

**CHANGES IN METABOLIC RATE AND ENERGY RESERVE
UTILISATION DURING STARVATION: ADAPTATIONS TO
LONG-TERM DROUGHT PERIODS.**

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Summary

Specimens of traíra, *Hoplias malabaricus*, were starved for 0, 30, 60, 90, 150, 180 and 240 days. Two groups were refed after 90 and 240 days of food deprivation. Body mass (W_t) decreased during the starvation periods. Hepatic reserves were mobilized mainly after 30 days and perivisceral fat bodies were consumed gradually, being completely exhausted after 240 days of food deprivation. Length of starvation was associated with a significant decrease in the oxygen uptake ($\dot{V}O_2$). In spite of this reduction, the respiratory frequency (f_R) was kept nearly constant along the starvation periods. Traíra can survive food deprivation for periods of up to 180 days without reductions in metabolism and when they do become hypometabolic, normal metabolic rates are rapidly restored upon refeeding.

Introduction

Traíra (*Hoplias malabaricus* Bloch, 1794) represents about 25% of biomass in temporary ponds formed during seasonal drought periods in several South America environments (Bonetto et al., 1969). This species is widely adapted to survive the adverse conditions of these stagnant shallow waters.

During extreme situations, the species can be found buried in the mud aestivating (Paiva, 1974). The physiological responses of traíra to hypoxia have been studied extensively (Driedzic et al., 1978; Hochachka et al., 1978; Johansen et al., 1978; Rantin and Johansen, 1984; Rantin et al., 1992; 1993; Fernandes et al., 1993; Kalinin et al., 1993; 1996; Sundin et al., 1999). The ability of traíra to survive long periods of food deprivation is also well known (Paiva, 1974; Machado et al., 1989), however, data on the metabolic responses involved in this process are scarce.

In order to understand the physiological strategies employed by traíra to survive long-term starvation, the present study verified the metabolic rate ($\dot{V}O_2$), respiratory frequency (f_R), and energy reserve utilisation and restoration (hepatosomatic index - I_H , liposomatic index - I_L , and condition factor - K) of fish starved for periods varying from 30 to 240 days and refed after 90 and 240 days of food deprivation.

Material and Methods

Specimens of traíra (251.7 ± 6.5 g) were collected in the Mogi Guaçu River basin (São Paulo State, Brazil). During acclimation (20-30 days), fish were kept in normoxia ($PwO_2 \geq 130$ mmHg), constant temperature (25 ± 1 °C) and photoperiod (12/12), and were weekly fed on small live fish. After acclimation, fish received food at controlled levels (2% of biomass \cdot day⁻¹) during 30 consecutive days. The control group was sampled just after this period. Groups of 10 individuals were subjected to 30, 60, 90, 150, 180, and 240 days of food deprivation. Two other groups were refed for 30 days (2% of biomass \cdot day⁻¹) after 90 and 240 days of starvation. In the control and refed groups, food was withheld 2 days before the measurement of oxygen consumption to ensure a post-prandial state.

After the treatments, oxygen uptake ($\dot{V}O_2$) and respiratory frequency (f_R) were determined simultaneously. Fish were previously anesthetized with benzocaine (100 mg \cdot L⁻¹) after which a PE-100 catheter was inserted in the roof of the mouth. After surgery, fish were placed in individual respirometers where they remained for an overnight recovery (18 - 20 h) in normoxic water at 25 °C. Respiratory frequency (f_R – breaths \cdot min⁻¹) was measured by recording the buccal pressure variations during the ventilatory cycle, connecting the buccal catheter to a pressure transducer of a Narco Narcotrace 40 (Narco Bio-Systems, Houston, Texas) physiograph.

$\dot{V}O_2$ was determined by flow-through respirometry (Rantin *et al.*, 1992; Maricondi-Massari *et al.*, 1998). The $\dot{V}O_2$ of 5 animals was measured, in parallel and simultaneously, for each fish. The O_2 tensions of the ingoing ($PinO_2$) and outgoing ($PoutO_2$) water flows were measured continuously by siphoning water via polyethylene catheters through thermostatted cuvettes housing O_2 electrodes (FAC 001- O_2 , FAC - São Carlos, SP, Brazil), connected to O_2 analyzers (FAC-204A). Oxygen uptake ($mlO_2 \cdot kg^{-1} \cdot h^{-1}$) was calculated as:

$$\dot{V}O_2 = [(PinO_2 - PoutO_2) \cdot \alpha \cdot \dot{V}_R] / Wt,$$

where: \dot{V}_R = constant water flow through the respirometer, α = O_2 solubility coefficient in water, and Wt = total body weight (kg).

The condition factor (K) was determined according to Le Cren (1951):

$$K = (Wt/Ls^3) \cdot 100,$$

where: Wt = total body weight (g); Ls = standard length (cm).

Fish were killed by rapid decapitation. Liver and perivisceral fat bodies were removed and weighed. The hepatosomatic (HSI) liposomatic indexes (LSI) were determined according to the equation proposed by Collins and Anderson (1995) for the somatic index (I), as follows:

$$I = (Wr/Wt - Wg) \cdot 100;$$

where: Wr is the total weight of the reserve tissue (liver or perivisceral fat bodies), Wt is the total body mass, and Wg is the weight of the gonads.

One-way analysis of variance (ANOVA), complemented by the Bartlett's test for homogeneity of variances and Tukey-Kramer multiple comparisons test, were employed to analyze the data of each group (GraphPad InStat – GraphPad Software Version 2.01). Data were expressed as mean \pm SEM and considered significantly different when $P < 0.05$.

Results

Condition factor (K) reflected a 2 phases decrease in Wt during the period of starvation (Figure 1). In the first phase (after 30 days), fish showed a significant decrease ($P < 0.05$) in K in relation to control group. And during the second phase (after 180 days) K was again significantly reduced ($P < 0.05$) when compared to control group and also to the former periods. After refeeding, fish did not recover the weight lost during starvation for 90 days ($P < 0.05$) and 240 days ($P < 0.05$).

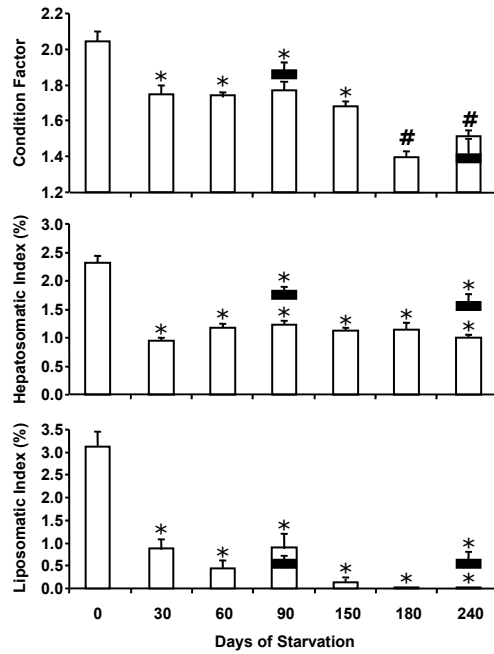


Figure 1. K , HSI and LSI of *Hoplias malabaricus*, in relation to days of starvation (white bars) and in refeed groups (black bars). * = $P < 0.05$ in relation to control; # = $P < 0.05$ in relation to control, 30, 60, 90 and 150 days (mean \pm SEM; n = 10).

In the first 30 days of starvation, a significant reduction in the weight of both tissues was observed ($P < 0.05$; Figure 1). However, the liver size did not change further as food deprivation continued, however the reserves stored in perivisceral fat bodies continued to decrease gradually being completely exhausted after 180 days. Refeed fish did not recover the hepatic and perivisceral reserves to the same levels of the control group ($P < 0.05$), however the liver mass of fish refeed after 90 days of starvation was higher than that observed for all starved groups ($P < 0.05$). In fish refeed after 240 days of starvation, the I_H was higher only when compared with fish starved for 30 and 240 days ($P < 0.05$).

The $\dot{V}O_2$ of fish deprived for 30 – 180 days was nominally the same as the control group (Figure 2A).

Nevertheless, after 240 days of starvation, fish displayed a large decrease in $\dot{V}O_2$. Oxygen consumption in the fish starved for 240 days was, on average, 36.4 % lower than that of the control group ($P < 0.05$). Fish refed after starving for 90 days had $\dot{V}O_2$ rates similar to those of control group, yet the group refed after 240 days had a significantly higher $\dot{V}O_2$ when compared with fish starved for a similar period but not refed ($P < 0.05$). The respiratory frequency of all starved traíras was similar to those of the control group. Although the refed groups have also presented practically the same f_R of the control, they showed a significantly higher f_R in relation to the groups deprived for 180 and 240 days ($P < 0.05$; Figure 2).

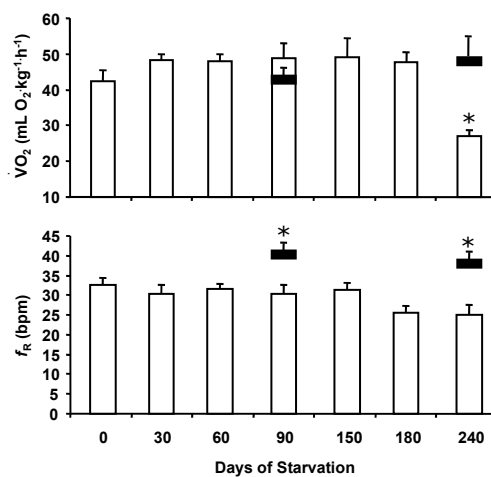


Figure 2. $\dot{V}O_2$ and f_R of *Hoplias malabaricus*, in relation to days of starvation (white bars) and in refed groups (black bars). * = $P < 0.05$ in relation to control group (mean \pm SEM; n=10).

Discussion

Periods of starvation shorter than 180 days did not affect $\dot{V}O_2$ of *H. malabaricus*. When compared to other species, traíra requires a much longer

period of starvation (240 days) before exhibiting hypometabolism. As an example, metabolic rate decreased markedly after only 2 days of food deprivation in the black bass, *Micropterus salmoides* (Glass, 1968), and after 3 days in *Salvelinus fontinalis* and *Catostomus commersoni* (Beamish, 1964). These authors attributed this reduction, at least partially, to the decrease in spontaneous activity. Many fishes subjected to longer periods of starvation have also shown significant decreases in their metabolic rate, such as rainbow trout starved for 15 days (Lauff and Wood, 1996), perch for 14 days (Mehner and Wieser, 1994), plaice for 35 days (Jobling, 1980), African catfish for 42 days (Hogendoorn, 1983), and the Atlantic salmon deprived of food for 23 days (O'Connor *et al.*, 2000) or 56 days (Cook *et al.*, 2000).

In this study, $\dot{V}O_2$ ($42.4 \pm 3.1 \text{ mL O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and f_R ($32.5 \pm 1.9 \text{ breaths} \cdot \text{min}^{-1}$) of fed traíras were very similar to those observed in other studies with the same species of similar sizes under normoxic conditions (Rantin *et al.*, 1992; 1993; Kalinin *et al.*, 1993). However, the $\dot{V}O_2$ of this species is very low when compared with other tropical fish such as *Oreochromis niloticus* ($\sim 60 \text{ mL O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, Fernandes and Rantin; 1989; 1994; Kalinin *et al.*, 1999), *Prochilodus scrofa* ($\sim 80 \text{ mL O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, Fernandes *et al.*, 1995) and *Piaractus mesopotamicus* ($\sim 125 \text{ mL O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, Rantin *et al.*, 1998). Reduced $\dot{V}O_2$ indicates relatively low needs for energy to maintain the metabolism of traíra. This may explain the low voracity and, consequently, high incidence of empty stomachs observed in traíras in natural conditions (Caramaschi, 1979). The low $\dot{V}O_2$ under normal feeding conditions suggests that a further reduction in metabolic expenditures might impair normal physiological function. Yang and Somero (1993) suggested that *Sebastes alascanus*, in its natural habitat, is metabolically similar to starved individuals. These authors attributed the low metabolism of this species as an adaptation to environmental conditions, which is characterised by low oxygen and food availability. Similarly, Pusey (1986) suggested that the constant O_2 uptake showed by *Lepidogalaxias salamandroides* after 20 – 40 days of starvation is related to its capacity to aestivate and inherent low metabolic requirements. This could also explain the constant $\dot{V}O_2$ shown by the Japanese eel (*Anguilla japonica*) after 90 days of food deprivation (Inui and Ohshima, 1966).

The Australian freshwater fish *L. salamandroides* inhabit environments with similar characteristics of those where traíra lives in South America, and also

survives drought periods by aestivating burrowed in the substrate. The $\dot{V}O_2$ values recorded for traíra in the present study are very similar to those obtained by Pusey (1986) for *L. salamandroides*.

The constant f_R kept by traíra, even after 240 days of starvation, was probably necessary to overcome the reduced blood oxygen carrying capacity, caused by a possible anemic situation due to the long period of starvation. Fish refed after 240 days of starvation appeared to be still anemic and displaying higher f_R (Figure 2B). Increased f_R was also observed for the anemic goldfish, *Carassius auratus* (Houston and Murad, 1995). Interestingly, traíra possess one of the lowest metabolic costs of gill ventilation measured in teleosts: 3 % of the total $\dot{V}O_2$ in normoxia and 13 % under severe hypoxia (Rantin *et al.*, 1992). This could represent an important adaptation to hypoxic conditions and/or energy conservation during long periods of food deprivation.

The constant L_s and decrease in K of starved traíra indicate that growth was stopped by food deprivation and endogenous reserves were mobilized in order to generate metabolic energy for maintenance. Kooijman (2000) argued that if the rates of maintenance cannot be considerably reduced, the presence of reserves is essential. The capacity to survive periods of starvation then depends on the ability of the species to utilize its reserves (Wootton, 1990). In teleosts, the main sources of energy reserves are in the liver (e. g. Fujita *et al.*, 1986; Avila, 1986; 1987) and the perivisceral fat bodies (Collins and Anderson, 1995). Significant reductions in the weight of liver and exhaustion of perivisceral fat bodies in traíras were followed by a significant reduction in total W_t , suggesting that proteins were mobilized from muscles as an energy source. The decrease in $\dot{V}O_2$ after 240 days of starvation apparently occurred after the depletion of the main energy reserves, which probably imposed on the fish the adoption of a less expensive metabolic strategy to preserve their essential functions.

Blasco *et al.* (1992) verified two phases of fasting in carp, *Cyprinus carpio*. The first phase (until day 8 of fasting) was characterized by a reduction in the hepatosomatic index mainly due to glycogen mobilization, whilst decreased muscle levels characterized the second phase. This biphasic response was also observed in traíra. During the first phase (until 150 days), fish consumed the main endogenous reserves and sustained the $\dot{V}O_2$ at control rates (see Figure 2A). In this phase, the loss of weight, more intense

in the first 30 days, was probably due to the reduction on lipids and glycogen levels in the tissues. It is possible that the plateau observed in Figure 1A (between 30 and 150 days) reflects the water accumulation in the tissues as the lipids are metabolized (cf. Love, 1980). Intense mobilization of muscular proteins after exhaustion of the main energy reserves resulted in a further loss of weight characterizing the second phase of starvation (after 180 days). During this phase, traíra became anemic and the $\dot{V}O_2$ was significantly reduced after 240 days (Figure 2A).

Refeeding for 30 days after a long-term starvation (90 and 240 days) was not enough to replace the reserves consumed by traíra during starvation. The most likely explanation for this is that refed fish use the energy for restoration of other tissues and functions impaired during starvation. Refed fish, however, recovered their $\dot{V}O_2$ values to the same levels of the control group. This was also been observed in other species (Wieser *et al.*, 1992; O'Connor *et al.*, 2000). This increase in $\dot{V}O_2$ following re-feeding is probably related to the restoration of protein synthesis and turnover capacity (Lied *et al.*, 1983; Peragon *et al.*, 1999).

In conclusion, the low energy requirement of traíra probably allows its survival for long periods of food deprivation at normal metabolic rates while exploiting reserves accumulated during periods of food abundance. This low maintenance metabolic rate also makes it relative easy to acquire surplus energy for storage during periods of food abundance. Interestingly, traíra have among the greatest tolerances to food deprivation recorded. In addition, traíra can survive food deprivation for periods of up to 180 days without reductions in metabolism and when they do become hypometabolic, normal metabolic rates are rapidly restored upon refeeding. In lakes and pounds where traíra occur, the drought periods will only rarely be greater than 180 days. Severe food shortages persisting for longer than 240 days would be necessary to endanger this species survival.

Acknowledgements

The authors would like to thank FAPESP for providing a PhD fellowship and grant to Flavia Sant'Anna Rios (Proc. 98/06737-3), the field technician Nelson S. A. Matos for collecting the fish and José Roberto Sanches for his technical support in the laboratory.

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