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Allometric relationship between oxygen consumption and body weight of mosquitofish, *Gambusia affinis*

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Synopsis

The allometric relationship between body size and oxygen consumption of *Gambusia affinis* at 28° C was determined under controlled experimental conditions, using a manometric respirometer. The allometric exponent (b-value) was 0.64 ± 0.02 S.E. (n = 51). Oxygen consumption was not influenced by any time-related factors during the 17 days of measurements. Variance between replicated oxygen consumption trials on individual fish was negligible. Specific oxygen consumption rates of several *G. affinis* at 28° C that were determined by using a sealed vessel and O_2 electrode respirometry method were similar to the rates measured by the manometric respirometry method in similar-sized *G. affinis*.

Introduction

It is difficult to define a normal resting oxygen consumption rate, or even to define the scaling of oxygen consumption relative to body size for poikilothermic animals such as fish because so many factors affect their metabolic rate. For example, environmental conditions such as present temperature, thermal history, oxygen content of the water, feeding and nutritional state, and day length influence fish metabolic rate (Schmidt-Nielsen 1984). In addition, the experimental conditions imposed on the fish during the oxygen consumption measurement process may significantly affect fish metabolic rate. Examples of these experimental conditions include time of measurement relative to circadian cycles, time of measurement after introduction of fish into the experimental apparatus, size of the experimental chamber, degree of suppression of external stimuli, and the presence of other fish in the chamber (Schmidt-Nielsen 1984). Thus, in order to determine the relationship between metabolic rate and fish size for a given species, it is necessary to specify clearly the environmental and experimental conditions and then determine the dependence of oxygen consumption on fish body size under otherwise identical conditions.

There have been studies to define the relationship between oxygen consumption and size of several fish species (Fry 1957, Beamish 1964, Ultsch 1973, Brett & Glass 1973, Jobling 1982, Morris & North 1984). This relationship is expressed by the allometric equation:

$$\dot{V}_{O2} = aW^b$$
or
$$\log \dot{V}_{O2} = b(\log W) + \log a,$$

where $\dot{V}_{O2}$ = volume of oxygen consumed per fish per unit time, W = fish mass, b = the allometric

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exponent or slope of the line representing the allometric equation plotted on logarithmic scales, and a = the proportionality coefficient or intercept of the regression line at unity. However, data on the relationship of oxygen consumption with fish body size over a large intraspecific size range or for small size fish species are uncommon. Furthermore, although the mosquitofish, *Gambusia affinis*, is common and widespread throughout the southeastern part of the United States and has also been introduced to a variety of temperate and tropical regions worldwide for mosquito control, few data have been reported on its oxygen consumption. No data on the relationship between oxygen consumption and *G. affinis* size were found in the literature.

The purpose of this investigation was to determine the allometric relationship between oxygen consumption and body weight in *G. affinis* from a field population whose thermal history was known and whose body weights ranged over approximately two orders of magnitude. Oxygen consumption was measured using a manometric respirometer and uniformly-controlled experimental conditions. The study also was designed to assess whether the fishes' oxygen consumption changed, independent of fish size, as the length of time increased since their capture from the field. In addition, the study was conducted to measure whether the precision of replicated 60 min oxygen consumption trials for individual fish was acceptable using the manometric respirometer technique.

Materials and methods

Fish

Gambusia affinis (Baird & Girard) were collected on September 26, 1984 by netting from below a coal fly ash basin on the Savannah River Plant near Aiken, South Carolina, USA. The fish were selected from this location as part of a larger study investigating the allometric relationship between trace metal body burdens and body weight of *G. affinis*. Mean water temperature was 27.2° C ± 1.9° C (mean ± sd, n = 33) during the four months prior to fish collection (Environmental & Chemical Sciences, New Ellenton, SC, unpublished data), and was 27.5° C on the day of collection. Ash basin water was collected at the same time as the fish, filtered and used as the water source throughout the study.

Female and juvenile fish were sorted by length into six relative size classes and placed into corresponding glass aquaria with a washed gravel substrate. The six aquaria were kept in an Environator growth chamber, which maintained a mean water temperature of 28.1° C ± 0.6° C (mean ± sd, n = 102). The light : dark cycle was 12 h : 12 h, corresponding to the actual day length on September 26. The light : dark cycle included a 0.5 h dawn and dusk simulation created by illuminating approximately 25% of the lights inside the growth chamber. Fish were fed Tetra Guppy Special Diet food daily, but 24 h of fasting were allowed before performing the oxygen consumption measurements. The fish were acclimated to these laboratory conditions for seven days before oxygen consumption measurements were begun.

Oxygen consumption measurements

Oxygen consumption (expressed as μl dry O_2 at STP $fish^{-1}$ h^{-1}) was measured in a Gilson differential respirometer with 125 ml active and reference flasks. The gas manifold on the respirometer was not used due to frequent leaks; instead, each active flask was connected to its own reference flask. Oxygen consumption trials were performed on nine analysis days during a 17 d period (7, 8, 14, 16, 19, 20, 21, 22 and 23 days after capture). Mean water temperature during oxygen consumption measurements was 27.9° C ± 0.1° C (mean ± sd, n = 17). In order to minimize external stimuli to the fish, the respirometer was located in a quiet room with only one person present to perform the measurements and record data. All measurements were performed by the same person.

Approximately one milliliter of 10% (w/v) KOH solution and a Whatman chromatography paper wick were placed in the center well of each active flask to absorb CO_2. Each active flask contained 55 ml of filtered ash basin water and one fish. The

reference flasks contained 55 ml of deionized water. Each analysis day, one active flask containing no fish was used as a blank to measure any biological or chemical oxygen demand in the ash basin water. The flasks were connected to the respirometer and allowed to equilibrate for 45 min before measurements were begun.

Two sets of oxygen uptake measurements were conducted on five or six fish each analysis day. Sexes of the juvenile fishes (3.8 to approximately 15.0 mg dry wt) were unknown; otherwise only females were used. Oxygen consumption was always measured between 1130 and 1530 h. Micrometer readings (in μl) was taken at 10 min intervals for one hour. After the 60 min reading, the KOH solutions and paper wicks were replaced and air was bubbled through the water in the active flasks for 45 sec. The flasks were reconnected to the respirometer and equilibrated for 45 min. A replicate set of micrometer readings was taken at 10 min intervals for 1 h. Immediately after the oxygen consumption measurements were completed, fish were weighed and frozen. The frozen fish were later lyophilized and weighed.

Oxygen content of the water (P_{O_2} or dissolved O_2) was not measured during the manometric respirometry measurements. Therefore, a sealed vessel and Radiometer O_2 electrode respirometry method (Ultsch 1973) (hereafter referred to as the O_2 electrode respirometry method) was used to determine the changes in P_{O_2} at 28° C during oxygen consumption measurements of three *G. affinis* (79–205 mg dry wt). In addition, the O_2 electrode respirometry method was used to compare the specific oxygen consumption rates of the three fishes to the rates in similar-sized mosquitofish measured by the manometric respirometry technique.

Experimental design and statistical analyses

The experiment was a 3-level, unbalanced nested design with fish nested within analysis day, and replication nested within fish within analysis day. The analysis of variance was performed using the Statistical Analysis System (SAS) GLM routine (SAS Institute 1985). An analysis of covariance was conducted to adjust oxygen consumption means each analysis day for differences in fish weights. Least squares linear regression of log-transformed total oxygen uptake per fish versus log dry weight was performed using the SAS GLM routine. Corrections for bias in the regression estimates after logarithmic transformation were performed according to the method of Beauchamp & Olson (1973). Estimates of the variance components of log oxygen consumption between fish standardized to an adjusted weight and between replicated measurements in fish of the same weight were estimated by the method of moment estimators (Hocking 1985).

Results

The relationship between total oxygen consumption and body weight of *G. affinis* was described by the allometric equation:

$$\dot{V}_{O_2} = 309.4 \text{ (fish mass, dry)}^{0.64}$$

($r^2 = 0.926$; standard error of the exponent = 0.02; 95% confidence interval for the exponent = 0.60–0.68; n = 51). Total oxygen consumption increased as fish mass increased (Fig. 1). The range of whole body dry weights of mosquitofish used in the determination of the allometric relationship was 3.8 mg to 346.2 mg, representing a 91-fold range. The relationship between *G. affinis* dry weight and wet weight was described by the equation:

$$\text{dry wt} = 0.292 \text{ (wet wt in mg)} - 2.70$$

($r^2 = 0.988$; n = 51).

Smaller sized *G. affinis* had greater specific oxygen consumption rates (μl O_2 g^{-1} dry wt h^{-1}) than larger fish (Fig. 2). Mean specific oxygen consumption rates (mean ± sd, n = 2) ranged from 2645 ± 93 μl O_2 g^{-1} h^{-1} in a 3.8 mg dry wt fish to 407 ± 39 μl O^2 g^{-1} h^{-1} in a 346.2 mg dry wt fish.

The mean specific oxygen consumption rates (mean ± sd) measured by the O_2 electrode respirometry method in three *G. affinis* weighing 79 mg, 135 mg and 205 mg dry wt were 630 ± 187 μl O_2 g^{-1}

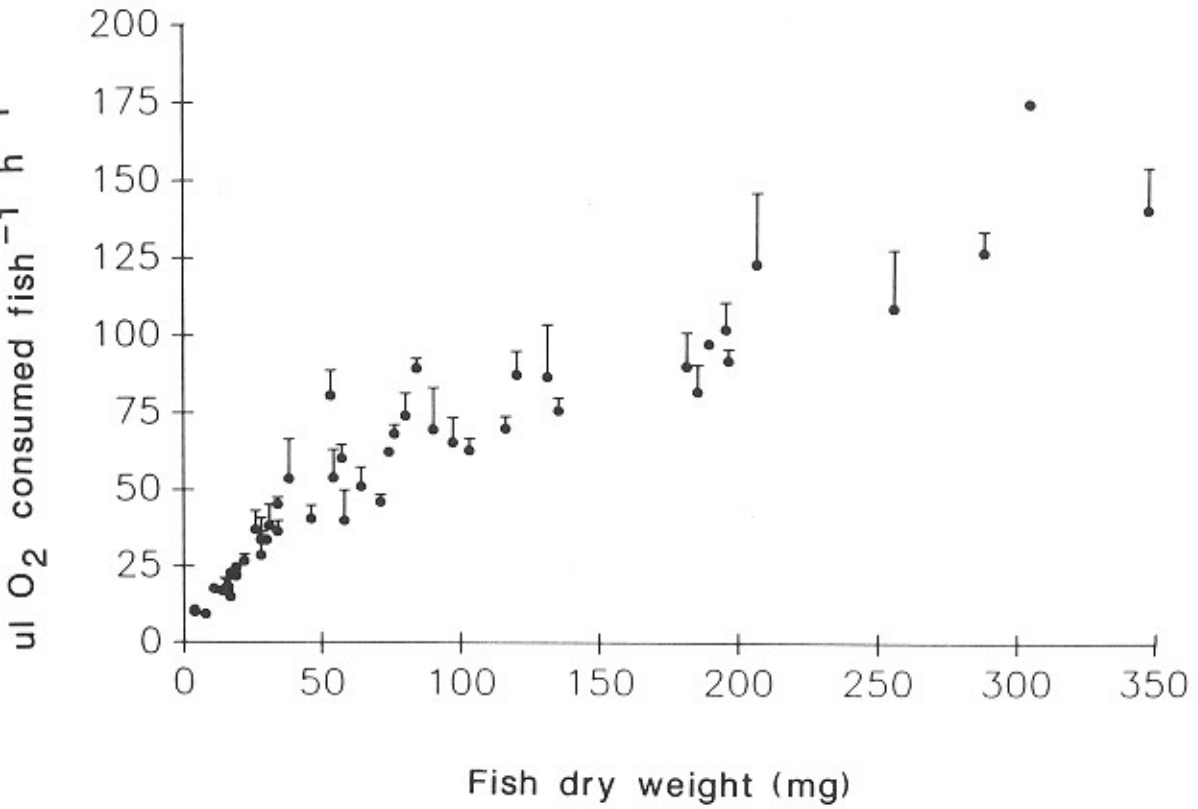

Fig. 1. Plot of the total oxygen consumption for each individual *G. affinis* (μl O_2 $fish^{-1}$ h^{-1}) versus body dry weight. Each point and error bar represents the mean of two replicate measurements ± sd. N = 51. Error bars are not shown on points representing means whose sd was less than 2 μl O_2 $fish^{-1}$ h^{-1}.

h^{-1} (n = 2), 770 ± 149 μl O_2 g^{-1} h^{-1} (n = 3), and 613 ± 40 μl O_2 g^{-1} h^{-1}, (n = 2), respectively. Initial P_{O2} in the flasks containing a single test fish were 134–139 torr, and decreased to 98–103 torr after 5 h.

Oxygen consumption by the fish did not change significantly as the time in captivity increased (covariance analysis, dry weight as covariate, $P>0.36$). When the residuals from the least squares linear regression model of log oxygen consumption versus log dry weight were plotted against the number of days since the fishes' capture from the field, the points fell within a horizontal band pattern, indicating that a long-term effect was not influencing the data (Draper & Smith 1981).

Moment estimators of the variance between replicated measurements of log oxygen consumption in fish of the same weight and variance between log oxygen consumption in fishes standardized to an adjusted weight were 0.00251 and 0.00528, respectively (0.050 and 0.073 standard error estimates, respectively). Variance between fish was significantly greater than the variance between replicated measurements (one-sided F-test of variances, $P<0.01$).

Discussion

Allometric relationship

Published allometric exponents (b-values) from the relationship describing size-dependent oxygen consumption in fish range from <0.40 to >1.00, but most often are 0.7 to 0.9 (Winberg 1956, Schmidt-Nielsen 1984). The b-value of 0.64 measured for *G. affinis* in the present study is within the range of published b-values for fish, but is lower

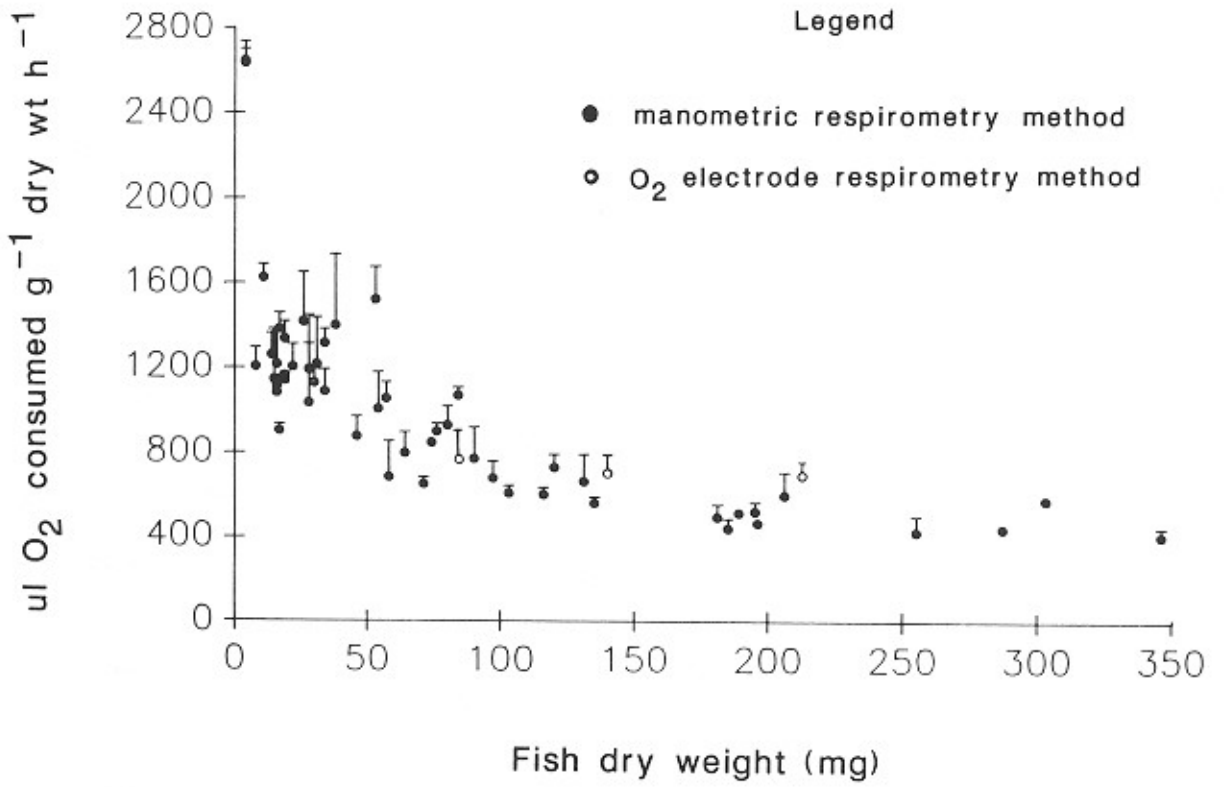

Fig. 2. Plot of the specific oxygen consumption rate (μl O_2 g^{-1} dry wt h^{-1}) versus dry weight of individual *G. affinis*. Each point and error bar represents the mean of two replicate measurements ± sd. N = 51. Error bars are not shown on points representing means whose sd was less than 12 μl O_2 g^{-1} dry wt h^{-1}.

than the 0.7 to 0.9 value typically reported.

Although the b-value observed in this study was lower than typical b-values reported for fish, we are confident in the results because of the following important considerations. First, a sample size of 51 *G. affinis* representing a 91-fold size range was utilized for the oxygen consumption measurements. Calder (1987) stated that the precision of the scaling exponent determination depends upon using a size range great enough to overcome the variability due to factors such as emaciation, obesity, ontogeny, age and experimental artefact within a size group. Kleiber (1961) observed that a ninefold size range was required to differentiate between b-values of 0.75 and 0.67. Secondly, replicated oxygen consumption measurement trials were performed on each experimental fish. Thirdly, the 95% confidence interval for the b-value was narrow (0.60–0.68). Fourthly, the specific oxygen uptake rates measured using the O_2 electrode respirometry method in three *G. affinis* were similar to the specific rates measured using the manometric respirometer technique with similar-sized *G. affinis* (Fig. 2). Finally, major environmental and experimental conditions were controlled or maintained as uniformly as possible throughout the experiment, thereby minimizing factors except body size that could influence oxygen consumption.

The wide variation in reported b-values makes comparison among studies difficult for two reasons. First, the variations in the b-values could represent actual intra- or interspecific differences, but could also merely reflect methodological differences or variations in the size ranges of organisms used in the studies (Calder 1987). Secondly, there is still no general agreement among physiologists regarding the physiological meaning, let alone actual values, of the allometric exponent (Heusner 1982).

Therefore, it is important to describe as thoroughly as possible the environmental and experimental conditions associated with the reported b-values in order to evaluate the consequence of size on oxygen consumption and to facilitate comparison between studies.

Specific oxygen consumption

There are few data on the oxygen consumption at 25–30° C by small size fish species weighing <2.0 g whole body weight, and the data are not expressed as specific oxygen consumption rates. We have taken data summarized by Cech et al. (1985) on fish wet weight and oxygen consumption (mg O_2 h^{-1}), and calculated the approximate specific oxygen consumption rates of the fish (μl O_2 g^{-1} h^{-1}) (Table 1).

The studies summarized in Table 1 report oxygen consumption data for fish only weighing approximately 200 mg wet wt. The specific oxygen consumption of similarly sized *G. affinis* (210–240 mg wet wt) in the present study were nearly equal to or approximately 3-fold less than those calculated for fish from the other studies. The present results are similar to the calculated specific oxygen consumption rate for *G. affinis* at 28° C as based upon the data of Cech et al. (1985). Also, the calculated specific oxygen uptake rates for *Oryzias latipes* at 25° C are similar to the rates observed in the present study for comparably sized *G. affinis* at 28° C. The 2-fold greater specific oxygen consumption of *G. affinis* calculated from the data of Maksudov (1940) may be partly attributable to the higher water temperature (31 versus 28° C), but may also result from methodological differences between the studies.

Long-term time effect on oxygen consumption

The acclimation period of seven days in the laboratory appeared to be sufficient for the *G. affinis* because no effects of analysis day on oxygen consumption were observed in the analysis of covariance (fish dry weight as the covariate). Thus, oxygen consumption in the fish did not appear to change significantly during the 17 d measurement period due to any time-related factors.

Precision of manometric respirometry technique

The manometric respirometry technique provided precise measurements of replicated oxygen consumption in *G. affinis*. Precision was exemplified by a 2-fold, significantly greater ($P<0.01$) variance

Table 1. Specific oxygen consumption rates that were calculated for 200–240 mg wet weight size range fish from several species at water temperature ≥25° C. Most data are approximate, as they have been calculated from the original regression equations or determined from graphical presentations and summarized by Cech et al. (1985).

Species	Temp °C	Body wet weight (mg)	Calc[1] dry wt mg	O_2 consumption fish^{-1}		Specific O_2 consumption μl O_2 g^{-1} dry h^{-1}	Reference
				mg h^{-1}	μl h^{-1}		
G. affinis	31	230	64	0.216	151	2274	Maksudov 1940 (as shown in Winberg 1956)
G. affinis	28	~200	56	0.092	64	1103	Cech et al. 1985
Oryzias latipes	25	200	56	0.08–0.14	56–98	971–1700	Umezawa & Watanabe 1973
Fundulus heteroclitus	25	240	67	0.28	196	2841	Targett 1978
G. affinis	28	210–240	True dry wt 54–76	–	54–68	684–1054	This study

[1] Dry weight calculated from the empirical relationship between *G. affinis* dry wt and wet wt: dry wt = 0.292 (wet wt in mg) −2.70.

component of log oxygen consumption between fish standardized to an adjusted weight, compared to the variance component of replicated measurements of the log oxygen consumption in fish of the same weight. Thus, the manometric respirometry method can be used to obtain consistent oxygen consumption data for *G. affinis* if the environmental conditions are uniform and carefully controlled throughout the study.

The manometric respirometry technique has several operational advantages over the O_2 electrode respirometry method. For example, oxygen consumption measurements are logistically easier to make and more quickly obtained using the manometric method. Also, an O_2 electrode requiring frequent, careful calibration is not required for the manometric technique.

Therefore, additional studies would be useful in which replicated oxygen consumption measurements for other small-sized fish species are determined by the manometric respirometry technique, and then are compared to oxygen consumption measurements determined by the O_2 electrode respirometry method. Such studies will provide further data on the precision and accuracy of the manometric respirometry method, thereby helping to define the utility of the technique in determining size versus oxygen consumption relationships for small fish.

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