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Specific oxygen consumption in two life stages of the cichlid fish *Cichlasoma nigrofasciatum* ☆

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Abstract

The specific oxygen consumption of the Convict cichlid (*Cichlasoma nigrofasciatum*) has been determined in two life stages, the young stage (before reaching the reproductive state) and the adult stage (reproductive state). Three clearly distinguishable oxygen consumption rates were observed in the young stage during the first 266 days of life. The oxygen consumption relative to body weight (BW) between these three phases differs significantly (average and standard deviation given): Phase 1, (0.407 ± 0.106) mg $O_2\,h^{-1}\,g^{-1}$ BW (age 40–60 days); Phase 2, (0.343 ± 0.113) mg $O_2\,h^{-1}\,g^{-1}$ BW (age 76–236 days); and Phase 3, (0.266 ± 0.057) mg $O_2\,h^{-1}\,g^{-1}$ BW (age 256–266 days). The oxygen consumption drops significantly to (0.132 ± 0.051) mg $O_2\,h^{-1}\,g^{-1}$ BW in the adult stage. No significant effect of body weight on the specific oxygen consumption was observed when the age was taken into account.

Keywords: *Cichlasoma nigrofasciatum*; Cichlid; Development; Fish; Indirect calorimetry; Oximetry

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1. Introduction

Indirect calorimetry is still the preferred method in comparison with direct calorimetry for metabolic studies in fish because it is relatively easy to handle, comparatively inexpensive, and gives reliable results within certain limits.

Animal development, growth and aging are accompanied by a decrease of the metabolic rate (MR) [1]. In most higher organisms the growth rate, maximum body weight and body size of an individual are limited, determined mainly by genetic factors and age. The variation of these parameters between individuals of the same species is low and the influence of environmental factors is rather limited. The major turning point in the growth rate occurs with the maturation of an individual. Because of their close link, the morphological parameters and MR can be used for age determination of an individual. However, there is one major exception. Fish generally have an indeterminate growth capability, which permits greater phenotypic adjustment to environmental factors such as food availability and crowding [2] and environment size [3]. Moreover, for certain species such as the Convict cichlid (*Cichlasoma nigrofasciatum*) a large variation in growth rate has been observed among individuals of the same age [4]. It is thus impossible to estimate the age of a fish based on the morphological parameters mentioned. The only parameter left which is linked to age is the total MR of the fish, as in any known living organism. The MR is expected to give a good estimate, not only for the determination of the overall physiological condition, but also useful information about the age of a fish. A prerequisite for this kind of age determination is a detailed knowledge and record of the MR and other growth parameters during the individual development of a certain species. The nature and significance of modifying factors have also to be determined.

In this paper, the oxygen consumption measured in the young stage (the growing state before reaching the reproductive state) and the adult stage (reproductive state) of the Convict cichlid are presented. One aim of this work is the acquisition of reference data and development of procedures under standardized conditions for further experiments investigating the influence of increased UV-A/UV-B intensities on the growth, development and general physiological parameters of aquatic organisms.

2. Experimental

2.1. Fish

The young fish used for measurement were offspring obtained in a single breeding in the laboratory from a single parent. The fish were grown in a 40 l aquarium equipped with a pump and physical–biological filters with a 14 h light period (TL 15 W, solar-spectrum imitation), aeration during the 10 h dark period, and a thermostat to give a constant temperature of $(28 \pm 1)^\circ$C. The number of fish at the beginning of our experiment (40 days old) was 30, and it declined to 13 at the end of experiment (266 days old). Size distribution from 159 observations varied from 0.02 to 4.98 g in weight and from 1.3 to 6.6 cm in total length. The adult fish, which were obtained from a local dealer, were kept in a 60 l aquarium with the same environmental conditions as for the young. The size of the adults varied from 7.5 to 11.7 cm in total length and 8.5 to 43.5 g in weight. Commercial dry food was given 2 or 3 times daily in sufficient amount. Water quality was monitored and kept stable.

2.2. Equipment

Two types of oximeter were used for the measurement of total oxygen consumption. A Drägerwerk oximeter (Drägerwerk, Lübeck, Germany) was used for measuring the total oxygen consumption on the 40–145 day old offspring in a 4.6 ml measuring chamber of diameter 22 mm. This unit measures the partial pressure of oxygen (given in Torr, 1 Torr = 0.133 kPa), and is designed mainly for medical applications. Total oxygen consumption of the 139–266 day old offspring and the adult fish was determined with a WTW-Oximeter (WTW, Weilheim, Germany), which measures the oxygen concentration in solution (given in mg l^{-1}). Three different sizes of cylindrical measuring chamber, with volumes of 39 ml (diameter 48 mm), 122 ml (68 mm) and 562 ml (117 mm) were used for the different sizes of fish measured with the latter type of oximeter. The animal chamber was designed to be large enough for the fish to move but small enough to limit the duration of the experiment (not more than 3 h).

2.3. Procedures

Zero calibration of the oxygen electrode membrane in water was carried out prior to measurement by adding ≈10% of $Na_2S_2O_4$ (0% O_2). Experiments were carried out in a closed system at room temperature (min–max 20.5–28.6°C; 87% of the data were obtained between 22 and 26°C). The fish were acclimatized in an open reference chamber at room temperature for 1–6 h before measurement. The last feeding was given a minimum of 1 h before acclimation. The average of the initial and final oxygen tension in the measuring chamber, as measured with the Drägerwerk oximeter, was (13.9 ± 5.4) and (2.9 ± 0.8) kPa, respectively. The initial and final oxygen concentration in the measuring chamber measured with the WTW oxymeter was (7.7 ± 1.1) and (1.1 ± 0.3) mg l^{-1}, respectively.

The oxygen consumption (mg O_2 h^{-1} g^{-1}) measured with the Drägerwerk oximeter was calculated from the formula

$$nO_2 = \frac{(pO_{2i} - pO_{2f})}{pO_{2i}} \cdot C^* \cdot V \cdot H^{-1} \cdot W^{-1}$$

where pO_{2i} and pO_{2f} are the initial and final partial pressures of oxygen in the measuring chamber, in kPa, C^* is the concentration of dissolved oxygen in water at a certain temperature, in mg l^{-1}, adopted from [5], V is the volume of the animal chamber in litres, H is the duration of the experiments, in h, and W is the body weight in g.

The oxygen consumption (mg O_2 h^{-1} g^{-1}) measured with the WTW oximeter was calculated from the formula

$$nO_2 = (cO_{2i} - cO_{2f}) \cdot V \cdot H^{-1} \cdot W^{-1}$$

where cO_{2i} and cO_{2f} are the initial and final oxygen concentrations in the measuring chamber, in mg l^{-1}, V is the volume of the animal chamber, in litres, H is the duration of the experiment, in h, and W is the body weight in g.

The oxygen concentration measured with the WTW oximeter is automatically corrected for the environmental temperature and the atmospheric pressure.

3. Results

All 159 observations reported here were made out on a pool of young fish from a single breeding event over the growth period of 40–266 days. The age of a young fish was thus exactly known at the time of measurement. In addition, the development of some individual fish could be followed for a certain period. The specific oxygen consumption rates of all observations are shown in Fig. 1. The specific oxygen consumption (shown as open squares and a line) varied from 0.118 to 0.938 mg O_2 h^{-1} g^{-1}, with an average value of 0.352 mg O_2 h^{-1} g^{-1}. The linear trend of the line of the specific oxygen consumption shows a tendency for a declining MR with increasing age. A large variation of body weight (shown as columns) was observed among individuals of the same age.

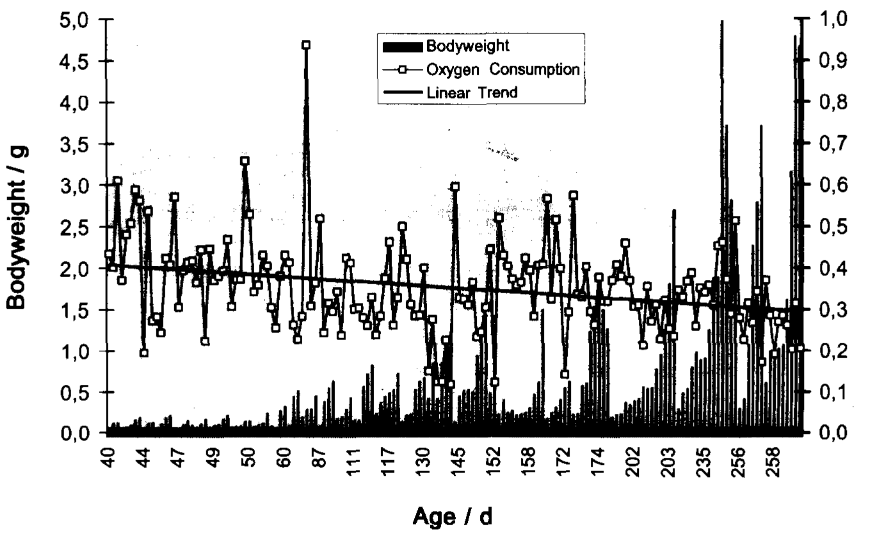

Fig. 1. Distribution of the specific oxygen consumption (represented by open squares and a line) and body weight (represented by columns) over 159 observations in the growing fish from 40 to 266 days of age. The straight line among the squares shows the linear trend of the specific oxygen consumption with increasing age.

Table 1
Summary output of multiple regression analysis to detect relationship between "specific oxygen consumption" and the independent variables "age", "body weight" and "temperature" in young and adult stages of the Convict cichlid

Variables	Young			Adult		
	Coefficients	Std. error	*t*-Value	Coefficients	Std. error	*t*-Value
Multiple regression with three independent variables:						
Intercept	0					
Age (days)	−0.0005	0.0002	3.272 [a]	nt [b]	nt	nt
Body weight (g)	−0.0055	0.0123	0.444	nt	nt	nt
Temperature (°C)	0.0177	0.0008	21.123 [a]	nt	nt	nt
Multiple regression with two independent variables:						
Intercept	0					
Body weight (g)	−0.0304	0.0100	3.047 [a]	−0.0009	0.0014	0.674
Temperature (°C)	0.0154	0.0005	33.028 [a]	0.0065	0.0012	5.561 [a]

[a] $P < 0.001$. [b] nt = not tested.

In adult fish, the specific oxygen consumption obtained from 10 fish (19 observations) varied from 0.046 to 0.259 mg O_2 h^{-1} g^{-1}. The average and the standard deviation obtained from all measurements was (0.132 ± 0.051) mg O_2 h^{-1} g^{-1}, ≈60% lower than the average value obtained in the young fish. For four females and six males examined, no significant difference of oxygen consumption between the sexes was observed.

Multiple regression analysis has been carried out to detect relationships between "specific oxygen consumption" and the variables "age", "body weight", and "experimental temperature" in both life stages. Because the age of adult fish is very difficult to determine (see above), this variable was excluded from calculation in the adult stage. The experimental temperature was included for analysis because of its strong influence on the MR, as shown previously [6]. The summary output data of the regression statistics are shown in Table 1. When we tested the relationships of the specific oxygen consumption with all three variables, the specific oxygen consumption of young fish was significantly affected by experimental temperature and age (t-test, $P < 0.001$ for both variables). A significant effect of body weight on the specific oxygen consumption was obtained in the young state (t-test, $P < 0.001$) when the variable "age" was excluded.

In the adult stage, the specific oxygen consumption was significantly affected by temperature ($P < 0.001$). No significant effect of body weight on the MR was observed.

The positive values of the regression coefficient of the variable "temperature" observed in both life stages indicated that the oxygen consumption increases with higher temperature. The partial regression coefficient of the variable "age" in the young stage was negative. This indicates that the specific oxygen consumption declines with increasing age.

Table 2
Oxygen consumption in young fish and statistical comparison for different age classes

Age class/ (days)	Oxygen consumption/ ($mg\ h^{-1}\ g^{-1}$)	Student's t-test					
		40–60	76–117	130–158	172–203	235–236	256–266
40–60	0.407 ± 0.106 ($n = 41$)	×	1.973	2.963 [a]	2.646 [a]	1.289	4.858 [b]
76–117	0.347 ± 0.142 ($n = 26$)		×	0.522	0.165	0.510	2.104 [c]
130–158	0.329 ± 0.119 ($n = 33$)			×	0.451	1.125	1.950
172–203	0.342 ± 0.097 ($n = 30$)				×	0.896	2.768 [a]
235–236	0.368 ± 0.069 ($n = 14$)					×	4.318 [b]
256–266	0.266 ± 0.057 ($n = 15$)						×

[a] $P < 0.01$. [b] $P < 0.001$. [c] $P < 0.05$.

The MR data collected continuously from the young stage were grouped into six age classes, each class covering a period of 20–40 days. The average oxygen consumption in each age class and the values of Student's t-test calculated between age classes are shown in Table 2. Significant differences (t-test, $P < 0.05$ to $P < 0.001$) were obtained in three out of five comparisons of the 40–60 day age class with the other age classes, and in four out of five comparisons between the 256–266 day and the other age classes. The t-values obtained from comparisons between 40–60 day and 76–117 day and between 130–158 day and 256–266 day fish were significant at the 90% confidence level. No significant differences were obtained from comparisons between age classes within the range 76–236 days. Also surprisingly, no significant difference was detected in the comparison between 235–236 days and 40–60 days.

We conclude that there are at least three distinguishable phases of oxygen consumption during the first 266 days of life in this fish. The first phase was observed at age 40–60 days with an average consumption of (0.407 ± 0.106) mg O_2 h^{-1} g^{-1}. The second phase, with an average of (0.343 ± 0.113) mg O_2 h^{-1} g^{-1}, ranged from 76 to 236 days. The third phase, with an average of (0.266 ± 0.057) mg O_2 h^{-1} g^{-1}, ranged from 256 to 266 days.

4. Discussion

Fish metabolism is influenced by several parameters, such as temperature [6–8], oxygen tension [9–11], food intake and crowding [12], starvation [13], activity [14]

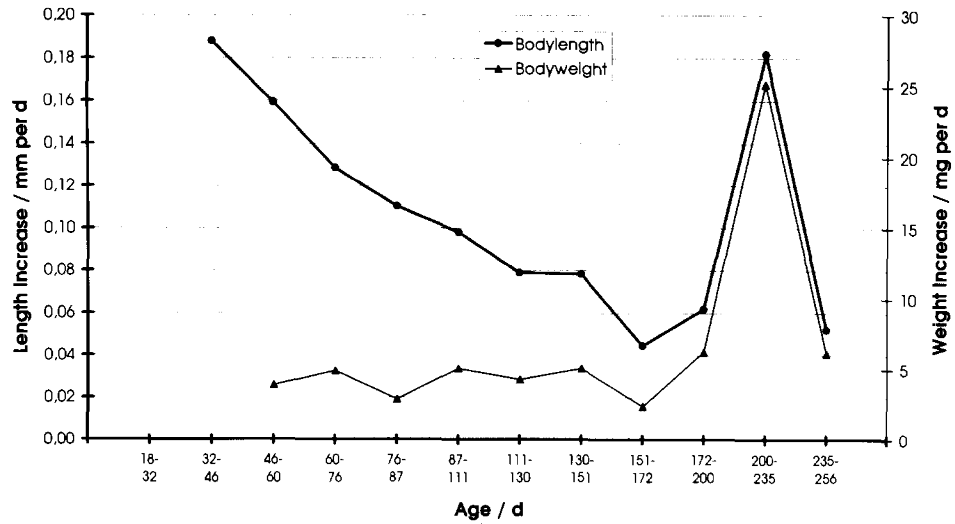

Fig. 2. Daily growth rate based on length (circles) and weight (triangles) in the growing cichlid from 18 to 256 days of age.

and body weight [7,14–16]. The present results confirm that the experimental temperature and the age have a significant influence on the MR of the Convict cichlid. A high variance of oxygen consumption observed in the present results might also be influenced by the physiological conditions and/or phenotypic differences of each individual, as previously discussed [6]. This fish is an oxyconformer with ≈45% MR reduction (measured at 23°C) with declining oxygen tension from 13.3 to 2.7 kPa [6]. We observed that this fish adapts rapidly to the experimental conditions.

Three significantly different phases of specific oxygen consumption could be distinguished in the growing cichlid before the fish reached the reproductive state. The first high level of oxygen consumption was observed between age 40 and 60 days. During this period we monitored the highest mortality rate of the young fish during the whole observation time [4]. The specific oxygen consumption declined afterwards with increasing age, as to be expected. In a similar pattern, the daily growth rate for the body length and body weight gradually decreased with increasing age during the first 230 days of life (Fig. 2) [4]. The mortality rate also declined and stabilized to nearly zero level. However, a sudden peak of high MR occurred at age 235 or 236 days, about 20 days before the MR dropped back to the third phase of even lower oxygen consumption than before. We suppose that this sudden peak, which is directly accompanied by a peak in the growth rate for body length and body weight (Fig. 2), reflects the major internal changes the individual undergoes immediately before maturation. During this period we noticed also the onset of typical breeding behavior, closely followed by successful breeding of some individuals.

The oxygen consumption in the adult fish (estimated to be 2–5 years old) was ≈ 50% of the MR of the 8–9 months old fish. This indicates that the MR drops rapidly in this fish after the reproductive state has been reached.

5. Conclusions

The specific oxygen consumption in both life stages (before reaching the reproductive state and in the adult stage) of the Convict cichlid as measured in the present study, was significantly affected by experimental temperature and by age. Further, we observed a strong relationship between specific oxygen consumption and growth rate.

There was no significant effect of body weight on the specific oxygen consumption in young fish when we included both the variables "age" *and* "body weight" in the regression analysis. However, a significant influence of "body weight" on the MR was observed in the young stage when the variable "age" was excluded. No significant effect of body weight on the specific oxygen consumption in adult fish was detected. Thus, we conclude that the declining oxygen consumption observed in both life stages is related primarily to the aging process. Only during periods of fast growth of the fish does the factor "body weight" seem to contribute significantly to the MR. More data on the adult fish are needed to establish whether and to what extent the specific oxygen consumption in this fish is influenced by reproductive activity, growth rate and/or the aging process.

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References

[1] A.I. Zotin, Thermodynamic Aspects of Developmental Biology, S. Karger, Basel, 1972, p. 43.
[2] F.W. Allendorf, N. Ryman and F.M. Utter, in N. Ryman and F. Utter (Eds.), Population Genetics & Fishery Management, University of Washington, USA, 1987, p. 1.
[3] R. Riehl and H.A. Baensch, Aquarien Atlas, Mergus-Verlag für Natur und Heimtierkunde Baensch, Germany, 1982, p. 630.
[4] L. Fidhiany and K. Winckler, J. Biol. Indonesia (submitted).
[5] H. Forstner and E. Gnaiger, in E. Gnaiger and H. Forstner (Eds.), Polarographic Oxygen Sensors, Springer-Verlag, Berlin, p. 321.
[6] L. Fidhiany and K. Winckler, J. Ilmu-ilmu Perairan Perikanan Indonesia, 2(1) (1994) 52.
[7] S. Fivelstad and M.J. Smith, Aquacult. Eng., 10 (1991) 227.
[8] K. Becker, K. Meyer-Burgdorff and U. Focken, J. Appl. Ichthyol., 8 (1992) 10.
[9] G. van den Thillart, Mol. Physiol., 2 (1982) 49.
[10] J. van Waversveld, A.D.F. Addink and G. van den Thillart, J. Exp. Biol., 142 (1989) 325.

[11] K. Becker, D. Noffz and L. Fishelson, Trop. Freshwater Biol. (Nigeria), 2 (1989) 141.
[12] E.H. Jørgensen, J.S. Christiansen and M. Jobling, Aquaculture, 110 (1993) 191.
[13] W.E. McLean, J.O.T. Jensen and D.F. Alderdice, Aquaculture, 109 (1993) 281.
[14] H. Swan, Thermoregulation and Bioenergetics, Elsevier, New York, 1974, p. 178.
[15] V.A. Grudnitzky and B. Schaarschmidt, in I. Lamprecht and A.I. Zotin (Eds.), Thermodynamics of Biological Processes, Walter de Gruyter, Berlin, 1978, p. 209.
[16] A.I. Zotin, Thermodynamic Bases of Biological Processes, Walter de Gruyter, Berlin, 1990, p. 69.