OXYGEN CONSUMPTION IN THE FATHEAD MINNOW (*PIMEPHALES PROMELAS* RAFINESQUE)—I.

EFFECTS OF WEIGHT, TEMPERATURE, GROUP SIZE, OXYGEN LEVEL AND OPERCULAR MOVEMENT RATE AS A FUNCTION OF TEMPERATURE[1]

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Abstract—1. A relationship is evident between oxygen consumption (OC in cm^3/hr) and weight (WT in g) such that:

$$OC = -0.580 \, (WT)^{1.053}.$$

2. Fathead minnows failed to exhibit immediate rate compensation as a result of acute temperature changes. Minnows showed an exponential increase in weight-specific oxygen consumption with temperature (in °C), resulting in a Q_{10} of over three. The equation is:

$$OC = 10^{0.0482} \, (Temp.) - 1.268.$$

3. Minnows acclimated to water temperatures of 5–21°C showed a steady increase in opercular movement rates (OMR) (ventilation rates) as expressed by the equation:

$$OMR = 0.1968 \, (Temp.)^2 + 1.064.$$

4. Grouping more than two fish per chamber resulted in an increase in oxygen consumption. The relationship of group size (GS) to oxygen consumption is:

$$OC = 0.04059 \, (GS)^2 - 0.2017 \, (GS) + 0.5353.$$

5. Oxygen consumption is a function of dissolved oxygen level. This relationship is shown by the equation:

$$OC = 0.003049 \, (OL)^3 - 0.06359 \, (OL)^2 + 0.4211 \, (OL) - 0.4020$$

where OL is oxygen level. Due to high variability, it is statistically impossible to determine oxygen level ranges in which they conformed and those in which they regulated.

INTRODUCTION

Many factors exhibit a marked effect on metabolic rates. Some of these include: ambient temperature, time of day, season, sex, age, level of activity, weight, light level, etc. Also, oxygen consumption of aquatic vertebrates may depend upon ambient pH, osmotic pressure, existing dissolved oxygen levels, group size, etc. This is the first in a series of papers dealing with effects and relationships of most of the above parameters to the bioenergetics of the minnow, *Pimephales promelas*.

Oxygen consumption has long been considered a valid measure of metabolism. It is also generally accepted that concentrations of dissolved oxygen affect the metabolic rates of aquatic animals (Kusins & Mangum, 1971).

Most energetics studies consist of only one or two variables. This is unfortunate when one considers that metabolism is a function of several simultaneous internal and external parameters. The purpose of this and subsequent studies is to account for as many of

these conditions as possible, in order to get a more complete picture of the organism's metabolism.

MATERIALS AND METHODS

Minnows (*Pimephales promelas* Rafinesque) used in this study were obtained commercially from the stock of a local bait shop, during March and April, 1977. They were maintained in aerated 30 gallon aquaria at room temperature (21°C ± 1°C), and were fed commercial fish food at regular intervals. After acclimation to these laboratory conditions for at least 1 week prior to testing, minnows were exposed to a cycle of 12 hr light and 12 hr dark (centered on 7:00 a.m.) at an average light intensity of 1.5 W/m², measured at the experiment vessel surface. All testing was conducted between the hours of 1.00 and 5.00 p.m. in order to avoid variation due to diurnal fluctuations. Essentially all of the water was obtained along with the fish from the bait shop, and used in the studies.

The amount of oxygen consumed was determined at the end of 2 hr runs by the Winkler method, as outlined by Hoar & Hickman (1967), using a Gilmont 2.0 ml micrometer buret (Roger Gilmont Industries, Inc., Great Neck, NY). In each study the parameters monitored were: pH, osmotic pressure, light level, weight, group size, original dissolved oxygen level, and temperature. This was done in order to make allowances for differences due to

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factors other than the variable being tested, and to provide information for a later multiple regression analysis. All experiments in this study were conducted at an average light intensity of 1.5 W/m². Weights were determined by difference using a Mettler model P1200 top loading scale. pH was determined with a Fisher model 420 digital pH/ion meter. Surface light levels were ascertained using a YSI-Kettering model 65A radiometer light meter. Temperature readings were taken from a standard Celsius thermometer. Osmotic pressure was measured with an Advanced Instruments Osmometer model 3W, using 0.2 ml samples of water. In each study, controls were run under identical conditions to determine natural oxygen use or increase due to microscopic organisms. Experiment vessels used were Mason quart jars of known volume. These transparent jars were arranged so as to isolate the fish from visual stimuli outside the vessel, which might have influenced activity. Regression analyses were performed on a Dual IBM 360/67 system. Statistical significance was defined as $P < 0.05$. The paragraphs following serve to explain in further detail the conditions of each specific test.

Weight

Minnows ranging in weight from 0.47 to 2.23 g were used.

Temperature

Six fish were used at each of six different temperatures, chosen in 5° increments from 2 to 27°C. All of the minnows had been previously acclimated to 21°C (and had not been fed for at least 12 hr prior to experimentation). Jars were preadjusted to the desired experimental temperature.

Group size

Six jars were filled with aerated aquarium water. Groups of 1–5 fish were then sealed within these jars. It was known that one fish would have sufficient oxygen to survive a 2 hr period, but the limited oxygen supply would not sustain a larger group for the 2 hr run. Consequently, the amount of time spent in the jar was reduced with increasing group size, so that fish-hr remained constant at two (thus, two fish spent 1 hr, three fish remained 40 min. etc.).

Oxygen level

Before the effects of oxygen level were tested, a standardization curve was constructed by bubbling oxygen and nitrogen through each of two separate 5 gallon containers. Oxygen content was calculated for samples taken every 30 min from each container. In the actual run, minnows were not acclimated to the new oxygen levels, but were introduced into jars filled with water that had been aerated with nitrogen or oxygen for varying time spans.

Opercular movement rate

Minnows were maintained in an aerated aquarium inside a water bath. Changes in the temperature of the aquarium water were effected by regulating the temperature of the water bath. Temperatures below ambient were accomplished by adding ice to the water bath. The fish were allowed to acclimate to a given temperature for 2 or 3 days. Testing originated at 5°C, with additional tests conducted at 10, 13, 18 and 21°C temperatures. Twelve fish, each chosen at random, were sampled at each temperature. Opercular movements were counted and recorded for 1 min.

RESULTS

For all additional studies reported in this paper, except those dealing with opercular movements and temperature, oxygen consumption has been weight-adjusted. In other words, a corrected weight-specific

oxygen consumption has been obtained as a function of the variable being studied.

Weight

Regression analysis of the data obtained yielded a linear relationship when transformed to log/log axes. The relationship between weight and oxygen consumption is illustrated by:

$$Y = -0.580 \, (X)^{1.053}.$$

where Y is oxygen consumption in cm^3/hr, and X is weight in grams. This transforms into:

$$\log OC \, (cm^3/hr) = -0.580 + 1.053 \, (\log WT) \, (g),$$
$$(\pm 0.003) \quad (\pm 0.204)$$

where values in parentheses are standard errors for the intercept and slope. ($F = 114.038$, d.f. 1 and 25, $P < 0.001$, and $r^2 = 0.82$.)

Temperature

Regression analysis of data resulted in a good curvilinear fit. Logarithmic transformation gave a linear relationship of weight-specific oxygen consumption vs temperature. The equation for this relationship is:

$$\log OC = 0.0482 \, (Temp.) - 1.268,$$
$$(\pm 0.051) \quad (\pm 0.071)$$

where OC is oxygen consumption $(cm^3/g^{1.053}$ per hr), and Temp. is temperature in °C. ($F = 381.270$, d.f. 1 and 33, $P < 0.001$, and $r^2 = 0.920$.)

Group size

As can be seen in Fig. 1, a possible optimal group size for fathead minnows was indicated by plotting the reduced data. Calculation of regression analysis gave the following equation:

$$OC = 0.04059 \, (GS)^2 - 0.2017 \, (GS) + 0.5353,$$
$$(\pm 0.012) \quad (\pm 0.074) \quad (\pm 0.0996)$$

where OC is again oxygen consumption (see above) and GS is Group Size ($F = 10.7$, d.f. 1 and 28, $P < 0.001$, and $r^2 = 0.4515$). Figure 1 shows the graph of the regression line. Analysis of OC (SNK test) showed no significant difference as a function of GS. Since the slope of the line contains a minimum, we are able to calculate an optimal group size. Taking the first derivative of the regression line will yield this optimum (Studier et al., 1975) as 2.48 fish/vessel or 1 fish/403 cm^3.

Oxygen level

Oxygen consumption decreased with a decrease in existing dissolved oxygen level, and increased as existing levels increased. The regression equation is:

$$OC = 0.003049 \, (OL)^3 - 0.0636 \, (OL)^2$$
$$(\pm 0.002) \quad (\pm 0.043)$$
$$+ 0.421 \, (OL) - 0.4020,$$
$$(\pm 0.246) \quad (\pm 0.393)$$

where OC is oxygen consumption and OL is oxygen level $(cm^3$/l). ($F = 4.818$ with 1 and 28 d.f.; $P < 0.05$; $r^2 = 0.3664$.) Based upon a graph of the regression equation (Fig. 2), the minnows appear to be conformers below 5 cm^3/l and regulators above. There are probably other factors that prevent a clear demonstration of a breaking point (see Discussion).
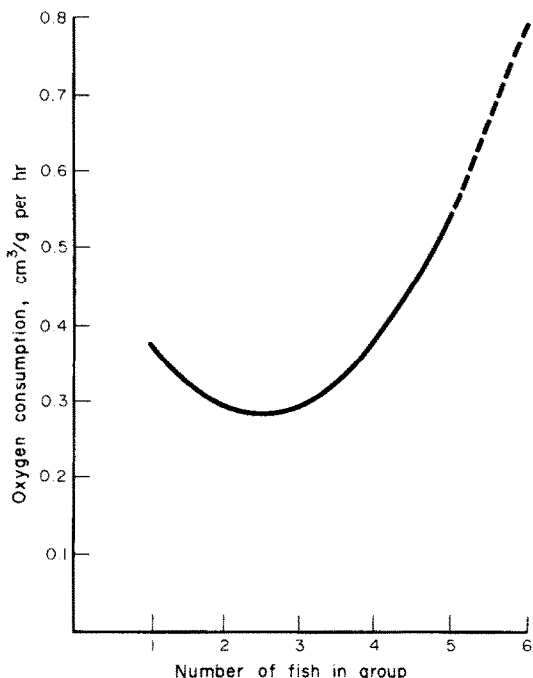
Fig. 1. Oxygen consumption of varying group sizes of fish (*Pimephales promelas*).

Opercular movement rate

An extremely strong direct relationship is indicated between opercular movement rates (OMR) and temperature. This is easily seen in Fig. 3. The equation obtained is:

$$OC = 0.197 \, (OMR)^2 + 1.084,$$

where OC is oxygen consumption ($cm^3/g^{1.053}$ per hr), and OMR is opercular movement rate (movements/min). ($F = 588$ with 1 and 58 d.f.; $P < 0.001$; $r^2 = 0.910$.)

DISCUSSION

These studies have attempted to determine metabolic rate of fathead minnows as a function of several parameters. In considering metabolic rate, it should
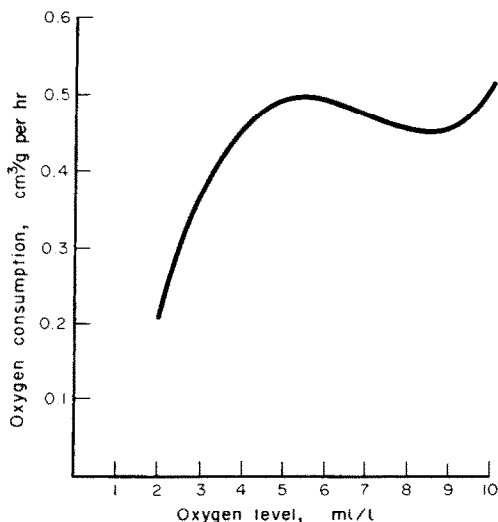

Fig. 2. Oxygen consumption of individual *Pimephales promelas* at various ambient oxygen levels.
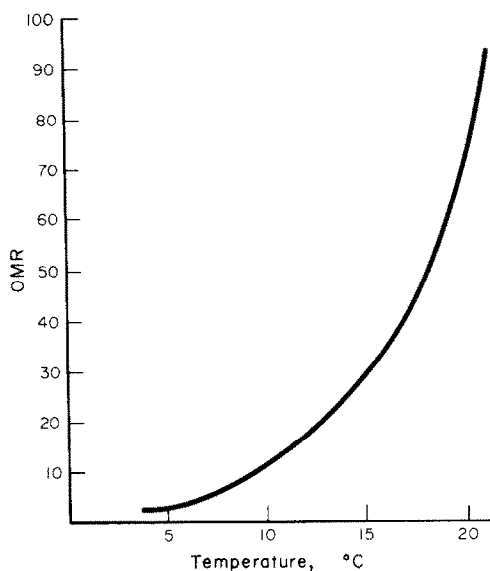
Fig. 3. Opercular movement rates of individual *Pimephales promelas* at varying ambient temperatures.

be noted that maximal metabolic rate represents a stressful situation, and not an optimal condition. An optimal condition is that which does not require the organism to expend a maximal amount of energy in maintaining its existence.

Weight

The results indicate the expected linear relationship of oxygen consumption to weight on a log–log coordinate system; however, the value of the respiration coefficient is higher than most reported values for fish (Table 1). It is also evident that weight-specific oxygen consumption would increase, rather than decrease, with an increase in weight.

Our data might seem to conflict with all of the values in Table 1. If one takes into account the confidence limits associated with the value of our respiration coefficient, though it is still at the upper end of the values reported, it is in closer agreement with those results.

It is not unusual to see extremely variable values for the respiration coefficient, even among related species (Fitch, 1975; Barnes & Barnes, 1969). It has been suggested by Brody (1945) and Zeuthen (1953) that a value of 0.67 is appropriate for homeotherms. Hemmingsen (1960) found 0.75 ± 0.015 (S.E. of slope) was not unusual for poikilotherms. While it can be seen that several of the values listed in Table 1 are in close agreement with Hemmingsen, it will also be noted that several are quite noticeably in conflict with his findings. Prosser & Brown (1961) make a broad generalization that values may range from 0.67 to 1.0. The lower value is an expression of the surface area to volume rule which suggests that surface area varies to the 2/3 power of body size. The upper limit of 1.0 would indicate a complete dependence of metabolic rate on body size. Almost all values have been found in this range, or below it, and a value that is less than 1.0 would show weight-specific metabolic rate decreasing with increasing size.

The only exception we were able to find in the literature was documented by Beamish (1964; see

Table 1. Value of the respiration coefficient

Organism	Temp. (°C)	Mean wt (g)	Resp. coeff. (k)	Reference
Brown trout	10	216	0.877	
Brook trout	10	102	1.107	
Brook trout	15	64	1.014	
Brook trout	20	112	1.036	
White sucker	10	72	0.994	
White sucker	15	70	0.828	
White sucker	20	95	0.770	Beamish (1964)
Brown bullhead	10	116	0.998	
Brown bullhead	15	147	0.903	
Brown bullhead	20	115	0.874	
Carp	10	174	0.983	
Carp	15	146	0.909	
Carp	20	106	0.876	
Goldfish			0.850	Beamish & Mookherjii (1964)
Starry flounder			0.86	
Lemon sole			0.84	Hickman (1959)
Sanddab			0.90	
Dogfish			0.74	Pritchard (1958)
Bacteria and Protozoa			0.70	Zeuthen (1953)
Small metazoa			0.95	
Large poikilotherms and homiotherms			0.75	Hemmingsen (1960)

Table 1), and he attributes it to the fact that one of the smaller fishes studied resulted in a respiration coefficient greater than 1.0, which to him indicated a phylogenetic increase in metabolic rate in smaller animals, even though it does not follow ontogenetically. Extrapolation of this argument would suggest that species as small as the fathead minnow would indeed show a larger respiration coefficient. Zeuthen (1953), however, asserted that it must be recognized that values greater than 1.0 are exceptions to the rule. He stated that values greater than 1.0 are only valid for a very narrow weight range. Our study used a weight range large enough that the largest fish weighed nearly five times more than the smallest. This aptly argues Zeuthen's precept. Since we did not age fish used in our study, ontogenetic differences may be implied. Zeuthen (1953) also contended that respiration coefficients greater than 1.0 could easily result from changes in factors other than size. We attempted to maintain all extraneous environmental conditions fairly constant to alleviate this problem. These factors will be examined in a final multiple regression analysis. One aspect which was not controlled, nor accounted for, was activity. Fox (1936) and Newell (1973) have shown oxygen consumption in relation to body size in intertidal invertebrates and fish to be affected by activity. The possibility of this being true for fathead minnows as well, could explain the discrepancy between this study and those of other authors. Fathead minnows appear to be normally quite active, swimming almost constantly, and due to their small size, their activity levels were not inhibited by the quart jar containers used. Thus, we consider that activity may be an important determinant of the fish's metabolism. A discussion of the effects of activity is planned for a later article.

If it is true that the respiration coefficient is greater than 1.0 because of activity, then we can consider two possible interpretations. We know that the increase is greater for each incremental increase in size and this would imply that the relative activity in-

creases with increasing size. Alternatively, a similar level of activity might result in larger weight-specific oxygen demands in the larger fish. We would also consider that with the range offered by our confidence limits, the respiration coefficient might well be 1.0 or slightly below. A larger sample size and more controls on activity will help to make this issue much clearer.

Weymouth et al. (1942), in studies on the kelp crab found 96% of the variance in oxygen consumption due to body weight, with only 4% due to all other factors.

Our study resulted in a large coefficient of determination, indicating a high degree of predictability. Therefore, by use of the equation produced, the effect of weight was eliminated as a variable in the other studies conducted.

Temperature

Prosser & Brown (1961) have stated that standard metabolism of fish increases continuously with temperature up to lethal levels, suggesting a rate change of 2.5 times per 10°C in the physiological range. Our study gives a rate change of 3.0 times per 10°C. A crude approximation yields a Q_{10} of about three. This is slightly higher than that suggested by Prosser & Brown (1961). Beamish (1963) stated that freshwater carp have an apparent overall Q_{10} of about two between 10 and 20°C. Brook trout show a similar Q_{10} over this same range (Graham, 1949). Even the saltwater fish used in Merriman's study (1970), Bathygobius ramosus, has an overall temperature coefficient only slightly larger than two. We are able to conclude from this that these minnows appear to exhibit a metabolic rate which is more temperature labile than has been observed in many other species of fish. Though there are some exceptions (see Roberts, 1964), temperature compensation is a rare phenomenon among the species of fish which have been studied. It seems apparent that the enzymes of the fathead minnow are notably resistant to thermal denaturation over a relatively broad temperature range.

Hochachka & Somero (1973) believe most biological reactions to occur in a range of Q_{10} values between two and four. Considering this, our Q_{10} of approximately three does not seem unreasonable, though remarkable.

At 27°C, the rate of oxygen uptake diminished as oxygen levels of the water decreased. This phenomenon is so general among fish as to be called "respiratory dependence". Several authors (Graham, 1949; Hall, 1930; Maloeuf, 1937) have reported this occurrence for several species of fish. For many fish, respiratory dependence occurs when the oxygen content drops below approximately 5 ppm (Gibson & Fry, 1953). A more detailed discussion will be found in the section dealing with effects of oxygen level on metabolism.

This study supports the belief that for most fish, and for many ectotherms, weight-specific oxygen consumption increases exponentially with increasing temperature, at least within the critical survival limits of the organism. In particular, fathead minnows exhibit no metabolic compensation over the temperature range studied, and indeed, show a relatively high Q_{10}.

In connection with temperature, an investigation of opercular movement rates (OMR) between 5 and 21°C was conducted. OMRs were found to be temperature labile. We assume minute ventilation volume to be equal to OMR times the volume per movement (considered to be some constant, k), and, therefore, OMR is directly proportional to minute ventilation volume. We also made the assumption that OMR was related to the partial pressure of oxygen.

It was expected that more opercular movements would be necessitated by the meager amounts of oxygen available in warmer waters. Temperature effects on oxygen solubility influence oxygen uptake adversely by two routes: first, by reducing the diffusion coefficient, and second, by reducing total oxygen content. Both demand an increase in ventilation. Increasing ventilation, however, reduces the thickness of the water film over the surface of the secondary lamellae, which provide the major resistance to gas exchange (Hills & Hughes, 1970). Increased rate of oxygen uptake during accelerated metabolic activity should be the result of a proportional increased oxygen gradient between the blood and water (Steen & Kruysse, 1964). Computation of oxygen consumption using the temperature equation, and calculation of OMR using that respective equation, gave a linear relationship when OC (oxygen consumption) was plotted as a function of OMR. Thus, ventilation volume appears to be connected with temperature and OC can be predicted from OMR.

Group size

Beyond two fish per jar, the number of fathead minnows contained in a constant volume of water affects the amount of oxygen consumed per gram of fish. These results are contrary to the bulk of previously reported research. Malyukina *et al.* (1962), Grigor'eva (1966) and Shtefanesku (1958) all reported an inverse relationship of group size to oxygen consumption. Several species were examined, and group effects were found even for those species not given to schooling in the natural habitat. Allee (1931) in summarizing research to that date, assumed group

effect to lower oxygen consumption of group members. Schuett (1933) found group effects in gold fish (*Carassius auratus*), not a noticeably social animal; he was, however, able to obviate the effect by maintaining constant volume per fish with increase in numbers. Bowen (1932) found no significant dissimilarity between grouped and isolated catfish (*Ameriurus melas*), but felt the results were due to increased activity in the grouped animals.

Here, the fathead minnow, a schooling fish by nature, is found to increase oxygen consumption when surrounded by schoolmates. This would indicate an increased activity on some level. They were not observed to be abnormally active when grouped. Conversely, isolated fish swam more in their vessels than did grouped individuals, intimating an increased demand for oxygen due to some unobserved parameter of metabolism.

Russian scientists (Grigor'eva, 1966; Shtefanesku, 1958) found in some species that olfactory cues are important in determining schooling behavior. Fish were observed to show the same lowered oxygen utilization when isolates were kept in water previously occupied by large numbers of conspecifics. Species exhibiting this effect most markedly were those whose habitat is relatively turbid and stagnant. In this study, while water and fish in all conditions were maintained in separate holding tanks between set-ups, water was not discarded between runs, nor replaced between this and other segments of this experiment. Residual olfactory cues from conspecifics could possibly have accumulated between runs, such that additional fish present in "sweaty" water could have been perceived as over-crowding. Isolated fish would, in this case, be most "uncrowded", and least likely to increase their metabolic rate.

Alternatively, reduction of range of oxygen uptake in response to increased group size could intimate that fathead minnows have an optimal metabolic rate greater than that shown by isolates, and that the presence of conspecifics elevates an individual's rate toward that level. In this event, isolates would have to vellicate much more, to increase their oxygen consumption, and some individuals would be more accomplished than others at this. Aggregates could diminish motor activity, in that stimuli provided by conspecifics trigger a rate increase directed at that level which enables the school to respond appropriately to this environment. The results of this study do not allow the conclusion that fathead minnows have been a schooling species long enough for it to be metabolically more efficient for the fish to school.

Oxygen level

Based upon Fig. 3, fathead minnows appear to make a transition between conforming and regulating at a dissolved oxygen level of about 5 cm³/l. Walshe (1947) subjected species of aquatic insect larvae to low oxygen levels and observed oxygen consumption to decline rapidly, the relationship between oxygen consumption and oxygen level being roughly linear. Walshe dealt with aerated water, as opposed to stagnant water. We assumed fathead minnows to inhabit an environment approximating that of these larvae. We therefore thought our findings would be correlative. Snails not exposed to meager oxygen levels exhi-

bited partial regulation at higher oxygen levels (Kusins & Mangum, 1971). These minnows suggested partial regulation at escalated oxygen levels. Our results showed fathead minnows to be neither true conformers nor regulators, but partial regulators and conformers, depending on the oxygen level.

Only information pertaining to partial pressures of carbon dioxide was found for fish, and not direct effects of oxygen levels. Beamish (1964) assumed respiratory volume of fish in elevated CO_2 situations to rise above that of fish in air-saturated water, consequently raising respiration costs and therefore the rate of oxygen consumption. Beamish (1964), however, demonstrated the standard rate of oxygen consumption to be insignificantly changed in various concentrations of CO_2.

Walshe (1947) found metabolism in trout directly related to current velocity. This seems plausible when one considers aeration as a function of velocity. This could well be related to the motor activity required to maintain position in a current. Activity will be studied as a separate variable in a later work.

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