

Effect of water temperature on the physiological stress and growth performance of tapah (*Wallago leeri*) during domestication

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Abstract. The objectives of the present study were to analyze the effect of temperature regimes on stress levels and to determine the suitable water temperature for the domestication of tapah, *Wallago leeri* Bleeker. A completely randomized method was used in this study, and three levels of water temperature were tested, namely, 27, 29, and 31°C, in seven replicates. A total of 500 experimental fish were collected from the Kampar River, Langgam, Riau, Indonesia, and transported to the Aquaculture Laboratory, Riau University. The fish were reared in 21 tanks at stocking densities of 70-100 fish tank⁻¹. The fish were fed rough fish three times a day at 08:00, 12:00, and 21:00 for 30 days. Blood samples were taken on days 1, 14, and 29, which were analyzed for cortisol, glucose, and plasma protein. Growth and survival rates were recorded at the end of the experiment. ANOVA showed that water temperature had a significant effect on the stress condition, growth, and survival rate of tapah. Lower blood glucose, protein, and cortisol plasma levels were recorded in fish acclimatized at 29°C with values of 14.44 mg dL⁻¹, 5.35 g dL⁻¹, and 6.90 mg mL⁻¹, respectively. Additionally, higher growth performance (32.30 g) and survival rates (100%) were also found in fish reared at 29°C. It was concluded that the best water temperature for the domestication of tapah is 29°C.

Keywords: blood protein plasma, blood glucose, cortisol plasma, growth rate, survival rate

Introduction

Tapah, *Wallago leeri* Bleeker is a commercial freshwater fish in Riau waters, Indonesia. This species is distributed in southeast Asia from Thailand to Indonesia (Froese and Pauly 2017). Tapah is the main target of local fishermen in Riau Province, Indonesia. Intense fishing of this species over the last decade has resulted in declining wild populations of *W. leeri* (Utomo 2016). In addition, the tapah population is also threatened by habitat degradation from deforestation that is prompting increases in temperature and turbidity and leading to lowered dissolved oxygen concentrations. Studies on tapah are scarce; the previous study on this species focuses only on morphometric characteristics (Nathasya and Elvyra 2014), photoperiod, and feeding regimes (Giri et al. 2002).

The cultivation of tapah has been initiated to prevent declines in tapah populations, and it is based on collecting fingerlings from the wild. However, tapah cultivation has not been optimal, because the breeding technology is not well understood. Therefore, it is crucial to develop breeding technology. For this purpose, the availability of high-quality broodstock is

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critically important. Therefore, the domestication process is the first step in overcoming this problem (Muchlisin 2013).

The domestication of tapah has been initiated in Riau Province, Indonesia. However, this attempt has faced several obstacles, including the fact that many broodstock candidates collected from the wild died during the domestication process from stress in the new environment in ponds. Stress was caused by environmental changes (Biron and Benfey 1994) especially temperature fluctuation, dissolved oxygen deficiency, pH, and high ammonia concentration during transport from the field to the domestication ponds (Brown 1993, Sandodden et al. 2001, Van Ham et al. 2003, Hoskonen and Pirhonen 2006, Rollo et al. 2006). Sudden temperature changes can cause difficulties in fish acclimatization that disturb physiological processes (Xafri et al. 2013) and causes cell damage and death (Affandi and Tang 2003). Changes in water temperature increase energy requirements from blood glucose for thermogenesis, which can be met if the glucose in the blood can immediately enter the target cells. The success of glucose transport to cells is specified by insulin activation and insulin-like growth factors (Fenn et al. 2016). Additionally, the role of thyroid hormone in controlling osmoregulatory and metabolic rates is crucial since osmoregulatory and metabolic rates are also affected by temperature (Peter 2011).

Studies of the effects of temperature on metabolic rates and growth performance have been conducted on several species, for example, *Rhamdia quelen* (Quoy & Gaimard) (Lermen et al. 2004), *Cyclopterus lumpus* L. (Nytrö et al. 2014), and *Polyprion oxygeneios* (Schneider & Forster) (Tromp et al. 2016). In addition, Long et al. (2012) studied temperature stress responses on the development of larval zebrafish, *Danio rerio* (Hamilton), and they found that the development of zebrafish larvae was significantly delayed under cold stress, while heat stress also exerted an inhibitory effect, which was lower than that of cold stress. Indeed, Giri et al. (2014) studied the role of HSP70 in cytoplasm protection against thermal stress in rohu, *Labeo rohita* (Hamilton), and they found that the LrHSP70 gene

plays an important role in response to thermal and cold stress in fish. However, there was no information on the effect of temperature regimes on the physiological performance of tapah during domestication. This information is useful when designating the optimum temperature for acclimatization during tapah domestication. Hence, the objectives of the present study were to analyze stress levels of domesticated tapah under various temperature regimes and to determine the optimum water temperature for tapah domestication.

Materials and methods

Experimental design

The experiment was conducted in the Laboratory of Aquaculture Technology, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia, from November 2014 to April 2015. A completely randomized design was used in this study. Three temperature regimes were tested, namely, 27, 29, and 31°C, and every treatment was conducted in seven replicates. A total of 21 aquarium tanks (80 cm × 40 cm × 40 cm) were used, and every tank was equipped with aerator and a heater which was adjusted to the respective temperature being tested.

Experimental fish collection

A total of 500 fingerling tapah with an average weight of 45.00 ± 3.98 g were collected from local fishermen at the Kampar River, Langgam Village, Riau Province, Indonesia. The fish were starved for 24 hours prior to transport to the laboratory. The fish were stocked into plastic containers (100 L) at a stocking density of 70-100 fingerlings, and every container was equipped with portable aerator, and then the fish were transported to the laboratory. The blood protein, glucose, and cortisol plasma levels were measured to analyze the stress condition of the experimental fish.

Acclimatization process and blood sample analysis

The fish were distributed into experimental tanks (96 L) at respective water temperatures. The fish were fed rough fish three times a day at 08:00, 12:00, and 21:00 at 3% body weight for 30 days, and weight gain was measured at bi-weekly intervals. Three fish from every treatment were chosen randomly, and blood samples were collected from the caudal vein at the ventral vertebrae using a sterile syringe. The blood was placed in Eppendorf tubes, and then centrifuged at 3000 rpm for 10 minutes (Alishahi and Buchmann 2006). The blood plasma was removed and placed in new tubes and kept at -150°C in a freezer prior to further analysis. The blood samples were collected three times during the study on days 1, 14, and 29.

Blood plasma cortisol analysis

Cortisol levels were examined with the radioimmunoassay (RIA) method based on Syawal et al. (2012). A total of 25 μL blood plasma was taken and placed in a polypropylene tube, and 1 ml of Coat-A-Count Cortisol reagent that contains radioactive cortisol was added to the tube, which was then centrifuged for 5 min. The solution was incubated for 45 min at 37°C . The cortisol level was calculated using a gamma counter (Anderson and Siwicki 1995).

Blood plasma glucose analysis

A total of 1000 μL glucose reagents were mixed with 10 μL blood plasma, the mixed solution was left for 15 min to become pink, and then the solution was transferred into a curved glass (1.5 mL) for spectrophotometry analysis at 546 wavelengths. A total of 10 μL standard solution and 1000 μL glucose reagents were mixed and used for spectrophotometry analysis at a similar wavelength for comparison. The blood glucose value was examined using the GOD-PAP Enzymatic Photometry Test (Thomas 1998).

Total plasma protein analysis

The total plasma protein was examined based on Anderson and Siwicki (1995) as follows: the blood samples were taken from the caudal fin with anticoagulant syringes rinsed in 3.8% Na citrate. The blood was centrifuged at 1000 rpm for 5 min. A total of 5 μL plasma was transferred into a microtube; then 25 μL reagent A and 200 μL reagents B (Bio-Rad, Hercules, CA) were added to the plasma solution and then stirred homogeneously. The mixed solution was incubated for 15 min at room temperature and then examined for plasma protein absorbance at wavelengths of $\lambda = 595\text{-}610\text{ nm}$.

Weight gain and survival rate

The survival rate was calculated based on Muchlisin et al. (2016) as follows: $\text{SR} = (\text{No} - \text{Nt}) \times \text{No}^{-1} \times 100$, where SR is the survival rate (%), Nt is the total number of fish that died during the experiment, and No is the total number of fish at the start of the experiment. Weight gain was calculated as follows: $\text{Wg} = \text{Wt} - \text{Wo}$, where Wg is weight gain (g), Wt is the average body weight of the fish at the end of the experiment (g), and Wo is the average body weight of the fish at the start of the experiment (g).

Data analysis

The data was subjected to the Analysis of Variance (ANOVA) test and followed by a comparison of means using the Duncan multiple-range test at a confidence level of 95% (Sofyan and Werwatz 2001). All statistical analyses were performed using SPSS software ver. 13.0.

Results

The blood glucose level of the tapah before transport was 18.22 mg dL^{-1} , and the blood glucose levels were increased to $33.29\text{-}43.83\text{ mg dL}^{-1}$ after transport from the field to the laboratory (Table 1); then the values decreased to $14.95\text{-}36.64\text{ mg dL}^{-1}$ after

Table 1

Total blood glucose levels of tapah (*W. leeri*) before and after transport. Mean values (\pm SD) in the same column followed by a different superscript are significantly different ($P < 0.05$)

Transport tank no.	Total blood glucose before transport (mg dL ⁻¹)	Total blood glucose after transport from field to laboratory (mg dL ⁻¹)
1	18.22	33.29
2	18.22	33.29
3	18.22	43.83

Table 2

Protein and cortisol plasma of tapah (*W. leeri*) acclimatized at three temperatures. Mean values (\pm SD) in the same column followed by a different superscript are significantly different ($P < 0.05$)

Acclimatization temperature	Total blood glucose after acclimatization (mg dL ⁻¹)	Protein plasma (g dL ⁻¹)	Cortisol plasma (mg mL ⁻¹)
27°C	24.27 \pm 5.68 ^b	4.05 \pm 0.90 ^a	20.32 \pm 1.84 ^b
29°C	14.95 \pm 2.30 ^a	4.99 \pm 0.79 ^a	6.90 \pm 4.52 ^a
31°C	36.64 \pm 5.08 ^c	2.77 \pm 0.44 ^b	88.10 \pm 4.22 ^c

Table 3

Weight gain and survival rates of tapah (*W. leeri*) in three water temperatures for 30 days of the experiment. Mean values (mean \pm SD) in the same column followed by a different superscript are significantly different ($P < 0.05$)

Water temperature	Average weight gain (g)	Average survival rate (%)
27°C	26.82 \pm 5.54 ^b	95.83 \pm 10.20 ^b
29°C	32.30 \pm 5.64 ^c	100.00 \pm 0.00 ^b
31°C	2.95 \pm 2.61 ^a	66.67 \pm 12.91 ^a

acclimatization. Lower glucose levels were noted in fish acclimatized at 29°C (Table 2). The protein and cortisol plasma levels of the tapah after acclimatization ranged from 2.77 to 4.99 g dL⁻¹ and from 6.90 to 88.10 mg mL⁻¹, respectively (Table 2). The acclimatization temperature had a significant effect on blood glucose, protein plasma, and cortisol plasma levels (ANOVA, $P < 0.05$). The lowest protein plasma level was noted at 27°C, but this value was not significantly different from that of the protein plasma at 29°C, while the lowest cortisol plasma was recorded at 29°C, and this value was significantly different from the other treatments ($P < 0.05$).

The average weight gain range was 2.95 to 32.30 g, while survival rate ranged from 66.67 to 100%. Water temperature had a significant impact on tapah

weight gain and survival rates (ANOVA, $P < 0.05$). The Duncan multiple-range test showed that the highest weight gain was noted in the fish reared at 29°C, and that this value was significantly different from 27 and 31°C, while the highest survival rate was also recorded at 29°C, but this value was not significantly different that at 27°C (Table 3). The results revealed that higher weight gain and survival rates were recorded in fish reared at 29°C; the conclusion is that this is the suitable water temperature for tapah during domestication. Lower weight gain and survival rates were noted in the fish reared at 31°C. This is probably due to stress that disrupted metabolic processes. Direct observations of the fish reared at 31°C showed that they were not responding to feed and passive swimming activity was detected.

Discussion

The study revealed that temperature plays an important role in acclimatization, with increased blood glucose and cortisol plasma levels during transport that decreased after acclimatization, and the best results were noted at 29°C. This finding concurs with the results of Kubulay and Ulukoy (2002), who report that water temperature is a crucial stress factor in rainbow trout, *Oncorhynchus mykiss* (Walbaum), and that higher temperature fluctuations cause acute stress in these fish.

Water temperature is an important immune system regulator in fish (Bozorgnia et al. 2011); low ambient temperatures increase pressure on fish immune systems, both specific and non-specific (Koeypudsa and Jongjareanjai 2010). Additionally, water temperature also significantly affects food intake, metabolic rates, enzyme activity, membrane function, and protein synthesis (Wedemeyer 1996); therefore, water temperature significantly affects fish growth performance as was indicated in the present study. This finding concurs with Nytro et al. (2014), who reported that the growth performance of lumpfish, *C. lumpus*, is affected by water temperature. A similar study has also been published on *P. oxygenation* (Tromp et al. 2016). Moreover, temperature stress inhibits larval development in *D. rerio* (Long et al. 2012). Water temperature also affects the activity of metabolic enzymes, for example, proteinase. These enzymes play an important role in catabolic and anabolic processes that provide fish with energy (Kuz'mina et al. 2008). According to Affandi and Tang (2003), metabolic rates increase with increases in the activity of metabolic enzymes resulting in an increase in the metabolic rate in the blood. When the metabolite level in the blood increases, fish become hungry; this stimulates the appetite and feed consumption increases. High feed intake increases the energy supply for basal maintenance and growth.

Blood glucose and cortisol levels are frequently used to assess stress in fish (Biron and Benfey 1994, Vijayan et al. 1997, Wendelaar Bonga 1997, Mommsen et al. 1999, Kucukgul and Sahan 2008, Syawal et al. 2012). The results of the present study

indicate that glucose levels increased during transport from the field to the acclimatization pond. Increased blood glucose levels are also accompanied by increased blood cortisol levels. However, glucose levels declined after acclimatization, and the best levels were noted at 29°C. High blood glucose levels can reduce appetite and inhibit growth and the reproductive development of fish (Cuban and Sen 2011). This is because energy needs are supplied by the blood glucose (Hesser 1960). In contrast, when blood glucose levels are low, the fish feel hungry and feed to meet increased energy needs. This phenomenon is crucial during weaning in the domestication process when fish have to switch from feeding on wild to domestic foods. The study showed that, at 29°C, the blood glucose levels decreased more sharply and were closer to those of wild fish. This suggests that the ideal temperature for the acclimatization of tapah was 29°C.

In general, the results revealed that the best protein and cortisol plasma levels were found in fish acclimatized at 29°C. At this temperature, blood protein was the highest and cortisol plasma was the lowest compared to other treatments. The direct measurement of cortisol plasma of unstressed (normal) fish ranged from 3.23 to 10.29 mg mL⁻¹, while in stressed fish it ranged from 77.36 to 135.34 mg mL⁻¹. This is an indication that the metabolic processes of fish run well at this temperature, because the fish utilize food effectively. Increasing blood cortisol levels causes declines in the blood plasma protein that negatively affects growth and survival rates as indicated in this study.

In addition to applying a suitable ambient temperature during acclimatization, as in the current study, the application of an active compound of raw materials in the diet also successfully reduced temperature stress and enhanced fish growth performance and survival rates. For example, Kazeem et al. (2017) used an aqueous extract of *Moringa oleifera* leaf in the feed to counter hyperthermal-induced stress effects on Nile tilapia, *Oreochromis niloticus* (L.), and they found that the survival rates of the fish were higher than in the control group. Yanti et al. (2013) and Dewi et al. (2013) used jaloh leaf, *Salix tetrasperma*, extract to reduce stress and enhance growth rates of Nile tilapia

and African catfish, *Clarias gariepinus* (Buchell), respectively.

Conclusions

Water temperature had a significant effect on the blood protein, blood glucose plasma, blood cortisol plasma, growth performance, and survival rates of tapah. The results indicate that the best water temperature for the domestication of tapah is 29°C.

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