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Responses of the teleost *Hoplias malabaricus* to hypoxia

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Synopsis

Oxygen uptake (VO_2) during graded hypoxia, rate of hypoxia acclimation, breathing frequency (f_R), breath volume ($\dot{V}_{S,\ R}$) and gill ventilation ($\dot{V}_G$) were measured in *Hoplias malabaricus*. Normoxia and hypoxia acclimated fish had similar and constant $\dot{V}O_2$ and $\dot{V}_G$ in a range of water PO_2 from 150 to 25 mmHg. Hypoxia acclimated fish showed significantly higher $\dot{V}O_2$ in severe hypoxia (PO_2 <15 mmHg). Normoxia acclimated fish showed symptoms similar to hypoxic coma after 1 h of exposure to water PO_2 of 10 mmHg whereas the same symptoms were observed only at PO_2 of 5 mmHg for fish acclimated to hypoxia. Fish required 14 days to achieve full acclimation to hypoxia (PO_2 ≥25 mmHg). Lowering of water PO_2 from 150 to 25 mmHg resulted in normoxic fish showing a 3–2 fold increase in $\dot{V}_G$. The increase was the result of an elevation in $\dot{V}_{S,\ R}$ rather than f_R. Among normoxia acclimated specimens, small fish showed a higher $\dot{V}_G$ per unit weight than the large ones in both normoxia ($PO_2 = 150$ mmHg) and hypoxia ($PO_2 = 15$ mmHg). A decrease in the ventilatory requirement ($\dot{V}_G/\dot{V}O_2$) with increased body weight was recorded in hypoxia ($PO_2 = 15$ mmHg).

Introduction

Hoplias malabaricus (Erythrinidae, Characoidei) is a widely distributed fresh-water predator. The species is normally found in streams and shallow waters in tropical and sub-tropical South America, where it represents an important species and high percentage of the biomass (Bonetto et al. 1969, Kramer et al. 1978, Fink & Fink 1979). Among other members of the family are two facultative air-breathers, *Hoplerythrinus unitaeniatus* and *Erythrinus erythrinus* (Kramer et al. 1978). *Hoplias malabaricus* is an exclusive water-breather (Wilmer 1934), but inhabits the same hypoxic waters as its air-breathing relatives. Biochemical evidence suggests that *Hoplias* has a high anaerobic capacity which aids survival in hypoxic conditions (Driedzic et al. 1978, Hochachka et al. 1978), but the respiratory responses of the species to hypoxia still remain to be elucidated.

The objective of the present study was to determine the O_2 uptake in relation to the partial pressure of dissolved O_2 and the rate of change in O_2 uptake during acute and chronic exposure to hypoxia. Additionally, the directly measured ventilatory response to acute and chronic hypoxia were studied.

Material and methods

Specimens of *Hoplias malabaricus* (Bloch) in a size range of 40 to 200 g were collected in Broa Reservoir and Universidade Federal de São Carlos Reservoir, State of São Paulo, Brazil, and shipped by air to Aarhus, Denmark. In the laboratory the fish were kept in holding tanks (120 liters) with continuously flowing and filtered well aerated (PO_2 >140) water at 20.0 ± 1.0° C. The aquaria were maintained on a 12 h photoperiod. Fish were fed weekly on small live trout, and all animals were postabsorptive (5–7 days after feeding) when used in experiments. Fish kept in well aerated water for 2 weeks were considered normoxia acclimated. Hypoxia acclimation was carried out in a specially prepared tank. Oxygen tension (PO_2 = 25 ± 3 mmHg) was kept stable by using an oxygen regulating system (Wood et al. 1975). The system consisted of an oxygen regulator module adapted to a Radiometer PHM - 71 MK2 analyser which controls an input of compressed air connected to the tank by means of a magnetic valve. The water PO_2 was lowered gradually during one week by the O_2 consumption of fishes until it reached a selected value of 25 mmHg. The acclimation tank was supplied with a closed filtering system and a low water flow through the tank was maintained assuring low PO_2 and water renewal.

Oxygen uptake was measured using a flow-through respirometer. A closed circuit pump was used to ensure uniform O_2 tension in the respirometer. Oxygen tensions of the ingoing (PiO_2) and outgoing (PeO_2) water were measured continuously by siphoning water via polyethylene catheters through thermostatted cuvettes housing O_2 electrodes (Radiometer E 5046). The electrodes were connected to a Radiometer PHM-71 MK2 acid-base analyser, and the output signals were recorded on Radiometer Servograph REC-51 recorder. The respirometer was kept in a water bath of controlled temperature and O_2 tension. A Lauda K2R-D thermostat was used to maintain constant temperature (20.0 ± 1.0° C) and circulation of water. Water PO_2 was maintained at different stable levels by bubbling nitrogen and compressed air into the bath. Water flow through the respirometer could be regulated according to the fish size and water PO_2 and was calibrated volumetrically by means of a stop-watch and a measuring cylinder. In all the experiments a difference of 20–30% in PO_2 was kept between the ingoing and outgoing water.

Oxygen uptake of normoxia acclimated fish in response to gradual hypoxia was measured at five levels of PO_2: 150, 100, 50, 25 and 15 mmHg. The same procedure was used on hypoxia acclimated fish but in this case an additional group was tested also at PO_2 = 10 mmHg. Five specimens were studied in each group. The fish remained in the respirometer at least 1 hour before collection.

In order to measure the time course of hypoxia acclimation, the oxygen uptake of five hypoxia exposed fish was studied at 7, 14, 21 and 28 days after the start of acclimation (PO_2 = 25 mmHg). The acute responses to hypoxic exposure was stud-

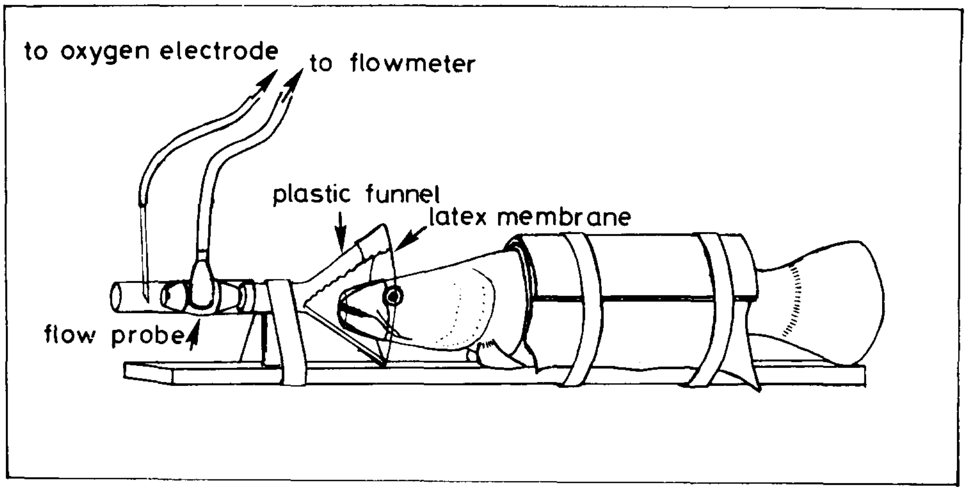

Fig. 1 Scheme of the system used to measure the breathing frequency and gill ventilation of *Hoplias malabaricus*.

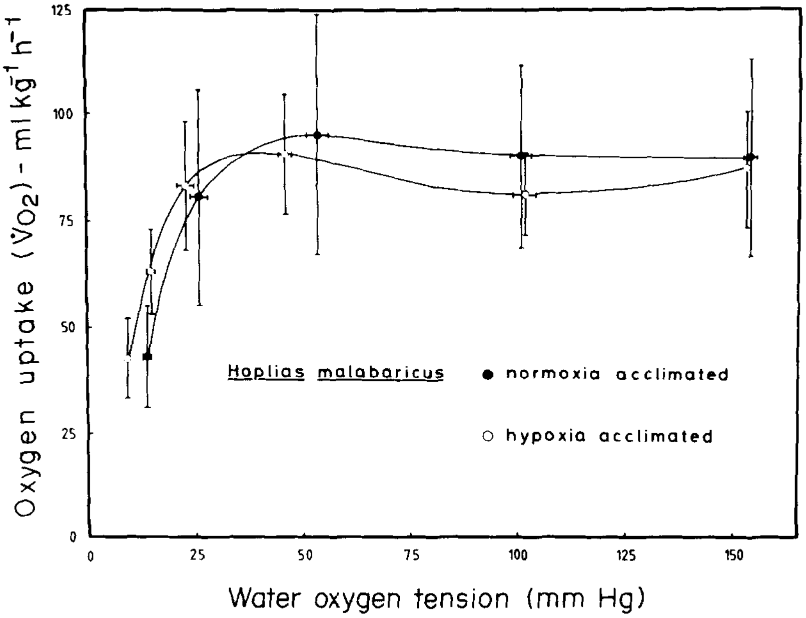

Fig. 2. The effect of decreasing water PO_2 on the oxygen uptake of *H. malabaricus*, normoxia and hypoxia-acclimated. Points are mean ± 1 S.D. Significant difference (t test; $P<0.05$) was detected at water $PO_2 = 15$ mmHg.

ied in another group of five animals by transferring them directly from normoxic to hypoxic water and measuring the changes in oxygen uptake every day for the first 7 days after transfer to hypoxic water.

Breathing frequency (f_R) and gill ventilation ($\dot{V}_G$) were measured directly by means of an electromagnetic flowmeter (Kerstens et al. 1979, Steffensen et al. 1982) in the same water PO_2 ranges and temperature as used in the O_2 uptake studies. A flow probe (diameter = 5 mm) was connected to a plastic funnel. The fish's head was inserted through a hole in a latex membrane resulting in a tight fit that sealed the anterior end of the fish from the rest of the body without disturbance of the respiratory movements (Fig. 1). Fish were held in a steady position by wrapping them in a piece of soft cloth (Gerald & Cech 1970). To reduce the effect of handling, recordings were begun after the fish had been quiet in this ventilation system for 30–40 min. Fish were kept about 10 min at each stable PO_2 and the ventilatory parameters were recorded only during this interval. The change to another PO_2 generally took 5 min to reach a stable level. Only results from fish remaining quiet during the entire experimental period were used.

Results

Figure 2 shows the oxygen uptake of normoxia and hypoxia acclimated *Hoplias* after 2 h of exposure to different levels of water PO_2. At PO_2 of 15 mmHg the hypoxia acclimated fish showed a significantly higher average $\dot{V}O_2$ than normoxia acclimated fish (t test, $P <0.05$). At 10 mmHg the hypoxia acclimated fish showed the same $\dot{V}O_2$ mean value as the normoxia acclimated did at 15 mmHg. Normoxic fish tested at 10 mmHg and hypoxic fish tested at 5 mmHg were abnormally restless and lost equilibrium after 40–60 min of exposure. An increase in physical activity followed by loss of equilibrium is a typical symptom of hypoxic coma in fish (Shepard 1955).

The mean oxygen uptake value of fish transferred to a water PO_2 of 25 mmHg measured at weakly intervals during the period of acclimation to hypoxia is shown in Figure 3. After one week of exposure the fish had a mean $\dot{V}O_2$ of 58.7 ± 12.4 ml $O_2\,kg^{-1}h^{-1}$. From the second week and later the group showed significantly higher values (t test, $P <0.05$). These higher stable values of $\dot{V}O_2$ showed no statistical differences between the 2nd and 4th week of acclimation.

Figure 4 demonstrates the changes in oxygen uptake during the initial 7 days of chronic exposure to hypoxia for fish of different weights. During the first two days of exposure there was a clear effect of size on the rate of reduction in oxygen uptake. Smaller fish (animals 1 and 2; 43.6 and 41.6 g) showed a 30% reduction while fishes of twice that weight (animals 3 and 4; 87.2 and 76.4 g) showed a 20% reduction. Fish in this size range showed a rise in $\dot{V}O_2$ after the 2nd day of exposure. The largest *Hoplias* (animal 5; 181.6 g) showed a decrease of 25% after the first day of exposure followed by a rise to 18% of the initial $\dot{V}O_2$ value on the second day of exposure.

Figure 5 represents a log—log plot of $\dot{V}_G$ against body weight for 10 normoxia acclimated specimens studied at water PO_2 of 150 mmHg (slope = -0.44; r = -0.56) and 15 mmHg (slope = -0.99; r = -0.90). There is no clear correlation between the ventilatory requirement ($\dot{V}_G/\dot{V}O_2$) and body weight for the fish group studied at 150 mmHg but a correlation became evident for the fish studied at 15 mmHg (slope = -0.52; r = -0.74).

The results obtained on breathing frequency, breath volume, gill ventilation and ventilatory requirement at each PO_2 level for both the normoxia and hypoxia acclimated groups are summarized in Table 1. Breathing frequency was unaffected by O_2 tension or acclimation. Mean breath volume of hypoxia acclimated fish was higher than the normoxic fish and when compared at 25 and 150 mmHg water PO_2 this gradient was statistically different (t test, $P < 0.05$). No statistical differences between mean values of ventilation and ventilatory requirement were observed between normoxia and hypoxia acclimated fish.

Discussion

The relationship between oxygen uptake ($\dot{V}O_2$) and water oxygen tension has been studied for many species. Among teleosts there are basically two different patterns of response to gradual hypoxia. Some species of 'conformers' (Hughes 1973) show a linear decrease in oxygen uptake with a reduction of ambient PO_2. This response has been

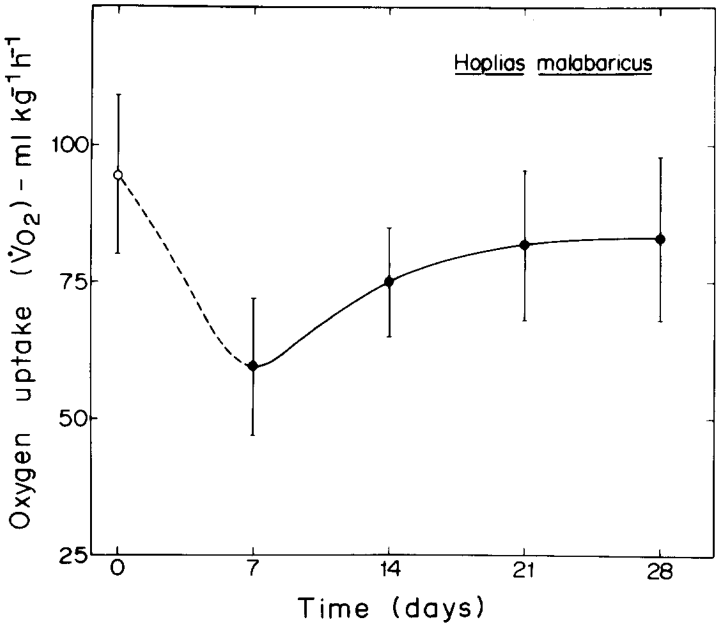

Fig. 3. The oxygen uptake measured 7, 14, 21 and 28 days after the start of hypoxia-acclimation (PO_2 = 25 mmHg). Points are mean ± 1 S.D. A significantly low $\dot{V}O_2$ (t test; $P<0.05$) was detected the 7th day. Open circle (time = 0) is mean (± 1 S.D.) of oxygen uptake of the same group of fish acclimated to normoxia measured just before the beginning of hypoxia acclimation.

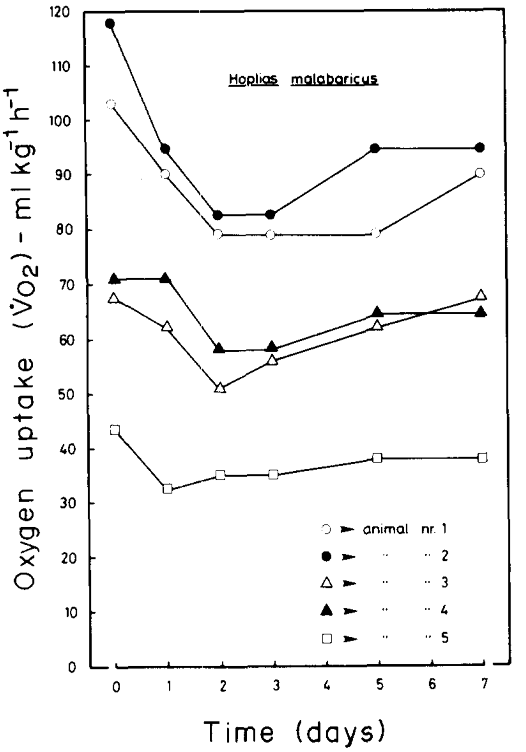

Fig. 4. The oxygen uptake measured 1, 2, 3, 5 and 7 days after the start of hypoxia-acclimation (PO_2 = 25 mmHg). Animal no. 1 = 43.6 g, 2 = 41.6 g, 3 = 87.2 g, 4 = 76.4 g, and 5 = 181.6 g.

observed in toad-fish, *Opsanus tau* (Hall 1929), brown bullhead catfish, *Ictalurus nebulosus* (Marvin & Heath 1968) and plaice, *Pleuronectes platessa* (Steffensen et al. 1982). Conversely 'non-conformers' or regulators show a stable or even an increased O_2 uptake in response to gradual hypoxia until the so called critical O_2 tension (Pc) is reached. Below that the $\dot{V}O_2$ also for them become dependent upon the ambient O_2 availability. Such a response has been demonstrated in goldfish, *Carassius auratus* (Prosser et al. 1957, Beamish 1964), carp, *Cyprinus carpio* (Beamish 1964), trout, *Salmo gairdneri* (Holeton & Randall 1967, Marvin & Heath 1968), bluegill, *Lepomis macrochirus* (Marvin & Heath 1968) and the flounder, *Platichthys flesus* (Steffensen et al. 1982). Thus, the critical O_2 tension denotes the loss in the ability to compensate for reduced ambient O_2 tensions. Teleosts show a considerable variation in Pc. Some species have a high Pc, like 75% of air saturated water (≈116 mmHg) for bluegill and 60% (≈93 mmHg) for trout (Marvin & Heath 1968) or 91 mmHg for flounders (Steffensen et al. 1982), but in most of the regulators the critical O_2 levels occur between 40 mmHg and 20 mmHg such as in the crucian carp, *Carassius carassius* (Blažka 1958), carp and goldfish (Beamish 1964), blackfish, *Orthodon microlepidotus* (Cech et al. 1979a) and the largemouth bass, *Micropterus salmoides* (Cech et al. 1979b). Normoxia acclimated *Hoplias* showed a stable $\dot{V}O_2$ within a wide range of water PO_2, with P_CO_2 near 25 mmHg (Fig. 2). The scarcity of data to both sides of that tension unfortunately does not allow a precise estimate of the critical O_2 tension, but the data show a relatively high capacity for regulation of $\dot{V}O_2$ even when ambient PO_2 was as low as 25 mmHg. This accounts for a low P_CO_2 for this species when compared with most of the published

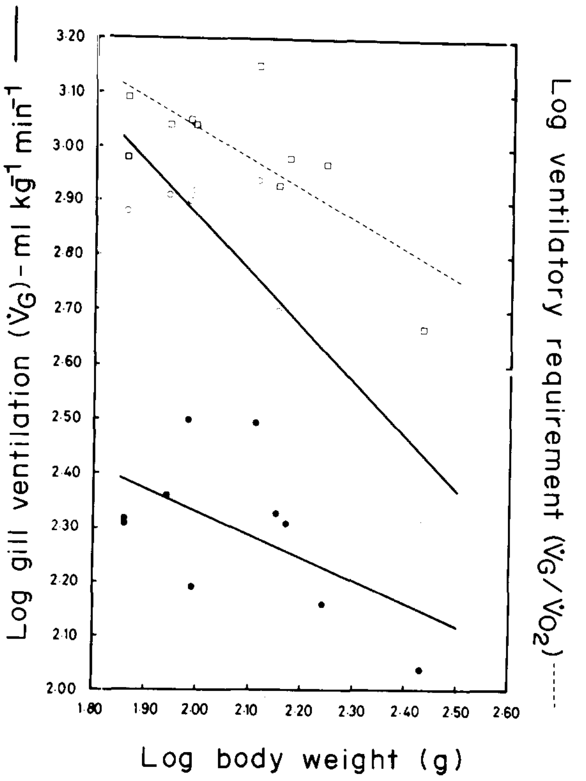

Fig. 5. The effect of body weight on the ventilation of *H. malabaricus* normoxia-acclimated exposed to normoxia (● – PO_2 = 150 mmHg; r = −0.56) and hypoxia (○ – PO_2 = 15 mmHg; r = −0.90), and ventilatory requirement of fish exposed to hypoxia (□ – PO_2 = 15 mmHg; r = −0.74).

values. Among characoid fish, the non-air-breathing lebiasinid *Piabucina panamensis* showed also a P_CO_2 of 25 mmHg (Graham et al. 1978). This species inhabits tropical shallow waters, apparently subjected to periods of hypoxia, similar to that of *H. malabaricus*.

Prosser et al. (1957) argued that hypoxia acclimated goldfish show a lowering in the average of O_2 uptake in well aerated water and a shifting of the critical O_2 tension towards lower values. Thus, during severe hypoxia, fish acclimated to low PO_2 displayed higher O_2 uptake than the normoxia acclimated fish. These findings were partially confirmed by Lomholt & Johansen (1979) who demonstrated that hypoxia acclimated common carp also show a higher O_2 uptake than for specimens acclimated to normoxia during exposure to severe hypoxia. Similarly, Kerstens et al. (1979) found that the $\dot{V}O_2$ of hypoxia acclimated flounders in hypoxic waters was two-fold higher than for normoxic fish acutely exposed to hypoxia. Normoxia and hypoxia acclimated *Hoplias* presented similar response curves when $\dot{V}O_2$ and water PO_2 were compared (Fig. 2). There is no indication of a shift in the P_CO_2, which remained nearly the same for both normoxia and hypoxia acclimated fish. Nevertheless, the significantly higher $\dot{V}O_2$ of hypoxia acclimated fish at water $PO_2 = 15$ mmHg and their $\dot{V}O_2$ at 10 mmHg (50% of the $\dot{V}O_2$ recorded in normoxic levels), must be attributed to an improvement in the capacity to take up O_2 after acclimation to hypoxia. But, in spite of these differences, the capacity of $\dot{V}O_2$ regulation presented by *Hoplias* must be emphasized since it can explain, at least in part, the occurrence of this species in hypoxic environments.

An important strategy of fish exposed to hypoxia is a reduction in activity and thus a low O_2 requirement. According to Davis (1975) compensatory responses of fish to hypoxia are costly in energy expenditure and consequently will reduce energy reserves for swimming, feeding, predator avoidance and other activities. Normoxia acclimated carp showed a two-fold higher fluctuation in the standard O_2 uptake than specimens acclimated to hypoxia (Lomholt & Johansen 1979). The same response pattern was observed in *Hoplias*. Although *Hoplias* is a very quiet fish, resting most of

Table 1. Values of breathing frequency, breath volume, gill ventilation and ventilatory requirement ($\bar{x} \pm$ S.D.) of 6 normoxia and 4 hypoxia acclimated *Hoplias malabaricus* at six dissolved oxygen tensions.

Normoxia acclimated (6):
Weight: 123.3 ± 35.2 g

Water oxygen tension (mm Hg)	Breath frequency (Breaths min^{-1})	Breath volume (ml kg^{-1} $breath^{-1}$)	Ventilation ($\dot{V}G$) (ml kg^{-1} min^{-1})	Ventilatory requirement ($\dot{V}G/\dot{V}O_2$)
150	39.1 ± 7.3	5.4 ± 0.9	210.2 ± 61.8	189.8 ± 44.7
100	42.3 ± 7.3	6.7 ± 1.6	284.8 ± 96.7	254.6 ± 60.9
50	48.9 ± 6.1	9.4 ± 2.6	458.8 ± 148.9	412.6 ± 104.5
25	53.9 ± 9.3	11.1 ± 2.8	595.8 ± 165.4	528.4 ± 73.3
15	53.5 ± 9.8	12.2 ± 2.1	650.5 ± 155.0	1010.4 ± 119.0

Hypoxia acclimated (4):
Weight: 121.0 ± 23.3 g

Water oxygen tension (mm Hg)	Breath frequency (Breaths min^{-1})	Breath volume (ml kg^{-1} $breath^{-1}$)	Ventilation ($\dot{V}G$) (ml kg^{-1} min^{-1})	Ventilatory requirement ($\dot{V}G/\dot{V}O_2$)
150	39.6 ± 5.7	9.5 ± 3.6	366.3 ± 152.4	319.3 ± 113.5
100	40.3 ± 8.2	8.8 ± 3.1	358.2 ± 160.6	311.3 ± 120.4
50	45.6 ± 9.7	11.6 ± 4.6	516.6 ± 189.3	450.6 ± 137.2
25	46.1 ± 2.8	15.3 ± 2.1	706.5 ± 108.8	621.4 ± 61.0
15	48.5 ± 10.2	16.4 ± 2.4	773.3 ± 132.9	960.9 ± 88.6
10	52.7 ± 10.4	15.6 ± 1.8	814.4 ± 133.9	1597.5 ± 123.9

the time near the bottom, it was possible to detect a markedly reduced activity level at a water PO_2 of 15 mmHg (Fig. 2). The literature is short of information about the time course of acclimation and its physiological mechanisms and ecological meaning. Shepard (1955) measured the rate of hypoxia acclimation for brook charr, *Salvelinus fontinalis.* A gradual increase in the ability to resist hypoxia was recorded and the fish were 95% acclimated after a period of 100–200 h (4–8 days). The data obtained on *Hoplias* (Fig. 3) pointed out a significant reduction in the $\dot{V}O_2$ during the first week of acclimation preceded by a return of $\dot{V}O_2$ to normal levels, after two weeks, suggesting that this is the time required by the species to achieve full acclimation. Details on the decline of O_2 uptake during the first week of acclimation were observed when daily measurements of $\dot{V}O_2$ were taken (Fig. 4). There was a critical period after 3 days when a marked decrease in $\dot{V}O_2$ was present. During this time the fish displayed a striking reduction in spontaneous activity. The avoidance of spontaneous activity resulting in a reduction in energy expenditure and O_2 uptake, must be an important compensatory response to hypoxic conditions, at least during the initial period of acclimation. During this time the fish are apparently relying upon anaerobic metabolism as observed in crucian carp (Blažka 1958) and goldfish (Beamish 1964). The capacity of *Hoplias* to survive for long periods of anaerobiosis must be attributed to the large reserves of glycogen of its red muscles (Hochachka et al. 1978), liver and heart (Driedzic et al. 1976). For these authors the reserves of glycogen probably are not utilized during the course of normal activity but may be mobilized in response to hypoxic and anoxic stress, providing the glucose for the anaerobic metabolic pathway.

The magnitude of gill ventilation which teleosts generally increase depends on the degree of hypoxia (Van Dam 1938, Saunders 1962, Gerald & Cech 1970, Lomholt & Johansen 1979, Steffensen et al. 1982). Flatfishes exposed to hypoxia presented a generally low increase (ranging 1.5 to 2.5 fold) as compared with normoxic values (Watters & Smith 1973, Steffensen et al. 1982). Normoxia acclimated carp in hypoxic conditions ($PO_2 = 40$ mmHg) showed a 5.8 fold increase in ventilation (Lomholt & Johansen 1979), while rainbow trout in low O_2 tensions ($PO_2 = 30$ mmHg) responded by an amazing 13 fold higher value than in normoxic water (Holeton & Randall 1967). When compared with other species normoxia acclimated *Hoplias* showed a moderate rise in ventilation during hypoxia, averaging a 3.2-fold increase (1.5 to 5.0) when water PO_2 was lowered from 150 to 15 mmHg.

The individual differences in gill ventilation of *Hoplias malabaricus* showed a correlation with body weight. Saunders (1962) suggested a higher ventilation for small fish than for large ones, and Gerald & Cech (1970) similarly proposed a decrease in ventilation with increasing size from a comparison of different species. The present data on *Hoplias* clearly show that smaller fish had a higher ventilation volume per unit weight than large fish. This tendency was clear during exposure to both normoxia and severe hypoxia (Fig. 5). In normoxic water no correlation between ventilatory requirement and body weight was observed. Nevertheless the ventilatory requirement clearly decreased as a function of body weight for the fish studied in severe hypoxia (Fig. 5). Such a correlation may suggest that larger fish have a higher gill surface area related to body weight than smaller fish. The size dependence of gill surface areas has been reviewed by Muir (1969) and Hughes (1970). Their conclusion was that among teleosts there is a general tendency for a reduction in the gill surface area with increased body weight. However, two species, *Trachurus trachurus* and *Callionymus lyra,* among the fourteen species studied by Hughes (1966) presented an augment in the gill surface area per unit weight with increased body weight, corroborating our conclusion for *H. malabaricus.*

Lomholt & Johansen (1979) demonstrated a higher O_2 uptake with a reduced ventilation in hypoxia acclimated carp when compared to normoxia acclimated fish when both groups were studied in hypoxic water. This acclimation change was not observed for *Hoplias malabaricus* since no effect of acclimation on breathing frequency or in ventilation could be demonstrated (Table 1).

The ventilatory requirement calculated for both normoxia and hypoxia acclimated fish gave notably lower figures in comparison with many other species studied at similar conditions. Gill ventilation and ventilatory requirement of the most relevant studies already published were reviewed by Lomholt & Johansen (1979).

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