A Rapid Method for Determining Metabolism of Fish

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Abstract: The utility of bioenergetics models for answering fisheries and ecological questions has often been hampered by the availability of data or resources for deriving species-specific models. Among the principal components of bioenergetics models are metabolism equations that historically have been derived from series of long experiments in which fish of different sizes are acclimated and tested at each temperature for extended periods. Acclimation may take several weeks to months and actual observation on metabolism (oxygen consumption) may take several days for each group of fish with several groups often needed to provide sufficient sample size. Here, I present a rapid method for determining metabolic rates by forcing the fish through a series of rapidly declining temperatures over a 1- to 2-day period. Metabolism data from pumpkinseed *(Lepomis gibbosus),* rock bass *(Ambloplites rupestris),* striped bass *(Morone saxatilis),* and yellow perch *(Perca flavescens)* compare favorably with those obtained via more standard methods. Variability in the rapid-derived metabolism models was low with $R^2 \geq 0.82$. Size-dependent exponents in the equations ranged from −0.23 to −0.26—well within the range of −0.2 to −0.3 reported in the literature by Winberg. Temperature-dependent exponents were also close to those reported by other methods. The rapid metabolism method permits development of metabolism models from measurements carried out over several days instead of months as is usually the case. This rapid method for determining metabolism may permit bioenergetic model development for many ecologically important species whose metabolism could not be determined before due to time or financial constraints.

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Bioenergetic models have been widely used in fisheries management and ecology (Kitchell et al. 1977, Stewart et al. 1983, Ney 1993, Hartman and Brandt 1995), particularly since the advent of user-friendly bioenergetics modeling software in 1987 (Hewett and Johnson 1987). Bioenergetic models had their origins in the balanced energy equation. Winberg (1956) proposed that energetics of fish must conform to the first law of thermodynamics and therefore all energy (C) consumed by a

fish must be accounted for in growth (G), metabolism (M), heat increment or specific dynamic action (SDA), or egestion (F), or excretion (U):

$$C=G+M+SDA+F+U \quad (1)$$

Kitchell et al. (1974) expanded the balanced energy equation to account for size- and temperature-dependent effects upon consumption and metabolism.

Bioenergetics models are data-hungry and not all data required to make a model are readily available for a species (Ney 1990). This has led some model developers to borrow information from other species to apply to their study species (Ney 1993). Ney (1993) points out that a typical bioenergetics model requires 15–30 model parameters. However, many of the model parameter values vary little from species to species (Hansen et al. 1997). Sensitivity analyses have shown that the egestion and excretion parameters also contribute little to errors, and that the allometric and temperature-dependent exponents (RB) and intercepts (RA) (see equation 2) are the most sensitive to errors (Bartell et al. 1987). Thus, it seems the best cost/benefit ratio for conducting experiments aimed at development of bioenergetics models should focus on species-specific parameters for consumption and metabolism.

The typical application of bioenergetics models involves the use of commonly collected fisheries data (e.g., temperature, weight of fish, diets) as inputs to the model to predict consumption from growth. When estimating consumption the importance of the consumption sub-model for a species is minimized, since it is not needed in estimating consumption from growth. What is needed, are accurate estimates of energy costs to the fish (metabolism). Thus, parameter estimation efforts can be further restricted to metabolic parameters when the intent is to use the bioenergetics model to estimate consumption from growth data.

During July 1993 as part of a classroom exercise, I noticed that thermal and weight-dependent metabolism data from a short experiment with rock bass *(Ambloplites rupestris)* produced metabolism model parameters that were similar to those developed for other centrachids by long-term testing (K. Hartman, pers. observ.). This was initially surprising because the rock bass were not acclimated to each test temperature prior to experiments. Further, metabolism measures were taken at all temperatures (on the same fish) over a period of 24–48 hours beginning with the warmest and proceeding to the coolest temperatures. I hypothesized routine metabolic rates measured in this "rapid" manner gives results comparable to more established methods for standard or routine metabolism where fish are acclimated to each test temperature for weeks or months. If metabolism measurements could be made rapidly, this would permit development of bioenergetic models for many more species, particularly species for which limited funds or societal interest are available. Thus, the objective of this study was to determine if "rapid" methods can be used to define metabolism parameters for use in bioenergetic models.

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Methods

Evaluations of the "rapid" metabolism method required measurements on fish with the rapid method and with traditional techniques. Metabolism of fish is estimated by measuring the oxygen consumption by fish in closed chambers. Traditional methods of estimating metabolism include routine and standard metabolism methods. "Routine metabolism" is estimated by measuring oxygen consumption by fish in closed chambers. Fish can swim within the chambers and thus, measures of metabolism include a standard, basal component and an additional active metabolism component. In measuring "standard metabolism," fish are placed in swimming chambers (Brett 1964) and forced to swim at a range of current velocities. Oxygen consumption is measured at each velocity and the standard rate is assumed to be at the intercept of the regression equation where swimming speed is 0. The rapid method more closely approximates routine metabolism as fish are able to swim freely within the chamber and thus, metabolism measures include some degree of activity. Activity may be less in the rapid method than the routine method simply because the fish may be sluggish due to the rapid decline in temperatures with the rapid method, but this was not evaluated here.

The rapid method was conducted on 4 species: rock bass, pumpkinseed *(Lepomis gibbosus),* yellow perch *(Perca flavescens),* and striped bass *(Morone saxatilis).* These species were selected because they represented 3 species for which metabolism measures had been made with traditional routine methods (Evans 1984, for pumpkinseed; B. Lantry, N.Y. Dep. Environ. Conserv. [NYDEC], pers. commun., for yellow perch; and Hartman and Brandt 1995, for striped bass) and 1 species (rock bass) for which no published metabolism data exist. Once rapid metabolism measures were made, they were compared with the species-specific traditional metabolism methods data with analysis of covariance. In comparisons between "rapid" and "traditional" metabolism, only the slopes were tested as differences in the intercepts could be corrected in the model-fitting growth experiments. Significance of all tests was set at the 0.05 level.

The Rapid Method

The rapid method consisted of a series of routine metabolism measures and immediate dropping of the temperature to the next colder experimental temperature. All measures were made with a 24- to 48-hour period. This method differs from traditional routine metabolism methods in that fish were not acclimated to each test temperature in this study, and an individual fish was run through all experimental temperatures within 24–48 hours. In traditional routine metabolism studies, groups of fish are acclimated to each experimental temperature for 2 weeks to several months and then metabolism is measured over a 24- to 48-hour period. To run traditional routine metabolism experiments on the same individual fish over the range of experimental temperatures fish might experience (e. g., 5–30 C, by 5 C increments for many temperate fishes) would take many months. Traditional experiments can be confounded by changes in body mass which are a significant influence on metabolism

rates of fish (Winberg 1956, Kitchell et al. 1977, Stewart et al. 1983, Hartman and Brandt 1995). Thus, the rapid method may save 4–6 months of time relative to traditional routine metabolism methods.

Rapid metabolism experiments were conducted in different chambers for different size- or shaped-fish. Experiments on all fusiform fishes and small (<30 g) deep-bodied fishes were done in 3–1 Ferback flasks. Experiments on larger deep-bodied fishes were done in 3.8-liter carboys. Placing a fish into a closed metabolic chamber at the warmest test temperature began an experiment. Fish had previously been acclimated to the initial test temperature for a period of at least 14 days.

Once a fish was inside the chamber the dissolved oxygen level was measured with a YSI model 58 oxygen meter. The meter was calibrated twice daily. Following initial dissolved oxygen (DO) measures the temperature was also recorded and chamber sealed to prevent gas exchange with the atmosphere. All chambers were immersed in a bath at the test temperature to minimize temperature changes in the static chambers. Fish were allowed to respire in the chambers for a period of 0.5 to 1.5 hours when, after a final DO temperature and time were recorded. Metabolism was measured through the oxygen depletion over time method (Hartman 1993). The time fish were allowed to respire depended upon fish size and temperature, but the target was a minimum of a 1.0 mg/liter change between initial and final DO measurements in the chambers.

A total of 6–12 fish of different weights were measured for each species at each test temperature. Metabolism was estimated as the quantity of oxygen used by fish in sealed chambers by a difference in initial DO and final DO following sufficient time for oxygen to be depleted at least 1.0 mg/l liter. As soon as final DO measures were made, chambers were aerated with forced air and airstones with ice added to the immersion bath to rapidly drop the test temperature to the next lower treatment level. Typically this temperature change was approximately 5 C and the change took place over 15–30 minutes. Aeration was then ceased and initial DO, temperature, and time measures were taken, then the next oxygen depletion and measurement took place as described above. This process was repeated until values were obtained at all test temperatures on each fish. At the conclusion of the metabolism measurements the fish was blotted and weighed on a portable balance to the nearest 0.1 g.

Data collected with the rapid metabolism method were transformed and analyzed with multiple regression to describe the least squares best fit of the equation:

$$M\ (g\ O_2/g/day) = RA*W^{RB}*e^{RQ*T} \quad (2)$$

where M is metabolism, RA is the intercept of the equation, RB is the slope of the size-dependent slope, RQ is the temperature-dependent exponent and T is temperature.

Results

As expected, for all species the metabolism increased with temperature (Fig. 1) and decreased with increasing size (Fig. 2). Regression equations describing rapid

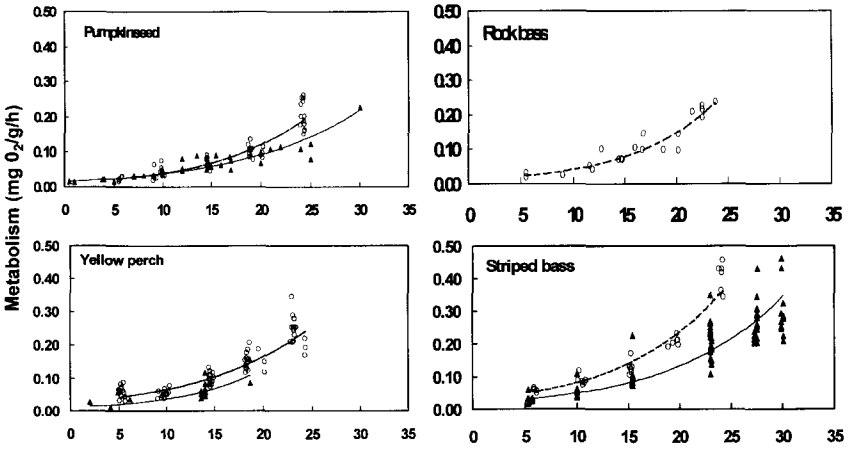

Figure 1. Temperature-dependence of metabolism (g O_2/g body weight/hour) for a 30-g wet weight pumkinseed, rock bass, yellow perch, and striped bass. Open circles represent individual fish data from rapid metabolism methods while solid triangles represent data from Evans (1984, pumpkinseed), B. Lantry (pers. commun., yellow perch), and Hartman (1993, striped bass).

metabolism as a function of fish size and temperature were highly significant ($P<0.001$). Equations described between 80 and 94% of variability in measured data (Table 1).

The rapid metabolism method provided model parameter values that were within the range reported for most species (Table 2). Intercept values for the metabolism equation *(a)* ranged from 0.0194 (pumpkinseed) to 0.0431 (yellow perch). Size-dependent exponents *(b)* ranged from −0.23 to −0.26. Temperature-dependent exponents (RQ) ranged from 0.088 to 0.110 (Table 1).

All metabolism model parameters measured during the rapid metabolism experiments were within the range reported for fish where authors used routine or standard metabolism in procedures requiring longer acclimation and experimental times. The pumpkinseed and rock bass parameters were similar to those reported for bluegill sunfish *(L. macrochirus)* and other centrarchids (Table 2 from Evans 1984 and Hansen et al. 1997).

Slopes of metabolism models developed with the rapid method were also similar to models developed from metabolism data in the literature using common, routine rate methodology (Figs. 1, 2). There were no significant differences in the size-dependence (ANCOVA, $F=2.54$, $P=0.113$) or temperature-dependence slopes of metabolism (ANCOVA, $F+2.52$, $P=0.053$) for striped bass between the rapid (this study) or traditional methods (Hartman 1993). Yellow perch metabolism from the rapid method did not differ significantly from data provided by B. Lantry (NYDEC, pers. commun.) with respect to size-dependence (ANCOVA, $F=1.11$, $P=0.294$), but the temperature-dependence slope was significantly different between methods (ANCOVA, $F=13.94$, $P=0.0003$).

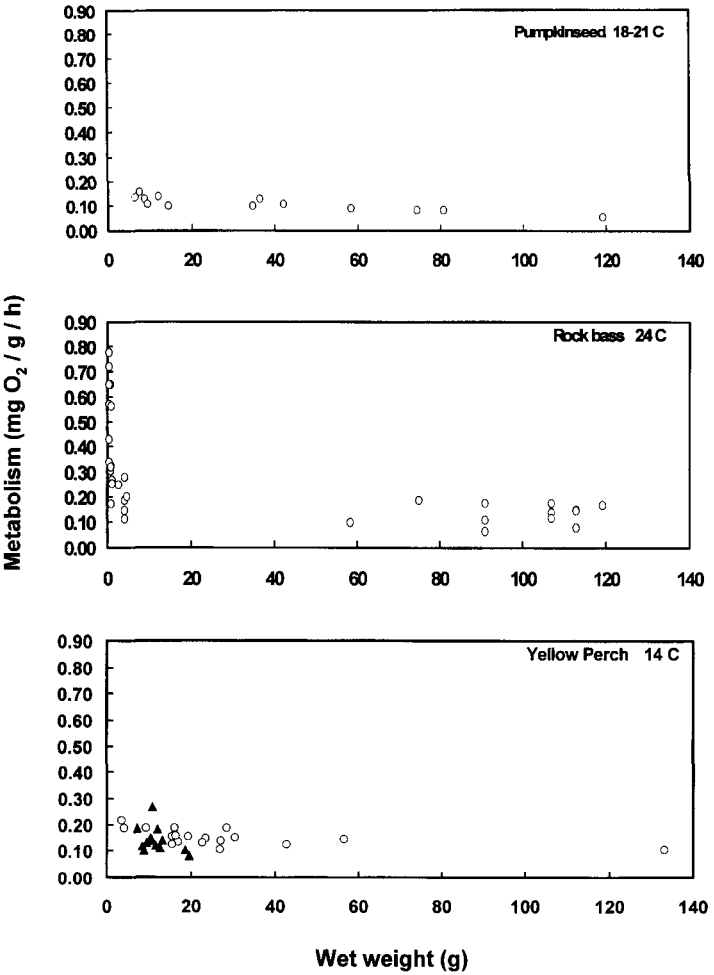

Figure 2. Size-dependence of metabolism for pumkinseed, rock bass, yellow perch, and striped bass. No graph is presented for striped bass, which lacked sufficient breadth of size (20–28 g) to warrant inclusion in this figure. Open circles represent data from an individual fish with the rapid metabolism method while solid triangles represent data from individual fish from B. Lantry (pers. commun., yellow perch).

Comparison of metabolism models for pumkinseed was possible only for temperature-dependence. Evans (1984) published a compilation of data from several sources on metabolism of this species, but all data were standardized to that for a 100-g fish. Thus, data from the rapid method for pumkinseed were transformed to that for a 100-g fish using the size-dependent exponent (-0.24) from this study and compared with data in Evans (1984). Slopes of temperature-dependent metabolism data from the rapid method and the Evans (1984) data did not differ significantly (ANCOVA, $F=2.99$, $P=0.089$) (Fig. 1).

Table 1. Multiple regression models describing the effects of size (weight, g) and temperature (C) upon metabolism (g O_2/g/day) measured using the rapid method. Model is of the form: Metabolism = Intercept * weight^{RB} $e^{(RQ*Temperature)}$

Species	*N*	R^2	Size range (g)	Temperature range (C)	Intercept	RB	RQ
Pumpkinseed	58	0.92	5.3–119.2	5.5–24.3	0.0194	−0.240	0.1045
Rock bass	29	0.82	0.5–113.0	5.5–23.9	0.0384	−0.240	0.1103
Striped bass	28	0.95	19.8–28.4	5.9–24.3	0.0389	0.228	0.0953
Yellow perch	84	0.85	3.5–133.2	5.0–24.3	0.0431	−0.263	0.0891

Discussion

The rapid metabolism method appears to be a promising alternative method to more standard techniques for measuring fish metabolism. In most cases, data obtained using the rapid method were not significantly different than data from the literature employing traditional routine metabolism methods. Temperature-dependence for yellow perch was significantly different from data provided by B. Lantry. However, overall values obtained with the rapid method were reasonable and differences may reflect differences in activity or handling between the rapid and routine methods. In both the rapid and the traditional routine method fish are able to actively swim. In all likelihood, more swimming occurs in the warmer temperatures in the rapid method since fish are essentially already acclimated to that test temperature. Striped bass metabolism from Hartman (1993) and the rapid method were not significantly different, nor were temperature-dependent metabolism data between Evans

Table 2. Model parameters reported in the literature for the effects of size (weight, g, RB) and temperature (°C, RQ) upon metabolism (g O_2/ g / day). All parameters below are reported in Hansen et al. 1997 from the primary literature except for pumpkinseed sunfish which are from Evans (1984).

Species	Intercept	RB	RQ
Alewife *(Alosa pseudoharnegus)*	0.0037	−0.215	0.0548
Bloater chub *(Coregonus hoyi)*	0.0018	−0.120	0.0470
Bluegill	0.0154	0.200	2.1[a]
Bluefish *(Pomatomus salatrix)*	0.0056	−0.264	0.0693
Dace *(Chrosomus spp.)*	0.0148	−0.200	2.1[a]
Herring *(Cluepea harengus)*	0.0033	−0.227	0.0548
Largemouth bass *(Micropterus salmoides)*	0.0028	−0.355	0.0811
Muskellunge *(Esox masquiniongy)*	0.0025	−0.180	0.0550
Pumpkinseed sunfish	0.0115	−0.240	0.0888
Smallmouth bass *(M. dolomieui)*	0.0090	−0.210	3.3[a]
Steelhead *(Oncorhynchus mykiss)*	0.0026	−0.217	0.06818
Striped bass (age-0)	0.0028	−0.218	0.0760
Striped bass (age-1 and older)	0.0015	−0.270	0.0834
Yellow perch and walleye *(Stizostedion vitreum*	0.0108	−0.200	2.1[a]
Weakfish (age-0) *(Cynoscion regalis)*	0.0009	−0.125	0.0912
Weakfish (age-1 and older)	0.0030	−0.155	0.0508

a. Some researchers used a different model form to assess temperature effects and thus, values are not directly comparable to those reported in this manuscript employing an exponential model.

(1984) (which included data from Roberts 1964, Brett and Sutherland 1965, Roberts 1967, O'Hara 1968, Burns 1975, and Evans 1984) and the rapid method for pumpkinseed. The lack of a significant difference in slope or intercept between datasets for striped bass are particularly important because any bias introduced by different researchers is controlled for in this case.

The metabolism model parameters derived with the rapid metabolism method are comparable to those reported in the literature for most species. Values for pumpkinseed and rock bass were very similar to other panfish in the literature (Hansen et al. 1997). The deep body shape of these species suggests that they are not highly mobile (Moyle and Cech 1988) and thus, similarities may be due to body morphology, taxonomic proximity, or low scope for activity in these species. Further research is needed to help identify the factors responsible for this observation.

In determining the metabolic component of bioenergetics models for fish, several authors have suggested testing in the lab (Hansen et al. 1993, Hartman and Brandt 1995) or field (Bevelheimer et al. 1985, Wahl and Stein 1991, Hansen et al. 1993), or fine-tuning of the model with lab experiments (Bevelheimer et al. 1985, Hartman and Brandt 1995) prior to general application of the bioenergetics model to the field. For all laboratory experiment-derived metabolisms, I recommend a series of laboratory or mesocosm growth experiments whereby all inputs to the balanced energy equation (C, G, T, energy density of fish and prey) are measured over a 7- to 30-day period (longer periods for slow growth conditions) at temperature treatments corresponding to metabolism temperature treatments. The model is then balanced for the activity multiplier of metabolism (ACT) by iteratively fitting the ACT required to balance the energy equation given observed C and G (see Hartman and Brandt 1995). The ACT at each temperature could then be used to establish the correct model intercept for the metabolism models. Regardless of the method of metabolism measurement, such experiments should be conducted anyway (Bevelheimer et al. 1985, Wahl and Stein 1991, Hansen et al. 1993, Hartman and Brandt 1995) and the rapid metabolism method can provide more metabolism data in several days than can reasonably be gathered in months using traditional, acclimated methods. Given the often overlooked need to quantify activity in bioenergetics models (Boisclair and Leggett 1989, Boisclair and Sirois 1993, Lucas et al. 1993) that necessitates these additional "balancing" experiments it would seem that the rapid metabolism method would expedite the model developing process.

Further research is needed to definitively determine if data obtained with the rapid method will produce reliable results in bioenergetics models. The results presented here seem promising, but they do necessitate the use of growth experiments to determine the anchor point (intercept) for the overall metabolism model. This anchor point exercise is really no more than adjusting the equations such that ACT is really 1.0. These experiments do add time to the rapid method. However, given the recommendation to use laboratory or mesocosm growth experiments to "tune" the models (Bevelheimer et al. 1985, Wahl and Stein 1991, Hansen et al. 1993, Hartman and Brandt 1995) regardless of metabolism method, it is clear that quicker calibrated metabolism models are possible using the rapid method.

Further research is needed to definitively determine if data obtained with the rapid method will produce reliable results in bioenergetics models as well as to see if these rapid methods will work with fragile species. Forcing fish through their environmental range of temperatures in 24–48 hours is stressful. Will fragile species be able to withstand this challenge, or will modifications of the method be required (such as splitting fish into 2 groups: one to run from warm to cool and one from cool to cold) to gather required data on these species? Additional tests are needed to evaluate whether the rapid metabolism method will provide realistic results for all species.

The importance of the rapid method is that it is quicker and therefore less costly than traditional metabolism methods. The rapid method appears to yield comparable information to other techniques, but the speed and low cost may permit development of bioenergetics models for less economically important species. Most species for which bioenergetics models exist are sport, game, or commercial species, or species which serve as food for the former (Hansen et al. 1997). The new, rapid metabolism method should make model development for these overlooked species much easier and should provide a viable alternative to the "species borrowing" that has plagued bioenergetics modeling to date (Ney 1993). It is hoped that the rapid metabolism method will provide fish biologists with a tool to help improve our understanding of fish ecology, trophic interactions, and managment.

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