The effect of acute handling stress on cortisol level, growth and reproductive performance in striped catfish (*Pangasianodon hypophthalmus*)

Wahyu Pamungkas, Jadmiko Darmawan, Evi Tahapari

Research Institute for Fish Breeding, Ministry of Marine Affairs and Fisheries, Subang, West Java, Indonesia. Corresponding author: W. Pamungkas, yhoe_pamungkas@yahoo.co.id

Abstract. An experiment was conducted to study the effect of an acute handling stress on the cortisol level, growth and reproductive performance in striped catfish (*Pangasianodon hypophthalmus*) during the rearing period. Thirty striped catfish were used and equally divided into two groups (A and B). Group A was subjected to acute handling stress, while group B was not subjected to stress (control group). Fifteen females with a bodyweight of 2,740±625.59 g for each group were used as experimental fish. The cortisol concentrations, growth and reproductive performance of the catfish were measured to evaluate the response of the fish specimens to the treatments. The research data was analyzed by an independent sample T-test. Cortisol levels showed an increase in the group A (218.75±64.21 pg mL^{-1}) compared with the Group B (181.01±35.64 pg mL^{-1}). The growth and reproductive performance in the group A were not significantly different from the group B. Overall data suggested that the acute handling stress during the research increased the level of cortisol but did not significantly affect the growth and reproductive performance of the striped catfish.
Key Words: striped catfish, blood sampling, stress, growth, reproductive performance.

Introduction. Striped catfish, *Pangasianodon hypophthalmus*, is one of the main products of aquaculture on the international market (McGee 2014). *P. hypophthalmus* is an important freshwater fishery commodity in South and Southeast Asia, including Malaysia (Asdari et al 2011), Vietnam (Bui et al 2010), Bangladesh (Ahmed & Hasan 2007), Indonesia (Griffith et al 2010) and have great economic value in India (Paniyar et al 2014). According to the Ministry of Marine Affairs and Fisheries, Republic of Indonesia (2013), the increase of striped catfish production in Indonesia from 2010 to 2013 reached 95.57%. The Ministry of Marine Affairs and Fisheries, Republic of Indonesia (2018) also reported that the price of this species ranged between USD 1.4-2 kg^{-1}.

Several studies have been carried out to increase the production of catfish including improving the reproductive performance through hormonal induction (Pamungkas et al 2019) and manipulation of the broodstock diet (Dewi et al 2017; Arfah et al 2018). Most sources of stress in aquatic research during the experiment and collecting data may be unavoidable. Fish handling, holding, measuring length and weight, inducing hormones and collecting blood samples are routine practices that can have significant effects on fish physiology and survival. They are included in the acute stress of handling fish, in a research activity. These effects can include decreased immune system, growth, swimming performance, reproductive performance and mortality (Barton & Iwama 1991; Barton et al 2002).

Stress can be defined as a condition where a fish's internal homeostasis is impaired from a stressor (Bonga 1997; Barton et al 2002). The stress response in teleost fish was classified as primary, secondary and tertiary responses (Figure 1). The release of catecholamines (e.g. epinephrine, norepinephrine) into the circulatory system from chromaffin cells is one of the primary stress response of teleost fishes. This stress response also stimulates the hypothalamic-pituitary-interrenal (HPI) axis to release corticosteroids (e.g. cortisol) from the interrenal tissue (Mazeaud et al 1977; Bonga 1997; Mommsen et al 1999; Barton et al 2002).

The stressed fish will show lower energy reserves, poorer conditions, reduced growth and impaired reproduction due to a decreased availability of energy (Smolders et al 2005). Disorders of eating behavior, such as reduced food intake, are common in the behavioral response to stress in fish (Bernier & Peter 2001; Bernier 2006). Stressful conditions are known to induce reductions in feed conversion efficiency (FCE) that can lead to decreased growth rates even when food intake levels are maintained (Barton et al 1987; Gregory & Wood 1999; Paspatis et al 2003; d'Orbcastel et al 2010). Under stressful conditions, fish eat less and grow more slowly than unstressed fish.

Based on the foregoing previous studies, a study on the physiological response of *P. hypophthalmus* to acute handling stress during the rearing period was carried out. The study aimed to evaluate the effect of acute handling stress on cortisol level, growth and reproductive performance in *P. hypophthalmus*.

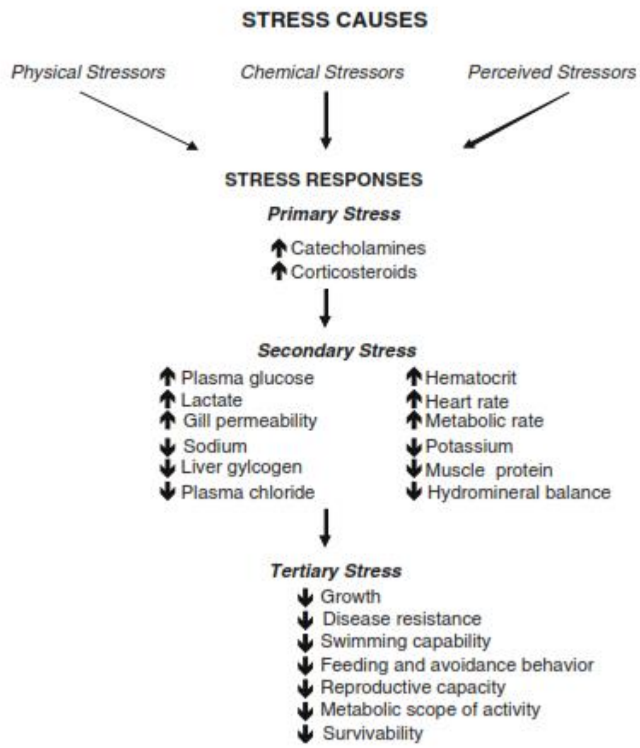

Figure 1. Physiological responses to stressors (Barton et al 2002).

Material and Method. This study was conducted from April to June 2019 at the Research Institute for Fish Breeding (RIFB), Ministry of Marine Affairs and Fisheries, West Java, Indonesia. This research does not require ethical approval from any institution and there are no such applicable laws in Indonesia.

Experimental fish. This study was carried out at the Research Institute for Fish Breeding (RIFB), Ministry of Marine Affairs and Fisheries, West Java, Indonesia. The fish used in the study was *P. hypophthalmus* obtained from broodstock populations at the RIFB. The striped catfish females (bodyweight 2,740±625.59 g) were used as the experimental fish. *P. hypophthalmus* were acclimated to experimental conditions for 2 weeks before treatment. Thirty fishes was kept into six net cages (3x5x1.5 m) located in the 6,000 m^2 of an earthen pond, at a stocking density of five fish per cage for 8 weeks. During the experiment, the fish were fed two times a day using commercial feed (38% of crude protein) at 3% of the body weight (BW).

The following experimental protocol was used: fish were divided into two groups (Groups A and B) of 15 fish each. Fish of Group A were subjected to acute handling stress (blood sampling every two weeks), while those of Group B were not subjected to stress (control group).

Cortisol concentration. Blood samples were collected for measurements of cortisol concentration on the intial and final study. 3 mL of blood was collected from each tested fish using a heparinized syringe containing phenylmethylsulfonyl fluoride (100 µL, 1 mM), PMSF (Roche, Germany). The blood obtained was stored in ice and centrifuged at 3,000 rpm for 15 minutes at 4°C. The supernatant (plasma) was stored at -20°C before analyzing by ELISA (EIA1561 DRG International Inc., Marburg, Germany) to measure the cortisol level. Five plasma samples from each experimental group were mixed and homogenized and the mixed plasma was used to measure the cortisol concentrations. Because the data represent the averages of pooled samples for each group and do not account for the variation among individuals, they were not analyzed for statistical significance.

Growth performance. Observations on the growth performance of striped catfish broodstock were done on the initial and final study including initial weight, final weight and daily growth rate.

Reproduction performance. Reproductive parameters of striped catfish during the experiment were obtained at the end of the research. These parameters were fecundity, ovi somatic index, fertilization rate and larval production.

Data analysis. The data of cortisol concentration, growth and reproductive performance were analyzed statistically using Microsoft Excel 2016 and the SPSS program (ver. 25). Data were analyzed using the Independent Sample T-test.

Results

Cortisol concentration. The cortisol concentration in the blood plasma of striped catfish were 61.92±3.17 at the beginning of the study and 218.75±64.21 pg mL^{-1} at end of the study, for the Group A), and 59.68±6.34 at the beginning of the study and 181.01±35.64 pg mL^{-1} at end of the study, for the Group B as represented in the Figure 2. Blood sampling every two weeks increased cortisol level in the blood plasma of striped catfish. It showed that the tested fish was under stress during the rearing period.

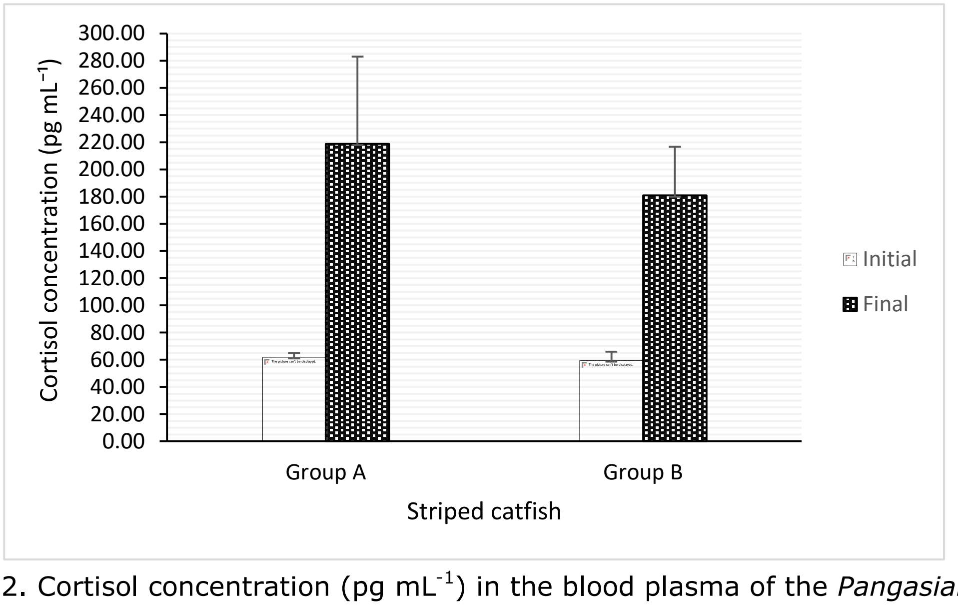

Figure 2. Cortisol concentration (pg mL^{-1}) in the blood plasma of the *Pangasianodon hypophthalmus*.

Growth performance. The growth performance of the striped catfish broodstock are shown in Table 1. The result showed that the blood samples collection every two weeks affected the fish growth. There is significant difference in the daily growth rate of fish between the two groups.

Table 1

Initial weight, final weight and daily growth rate of *Pangasianodon hypophthalmus* broodstock

Parameter	*Group A*	*Group B*
Initial weight (g)	2,709±762.56 [a]	2,653±248.71 [a]
Final weight (g)	3,879±1,129.8 [a]	4,260±424.76[a]
Daily growth rate (%)	0.60±0.06 [a]	0.79±0.05 [b]

Mean values in the same row with different superscript letters show significant differences between the groups ($p<0.05$).

Reproductive performance. Reproductive parameters of striped catfish collected blood samples are presented in Table 5. The results showed that the values of fecundity, ovi somatic index, fertilization rate and hatching rate had no significant differences between the groups. It means that the blood samples collection every two weeks during the study does not affect on the reproductive performance of the striped catfish females.

Table 2

Fecundity, ovisomatic index, fertilization rate, hatching rate and larval production of *Pangasianodon hypophthalmus*

Parameter	*Group A*	*Group B*
Fecundity (egg kg^{-1})	315,840±48,747[a]	395,300±49,011[a]
Ovi somatic index (%)	16.99±1.61[a]	18.15±2.63[a]
Fertilization rate (%)	85.13±3.16[a]	81.51±4.51[a]
Hatching rate (%)	85.24±5.80[a]	87.35±7.56[a]
Larval production (larva/female)	285,834±20,653[a]	343,084±22,531[b]

Mean values in the same row with different superscripts letter show significant differences between the group ($p<0.05$).

Discussion. The results of the study demonstrated that the handling stress affected the cortisol level of the striped catfish female. The "group A" fish specimens (blood samples collected every two weeks) showed a higher cortisol concentration than group B specimens (no blood samples collection). The increased levels and production rates of cortisol in the striped catfish is a response to the stressor. In fish, cortisol acts as a regulatory factor for a variety of physiological functions under normal conditions and also allows for rapid physiological adjustment in theface to a stress exposure. Cortisol is involved not only in the stress response but also in several processes such as regulation of metabolism, osmoregulation, behavior, growth and reproduction (Mommsen et al 1999). Given its importance as a modulator of various physiological processes, changes in plasma cortisol are considered to be the primary response to potential stressors. Cortisol increases dramatically during stress and is a major mediator of the stress-related responses. Studies on fish stress responses were conducted in order to evaluate the changes of cortisol levels due to the rising water temperatures (Sweeney et al 2015), to the catching and transporting (Bolasina 2011) and the acute stress exposure, especially on zebrafish (Ghisleni et al 2012). Cortisol plays an important role in stimulating several processes, such as the energy metabolism, oxygen uptake, gluconeogenesis and it also inhibits the glycogen synthesis (Bonga 1997; Mommsen et al 1999; Barton et al 1987; Morgan et al 1996; Tort 2010). Cortisol also modulates tissue inflammatory responses through an inhibitory effect on the cytokine production (Aluru & Vijayan 2009) and attenuates the heat shock protein response to thermal insult (Ackerman et al 2000; Basu et al 2001).

The blood sampling caused a stress in the striped catfish and influenced their growth. The daily growth rate of the group A specimens was lower than in group B (with a significant difference). Causes for stress-induced growth retardation are diverse. A reduction in the food intake levels and disruptions of the feeding behavior are common features of the fish behavioral response to stress (Bernier & Peter 2001; Bernier 2006). Under stressful conditions, fish eat less and grow more slowly than in the absence of stress. Stressful conditions are known to also reduce the feed conversion efficiency (FCE), leading to decreased growth rates even when food intake levels are maintained (Barton et al 1987; Paspatis et al 2003; d'Orbcastel et al 2010; Gregory & Wood 1999).

There are several pathways through which stress may affect growth. Pickering (1993) states that most of the anabolic and catabolic hormones are involved in regulating the growth changes induced by stress. The cortisol levels increase, following an acute or a chronic stress, has been strongly linked to higher plasma glucose and energy mobilization following stress (Schreck 1981; Gamperl et al 1994). Cortisol is essential for normal growth, but an increase above the 'resting' rate that occurs after stress can reduce growth (Schreck 1993). Many studies have reported that the cortisol administration can reduce the growth in rainbow trout and channel catfish *Ictalurus punctatus* (Davis et al 1985; Barton et al 1987), providing a direct relationship between this hormone and stress-related changes in growth.

The reproductive performance of fish showed that the group A specimens (that received a stressful treatment) had a lower fecundity, ovi somatic index and hatching rate values than group B. However, statistically, the range of values obtained between group A and B was not significantly different. There is a significant difference between larval production in groups A and B. The fish in group A produced fewer larvae than group B.

Besides having an effect on the growth, stress can also negatively affect the reproductive fitness of adult females (Schreck et al 2001), by either lowering their fecundity (number of eggs ovulated) or by decreasing the quality of the ovulating eggs. Females fish under stress must divide the available energy between recovery from stress or tolerance of stressors and energy investment in the eggs maturation at an acceptable level of quality, before ovulation (Schreck & Tort 2016). A stressed female may not invest in maturing eggs and spawning, but it might delay spawning and potentially lose any investment already made in the eggs production. This causes the production of atretic (nutritionally deficient) eggs and of a high numbers of lower fitness offspring. Corticosteroid hormones may highly participate in the modulation of the reproductive endocrine control in both sexes (Milla et al 2009), targeting a maximal reproductive investment into a smaller number of eggs, but with a higher nutritional content and fitness.

Based on the results obtained, it can be stated that the acute handling stress increases the cortisol levels and induces a decrease in the growth and reproductive performance of fish. Stress conditions in fish not only affect physiological conditions but also affect the quality of fish production.

Conclusions. The results of this study show that the acute handling stress affects the cortisol level, growth and reproductive performance and suggest that the changes of these parameters are particularly useful for monitoring the stressful conditions of the aquaculture production, which affect not only animal welfare but also the quality of the fish products.

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Conflict of interest. The authors declare no conflict of interest.

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Authors:
Wahyu Pamungkas, Research Institute for Fish Breeding, Ministry of Marine Affairs and Fisheries, Jl. Raya 2 Sukamandi, Subang 41256, West Java, Indonesia, e-mail: yhoe_pamungkas@yahoo.co.id
Jadmiko Darmawan, Research Institute for Fish Breeding, Ministry of Marine Affairs and Fisheries, Jl. Raya 2 Sukamandi, Subang 41256, West Java, Indonesia, e-mail:micho_jad@yahoo.co.id
Evi Tahapari, Research Institute for Fish Breeding, Ministry of Marine Affairs and Fisheries, Jl. Raya 2 Sukamandi, Subang 41256, West Java, Indonesia, e-mail:evitahapari@yahoo.co.id

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