Effects of stress and food deprivation on catfish, *Heteropneustes fossilis* (Bloch)

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No significant changes in plasma cortisol and plasma osmolarity (the indicators of primary and secondary response respectively) were observed when the blood samples were obtained from unanaesthetized, anaesthetized and stressed catfish, *H. fossilis*. The results suggest that the catfish is fairly hardy and not easily susceptible to stress by routine laboratory handling. The sustained plasma glucose levels and decreased liver and muscle glycogen concentrations during cessation of feeding of the catfish suggest that during period of food deprivation, it draws its energy through glycogenolysis. Hence, in any study dealing with carbohydrate metabolism, the catfish needs to be fed during acclimation and experimental periods.

Routine laboratory and experimental methods such as handling\(^1\)\(^-\)\(^2\) and anaesthetization\(^3\)\(^-\)\(^4\) are known to be extremely stressful to the fishes. Several strategies have been proposed to counter stress caused by these routine laboratory manipulations. However, the method which is used commonly is the anaesthetization of the fish mostly with MS222 (ethyl m-aminobenzoate methanesulphonate) before blood sampling. This clearly underlines the need for obtaining blood samples from quiescent and unstressed fish. It is then only that the effect of any particular physiological stimulus can be monitored with certainty and without being modified by stress-induced changes.

Nutritional status of the fish is yet another variable which is likely to influence the physiology of the fishes\(^5\)\(^-\)\(^6\). Evidences show that energy deficiency due to cessation of feeding may lead to disorders in parameters such as plasma osmolarity/plasma electrolyte\(^7\)\(^-\)\(^8\) and in major carbohydrate fuels such as plasma glucose, liver and muscle glycogen\(^8\)\(^-\)\(^10\). Hence, while performing studies dealing with any aspect of physiology, it is of utmost importance that the experimental animals are in a nutritionally sound state and adequate time interval of feeding during acclimation as well as experimental period has to be decided with great care so as to ensure that animal is in a stress-free state.

The catfish, *Heteropneustes fossilis* is an economically important airbreathing fish and constitutes an important component of culture and capture fishery of Indian subcontinent. Extensive work has been done on its osmoregulatory physiology\(^11\)\(^-\)\(^15\), bioenergetics\(^6\)\(^,\)\(^7\) and toxicological studies\(^16\)\(^,\)\(^17\). However, no systematic effort has been made to evaluate the effect of laboratory induced stress and nutritional state of the fish.

Therefore, the present study has twin objectives to:

(a) evaluate the effect of handling stress on the catfish by monitoring the changes in the plasma cortisol, an indicator of primary response and the plasma osmolarity, a hematological indicator of secondary response;

(b) study the nutritional state of the catfish following cessation of feeding by assessing changes in the major carbohydrate fuels such as plasma glucose, liver and muscle glycogen and plasma osmolarity which is a resultant factor of all adaptation mechanisms.

Materials and Methods

*Collection and care of catfish*—Adult specimens of the catfish, *H. fossilis* were obtained locally during February. They were kept in glass aquaria (60 x 25 x 30 cm) containing dechlorinated tap water and the lighting schedule at 12 hr of light (0800 to 2000 hrs) alternating with 12 hr of darkness (2000 to 0800 hrs). Catfish (body weight range 50 g) were acclimated to laboratory conditions for 15 days prior to initiation of experiments. During this period, they were fed *ad libitum* daily with Hindlever laboratory animal feed (Hindustan Lever Limited, Bombay, India) and the water in the aquaria was renewed daily with stored tap water adjusted to the laboratory conditions.

*Plasma samples*—Blood was drawn from the caudal artery using heparinized glass syringes fitted
with 24 gauge disposable needles. Immediately after collection, the blood was centrifuged for 10 min at 3000 rpm (Remi Ltd., India, model RSC), plasma was separated and stored at -20°C until used for estimation.

Plasma cortisol—Plasma cortisol levels were estimated by radioimmunoassay (RIA) according to the technique described and validated for *H. fossilis* by Lamba et al.²⁰

Plasma osmolarity—Plasma osmolarity was measured in 10 µl samples with a Vapour Pressure Osmometer (Wescor 5500, Utah, USA) and expressed as mmol/kg.

Plasma glucose—Plasma glucose was assayed by glucose-O-toluidine method.²¹

Liver and muscle glycogen—Glycogen, both in muscle and liver was estimated by anthrone method.²²

Statistical analysis—Data for all parameters were expressed as mean ± SE. Statistical comparisons between experimental and control groups were made by Student's *t* test.²³

Experiment 1—Effect of stress and anaesthetization on plasma profiles of osmolarity and cortisol of *H. fossilis*—Fish were allocated in three groups each with 4-9 specimens and were kept in glass aquaria containing 20 l tap water. Fish from first group were gently netted out from the aquarium and the blood was collected from the caudal artery as described earlier. In the second group the blood samples were obtained from the fish anaesthetized with MS222 at a dose of 100 mg/l (this concentration of MS222 was enough to immobilize the fish within 1 min), while in the third group, the blood was drawn after giving 2-3 min stress by vigorously chasing the fish with the hand net. Plasma osmolarity and plasma cortisol levels were analyzed according to the methods described earlier.

Experiment 2—Changes in plasma levels of osmolarity and glucose and liver and muscle glycogen contents following cessation of feeding of *H. fossilis*—The catfish were either fed daily in the morning (control group) or feeding was terminated (experimental group) for 2, 3, 6, 10 and 15 days and then various tissue samples (blood, liver and muscle) were obtained from each group containing 4-5 fish. A control group was sampled separately with each time point. The blood was collected from the caudal artery as described earlier and the plasma thus obtained was utilized for estimation of plasma osmolarity and glucose. A small piece of muscle was excised from just beneath the dorsal fin and liver was dissected out to estimate glycogen content.

Results

Experiment 1—Plasma cortisol and osmolarity—No significant difference was seen in plasma cortisol concentration and osmolarity in anaesthetized and unanaesthetized and stressed groups (Fig. 1).

Experiment 2
Plasma osmolarity—Cessation of feeding for up to 15 days did not significantly change plasma osmolarity of the catfish. However, there was a decreasing trend up to 6 days of cessation of feeding beyond which the values became almost similar to that of the fed control (Fig. 2).

Plasma glucose—Cessation of feeding for up to 15 days did not show any significant effect on the plasma glucose profile (Fig. 2).

Liver glycogen—There was a significant decline in the liver glycogen content during cessation of feeding. The values significantly declined from 2 to 6 days of cessation of feeding \( (P<0.001) \), showed some increase on day 10, but were still significantly lower \( (P<0.05) \) compared to fed control. However, the levels became nearly equal to the control after 15 days of cessation of feeding (Fig. 3).

Muscle glycogen—Muscle glycogen content significantly declined up to 6 days \( (P<0.025; \text{Fig. 3}) \), most significant decrease was observed on day 2 \( (P<0.01) \) and the parity with fed control was obtained from day 10 onwards. However, the extent of decline was less compared to that seen in liver glycogen (Fig. 3).

Discussion
It is apparent from the present results that routine laboratory handling does not cause any appreciable stress on \( H. \) fossilis. This corroborates the findings on yearlings of chinook salmon, *Oncorhynchus tshawytscha* \(^ {24} \) and those on rainbow trout, *Salmo gairdneri* \(^ {3} \) where no significant elevation in the cortisol concentration in anaesthetized and non-anaesthetized group was observed. However, Wedemeyer \(^ {25} \) observed that anaesthetization with MS222 at a dose of 80 mg/l induced a progressive depletion of interrenal ascorbate levels implying that the anaesthetization itself at that dose was a physiological stressor in this fish. Even in the present study, a moderate, though statistically insignificant, increase in the plasma cortisol following anaesthetization of the catfish at a dose levels of 100 mg/l which is relatively higher than the one used by Wedemeyer \(^ {25} \) was observed. It is likely that the stress susceptibility of the catfish is relatively less than that of the rainbow trout, *S. gairdneri* \(^ {25} \). This is also borne out by the observation when sustained physical stress caused to the catfish failed to evoke any significant rise in plasma cortisol. That the mild handling stress does not cause any significant change in the plasma levels of cortisol has been observed earlier. For instance, Fagerlund \(^ {26} \) observed no increase in cortisol concentration after 4 min of mild restrain and recorded an increase only after 15 min of the gentle agitation. Similarly, goldfish, *Carassius auratus* also did not exhibit any change in plasma cortisol levels.
until 10 min of mild restraint\textsuperscript{27} and until 15 min after exposure to a handling disturbance\textsuperscript{28}. Barton et al.\textsuperscript{29} observed that serial removal of cohorts from the same aquarium also did not cause any significant change in remaining fish. On the other hand, many studies have shown that even the disturbances of minor magnitude such as removal of fish from aquaria or a person entering the room housing the aquaria can elevate plasma cortisol levels\textsuperscript{30-32}. This clearly suggests that the degree of susceptibility to stress is a species-specific phenomenon. Fishes may, therefore, be categorized into two groups. First group includes species such as\textit{H. fossilis}; sockeye salmon, \textit{O. nerka}\textsuperscript{26}; goldfish, \textit{C. auratus}\textsuperscript{27,28}; rainbow trout, \textit{S. gairdneri}\textsuperscript{3-29} which are hardly and not easily susceptible to the minor stresses. Conversely, the second group comprising of the species which are extremely sensitive even to minor stress is exemplified by common carp, \textit{Cyprinus carpio}\textsuperscript{30}; brown trout, \textit{S. trutta}\textsuperscript{31}; coho salmon, \textit{O. kisutch}\textsuperscript{32}.

Interestingly, in the present study, handling stress did not cause any change in plasma osmolarity which further substantiates the hardy nature of the catfish and is in keeping with the similar observations on Atlantic salmon, \textit{S. salar}\textsuperscript{8}.

Suspension of feeding for up to 15 days does not affect plasma glucose levels and plasma osmotic pressure of the catfish, \textit{H. fossilis} which are in accordance with those reported in \textit{C. auratus}\textsuperscript{13}, \textit{Essex lucius}\textsuperscript{34}, \textit{Ophioccephalus maculatus}\textsuperscript{35}, \textit{Limanda limanda}\textsuperscript{46}, \textit{O. mossambicus}\textsuperscript{37}. They, however, differ from those reported in \textit{Notopterus notopterus} and \textit{Anguilla anguilla}, where blood glucose levels exhibit change after cessation of the feeding\textsuperscript{38,39}. The sustained plasma glucose levels in unfed fish may be either due to gluconeogenesis\textsuperscript{40} or glycogenolysis\textsuperscript{38}.

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\caption{Changes in liver and muscle glycogen concentrations following cessation of feeding of the catfish, \textit{H. fossilis}.}
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The present results show that termination of feeding induced highly significant decline in liver glycogen from 2 to 10 days beyond which the values became similar to the fed control. It is, therefore, logical to assume that the sustained blood glucose levels without any dietary input may possibly be through the breakdown of liver glycogen into glucose i.e. hepatic glycogenolysis which has also been shown to occur in many other fishes such as N. notopterus; juvenile perch, Perea fluviatilis and golden perch, Macquaria ambigua. The present data, however, differ from those reported in pike, E. lucius; snake head, O. maculatus; mudskipper, Boleophthalmus boddarti; coho salmon, O. kisutch where no significant decrease in the liver glycogen was observed after fasting.

A significant decline was observed in the muscle glycogen levels from 2 to 6 days following cessation of feeding after which the levels obtained parity with fed control which is in keeping with the observation in N. notopterus, B. boddarti, P. fluviatilis and M. ambigua. Simultaneous decrease in muscle and liver glycogen up to 6 days of suspended feeding in the catfish may presumably indicate that in this fish the hepatic and muscle glycogenolysis proceed simultaneously and that during early period of suspended feeding catfish draws its energy from both tissues. This agrees well with the observations of Narasimhan and Sundararaj, Black and Love and Mehner and Wieser, where liver and muscle glycogen levels occur simultaneously. However, according to Collins and Anderson, muscle glycogen is utilized after the depletion of liver glycogen stores. In mudskipper, B. boddarti while muscle glycogen decreased significantly during the period of food deprivation, the liver glycogen remained constant which indicates that the muscle glycogen may be the immediate source of energy. A sharp decrease was observed in liver and muscle glycogen contents following cessation of feeding which showed a steady increase for 15 days. Liver and muscle glycogen reserve may serve as an immediate source of energy in the catfish which in later stages may switch over to the mobilization of protein and fats through the process of gluconeogenesis. This assumption, however, needs to be experimentally verified.

Thus, it may be concluded that the catfish, H. fossilis is fairly hardy and is not susceptible to stress by routine laboratory handling and that in any study dealing with carbohydrate metabolism the catfish needs to be fed continuously. Feeding may, however, not be critically important in osmoregulatory studies particularly those relating to studies dealing with the effects of deionized or hypoosmotic environment on the osmoregulatory physiology of the catfish.

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