Cortisol and glucose responses in juvenile striped catfish subjected to a cold shock

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Abstract

Cold-shock stress happens when a fish had been adjusted to a specific water temperature or range of temperatures and is consequently exposed to a rapid drop in temperature, resulting in a cascade of physiological and behavioral responses and, in some cases, death. In the current study, the stress response of striped Catfish (*Pangasianodon hypophthalmus*) was studied by evaluating serum cortisol and glucose level following an abrupt reduction in water temperature (from 28°C to 15°C) at different time points (prior to, and after 1h, 12h and 24h cold treatment, respectively). Regardless of some mortality occurred in cold challenged fish, none of the physiological parameters changed during evaluation period. The results, suggesting that despite of necessity of cortisol and glucose evaluation in any of stress assessment, yet, due to their high variability in different fish species, additional complementary tests such as measurement of other stress hormones *e.g.* heat shock proteins as well as blood-cell counts (preferably in chronic experiments) should also be included.

Introduction

Among the natural stressors fish can experience throughout their life cycle are thermal changes. Fluctuations in water temperature either resulting from a transient (daily change) or a seasonal change is generally associated with disease and fish mortality.[1] Cold-shock stress occurs when a fish had been acclimated to a range of water temperature and is subsequently exposed to a rapid decrease in temperature, resulting in a cascade of physiological and behavioral responses and, in some cases, death.[2] To deal with the environmental changes, fish respond by altering physiological functions including those associated with the stress response.[3] The physiological stress response in fish is mediated by the neuro-endocrine system and includes the release of hormones such as cortisol and adrenaline.[3] In response to most stressors fish will exhibit an increase in plasma cortisol levels, which is generally followed by an elevation in plasma glucose concentration. Although some e ects of temperature and (gradual) temperature changes on the stress response have been investigated in fish species,[4-6] however, little is known about the impacts of rapid temperature drops on the stress response.[7]

Temperature shock can hamper fish life by reducing metabolic rates,[8] impairing swimming performance,[9] reducing the ability to capture prey,[2] impeding predator avoidance,[10] altering rates of recovery from exercise[11,12] and disrupting physiological homeostasis.[8,12,13] Some studies have shown an endocrine stress response change in fish exposed to cold shock.[7,14-16] Cortisol and glucose are two of the most common stress indicators.[17] Increased plasma cortisol levels were observed in rainbow trout, common carp (*Cyprinus carpio*) and tilapia aurea (*Oreochromis aureus*), respectively, exposed to cold shock (in different experimental conditions).[16] Striped catfishes play an important role in Asian aquaculture and commercial fishing.[18] *Pangasianodon hypophthalmus* formerly referred to as *pangasius sutchi* is native to the Chao Phraya River in Thailand and the Mekong in Vietnam. It is abundantly available in the Amazon River, in parts of Russia and in other places of the world under different names.[19] Moreover, fingerlings of the species are often collected and transported to pet fish shops to several countries.[20] Nowadays, this species emerged as a promising species for aquaculture purposes even outside of tropical regions of Southeast Asia. However, development of culture industry for this species has faced difficulties mainly due to the limited knowledge of biology, ecology, and physiology in cultivated stocks.[21] In the current study, the stress response in a tropical fish during and after exposure to an acute cold shock (13°C decrease in water temperature) was investigated. The levels of cortisol and glucose as well as death rate prior to and during cold stress at several time intervals (over 1h, 12h and 24h cold stress) were studied. No recovery was appointed in this study.

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Materials and Methods

Experimental design

Juvenile Striped catfish (average initial weight 1.27±0.24 g and initial length 5.55±0.45 cm) were purchased from a local commercial pet fish shop and held in 1000 L glass tank for three weeks to be acclimated to the experimental conditions. In the beginning of the experiment, the fish were fasted for 24h and then weighed. Two hundred and ten fish of similar sizes were divided into two treatment groups (cold shock and control group). Each group had three replicates and completely randomized design (CRD) was followed to set up the experiment.

Fish were handfed a commercial diet (Table 1) at 2-3% of body weight to apparent satiation twice daily. Water temperature (27.56±0.86°C), pH (7.82±0.08) and dissolved oxygen (5.20±0.34 mg/L) were constant throughout this period.

Stress tests and sampling

The cold shock treatment consisted of transferring directly the fish from each replicate to 150 L tanks in which the water temperature was kept at 15°C by adding ice to the tanks. During the cold shock treatment (max. 24h) the temperature in the chilling tank was monitored and held stable by adding ice if necessary. An YSI model 55 probe was used during the cold shock to monitor water temperature and dissolved oxygen concentration. To account for handling procedures, fish from all treatments (the test and control groups) were transferred to tanks with the same initial water temperature (27.56±0.86°C). Food was withheld 24h before the onset of the cold shock. At each sampling point (prior to and after 1, 12 and 24h cold treatment), 3 fish were sampled at random from each experimental group and anesthetized with clove oil (50 mg/L). Blood samples were collected immediately after caudal vein amputation and transferred into sterile tubes and allowed to clot at room temperature for 1 h and then kept at 4°C for 5 h. Afterwards, serum was separated by centrifugation at 3000 g for 10 minutes and stored at –20°C until required.

Assays for determination of stress

Serum cortisol levels were measured by radioimmunoassay (RIA) and expressed as ng/mL.[22] The quantitative determination of glucose was carried out using commercially available diagnostic Experimental Protocols kits (Pars Azmun, Iran, 1 500 0178),[23] at 546 nm and 37°C according to the glucose oxidase method suggested by Trinder.[24]

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using the statistical software SPSS, version 11.0. All the measurements were made in triplicate. Significant differences between means were delineated by Duncan test. P<0.05 was considered significant.

Results

No differences in serum cortisol or glucose levels were found between fish from control and cold challenged fish at several time points of sampling (Figure 1).

No fish mortality was observed throughout 1h cold shock treatment in all experimental groups. However, the cumulative mortality reached to 50% after 12h and to 65% by the end of cold shock treatment (Figure 2). Nonetheless, the intensity of mortality was significantly reduced in second half compared to first half of 24h cold shock treatment (Figure 2).

Discussion

In the current study, none of the physiological parameters (cortisol and glucose values) measured in striped catfish changed at several time points of cold stress. Nevertheless, some of these parameters have been shown to change when fish are exposed to cold shock.[7,18,19,25,26] However, in a similar study, no significant changes either in cortisol or in glucose rate was detected immediately after 1h sudden cold exposure on the warm-water fish matrinxã (*Brycon amazonicus*).[20] Yet, after fish had been returned to the conditions prior to cold shock, a clear increase in plasma cortisol and glucose occurred in the cold-shock group. However, unlike this study, no recovery was arranged in our experiments, as fish were

Table 1. Proximate chemical composition of experimental diets.

Feed proximate composition	%
Dry mater	91.6
Protein	29.27
Fat	6.4
Ash	10.66
Carbohydrate	45.27

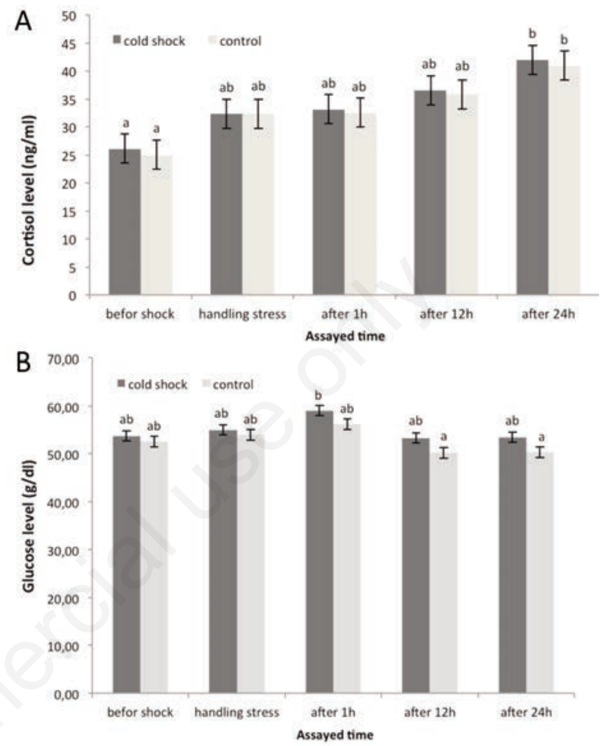

Figure 1. Values of serum cortisol level (A) and serum glucose concentration (B) of striped catfish challenged with a cold shock and sampled at time-matched sampling points (prior to, and at the end of 1, 12 and 24 h cold shock treatment). Data are expressed as mean ± standard error. Significant differences between values are indicated by different letters.

Figure 2. Cumulative mortality percentage recorded over a 12 and/or a 24h cold shock treatment in striped catfish. Data are expressed as mean ± standard error. Significant differences between values are indicated by different letters.

exposed to a constant cold stress for 24h.

In the present study, no mass mortality occurred in cold challenged fish as the highest rate of mortality reached to 65 percent detected by the end of 24h cold shock treatment. However, the intensity of mortality significantly decreased after 12h of imposing stress, likely due to a long-term acclimation to lower temperature, indicating that fish are really stressed despite no endocrine response. In fact, the lack of response would evidence the inability to adapt to cold, which could eventually lead to fish death. Indeed, in contrary to our results but in a similar condition, mass mortality of matrinxã due to sudden decrease of water temperature has been reported.[20]

It is equally difficult to explain the lack of endocrine response. One possibility is that the activity of the enzymes involved in steroid and glucose synthesis were altered (possibly down-regulated) by the low temperature.[20,27] Roach *Rutilus rutilus* L., which were confined during winter (5°C) had much lower post-stress plasma cortisol levels than fish confined during the summer (16°C).[28] Other studies in striped bass (*Morone saxatilis*) and sunshine bass (*Morone chrysops* × *Morone saxatilis*) have shown that cold water temperature had no effect or lowered plasma cortisol.[29]

The rate of cortisol clearance is another step in the cortisol cycle that may be influenced by environmental factors. Liver is the key organ for cortisol disposal with the hepato-biliary system as the main biochemical pathway for cortisol clearance.[30,31] However, the efficiency of that process is reported to be altered by stress, salinity, maturity, nutritional state, etc.[32]

Conclusions

In conclusion, reasons for the apparent low responses to cold stress in striped catfish are not known but may relate to their evolutionary history, neuroendocrine mechanisms involved in their corticosteroid responses, or anatomy of their interrenal tissues structure. Similar to our work, previously many studies utilized cortisol and glucose as sole stress indicators in fish, however, regarding the several factors that can affect these responses, one should consider that cortisol and glucose are not enough as stress indicators.[21] In fact, there are some inconsistencies in the results of various experiments that in some cases would be attributed to unknown situations.[21] Iwama *et al.*[33] argued that *none of the current indicators of stress are 100% suitable in reflecting stressed states in fish and recommended to complement cortisol and glucose with other stress indicators to establish a more complete profile of the experimental organism*. For example, glutamine synthase has been observed to increase even with small response of cortisol.[34] Moreover, there are some other important stress indicators such as catecholamines,[35] melanocyte stimulating hormone (α-MSH),[36-39] lactate,[40] lysozyme,[41,42] as well as heat shock proteins that should be taken into account to study fish stress responses.[33]

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