Although hemoglobin is also present in the samples, it separates from the myoglobin peak because it is larger than myoglobin and exits the column earlier; standards containing both myoglobin and hemoglobin were run, and absorbance spectra from 340 to 800 nm were examined, to confirm that hemoglobin did not interfere with the detection of myoglobin. Further details of the biochemical assay procedures can be found in Herrick (1999).

TABLE 2. *Coefficients of correlation between each biochemical variable and the maximum sustainable swimming speed (U_{sust}) and fork length (mm) in juvenile kawakawa tuna* (Euthynnus affinis) *and chub mackerel* (Scomber japonicus).*

Biochemical variable	Kawakawa tuna		Chub mackerel	
	U_{sust}	Fork length	U_{sust}	Fork length
Red Muscle CS	**0.743** (8)	**0.717 (8)**	0.106 (9)	−0.072 (9)
Heart CS	0.048 (7)	−0.355 (7)	0.556 (9)	0.237 (9)
White Muscle CS	0.583 (8)	0.491 (8)	−0.002 (9)	0.167 (9)
Red Muscle HOAD	0.003 (8)	0.145 (8)	−0.060 (9)	−0.081 (9)
Heart HOAD	−0.355 (7)	−0.578 (7)	−0.290 (9)	−0.350 (9)
Red Muscle [Mb]	**0.754** (7)	**0.845** (7)	**0.748** (9)	**0.794** (9)
Heart [Mb]	0.711 (5)	0.580 (5)	0.484 (7)	0.616 (7)

* Tissue citrate synthase (CS) activity (I.U. g^{-1}), 3-hydroxy-o-acylCoA dehydrogenase (HOAD) activity (I.U. g^{-1}), and myoglobin concentration ([Mb]; mg Mb g^{-1}) were used as biochemical indices of aerobic capacity. Number of individuals used for each correlation is indicated in parentheses; significant correlations ($P \leq 0.05$) are shown in bold.

RESULTS AND DISCUSSION

In both of our studies, correlation analysis revealed that several muscle biochemical variables were positively correlated with fish swimming performance (Gibb and Dickson, 1998; Herrick, 1999). However, many of these variables also correlated with fish length. Thus, to remove size effects, we performed an analysis of residuals from the regression of each variable with fish length for all variables that showed a significant positive correlation with body size. Then, we tested for significant correlations ($P \leq 0.05$) between the residuals of swimming performance variables and the residuals of biochemical indices. All enzyme activities analyzed this way were in I.U. per g tissue because we found no significant correlation between soluble protein concentration and enzyme activity.

Results from the halibut study indicated that both U_{max} and white muscle LDH activity increase with body size (Fig. 4A, B), although this relationship is stronger for swimming speed ($F = 322$, $P < 0.001$) than for LDH activity ($F = 27$, $P < 0.001$). However, when size effects are removed (Fig. 4C), there is no correlation between swimming speed and LDH activity ($F = 1$, $P = 0.25$). These results corroborate the findings of a recent study in which burst swimming performance in juvenile sticklebacks did not correlate with any of several biochemical indices, including muscle LDH activity, based on residuals analysis (Garenc *et al.*, 1999). Although Garenc and coworkers (Garenc *et al.*, 1999) found several significant biochemical correlates of burst swimming performance in adult sticklebacks, they found no consistent predictive patterns across both juveniles and adults. Thus, we have yet to identify an accurate biochemical predictor of burst swimming performance.

Results from the study of aerobic performance in the juvenile scombrids indicated that, of many potential indicators, only red muscle myoglobin concentration showed a consistent relationship with maximum speed powered by the red myotomal muscle in both species (Table 2). After size effects are removed (Fig. 5), there is trend that suggests a correlation between red muscle myoglobin concentration and maximum sustainable swimming speed. However, this trend is not significant ($F = 4$, $P = 0.06$). These findings reveal why there may be no consistent results across teleost taxa as a result of training for many potential biochemical indices of performance (Davison, 1997).

How do we reconcile these results with the strong correlations observed in the data from comparative studies? The interspecific studies are complicated by variations in temperature, body size, ontogenetic stage, growth rate, and phylogenetic relationships, all of which can have confounding effects on biochemical and performance data. Furthermore, few of those studies have quantified the swimming activity or performance of the fishes. These factors make it difficult to identify and test a causal relationship between any given pair of variables.

We propose that it will be possible to find useful biochemical correlates of individual locomotor performance that apply across species, by using a carefully designed experiment with a model group of teleosts. Ideally this would be a teleost family with a relatively small number of species; the species within the family should have different locomotor habits, but live at similar environmental temperatures. These fishes would be held and tested in a ''common garden'' experiment, and then individual performance and biochemical indices would be measured. After these experiments are conducted, phylogenetically based analyses such as independent contrasts (Garland *et al.*, 1992; Martins, 1996) would be used to identify correlations with locomotor habit that can be separated out from other confounding variables. We emphasize the need to measure locomotor performance variables directly, and we suggest some biochemical variables (Table 1) on which to focus in such future studies.

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