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Original Article

Impact of water temperature and sodium chloride (NaCl) on stress indicators of hybrid catfish (*Clarias gariepinus* Burchell x *C. macrocephalus* Gunther)

Weena Koeypudsa* and Malinee Jongjareanjai

Department of Veterinary Medicine, Faculty of Veterinary Science,
Chulalongkorn University, Pathum Wan, Bangkok 10330, Thailand.

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Abstract

This research was composed of 2 experiments. Short-term (1 day) and long-term (30 days) exposure were conducted in the laboratory. Each experiment had 2 temperature levels, high (Ht, 29.5 ± 0.5°C) and low temperature (Lt, 19.5 ± 0.5°C). Initial weight and length of catfish were 7.54±1.82 g and 9.90±0.96 cm respectively. Experimental catfish were subjected to 4 conditions as follows: high temperature with 0.1% sodium chloride (HtWs), high temperature without 0.1% sodium chloride (HtW/s), low temperature with 0.1% sodium chloride (LtWs), and low temperature without 0.1% sodium chloride (LtW/s). Blood was taken from caudal vessel of anaesthetized fish to investigate blood clotting time, cortisol, glucose, osmolarity, Na^+, K^+ and Cl^-. Ratios of Na^+/K^+, Na^+/Cl^- and $Na^+ + K^+ / Cl^-$ were also analyzed. In the short-term (1 day) experiment, values of all catfish blood parameters varied. These imply that catfish attempt to maintain internal balance, homeostasis. Osmolarity exhibited complete homeostasis in 2 h. From long-term (30 days) exposure, non-significant means of Na^+/Cl^- ratio (HtWs) and decreasing trend lines direction of blood clotting time (HtWs, LtW/s, LtWs) indicated that 0.1% sodium chloride and/or Lt helped stress reduction in catfish. Sum of Na^+ and K^+ to Cl^- ratio among 4 groups (HtW/s, HtWs, LtW/s, LtWs) revealed that catfish spent 10 days for adjustment themselves under stress circumstance (HtWs, LtW/s, LtWs) to natural situation (HtW/s). This information could be useful to improve the survival rate and health condition during rearing, handling and transporting aquatic animals.

Keywords: sodium chloride, stress indicator, stress reduction, fish health, fish blood

1. Introduction

Fish are subject to stress everyday. Changes in culture system, water quality, environment, fish physiology and social condition constitute stress factors. Stress disturbs the fine internal balance, homeostasis, and has further detrimental effects on behavior, growth, reproduction, immune function and disease tolerance (Tanck *et al.,* 2000; Goos and Consten, 2002; Chen *et al.,* 2004; Morales *et al.,* 2005). Fish have developed physiological and biochemical adaptations to cope with these constraints that minimize or eliminate the deleterious effects, which is called stress response.

The stress response is divided into primary, secondary and tertiary responses (Goos and Consten, 2002; Ham *et al.,* 2003; Davis, 2004). The primary response involves the release of stress hormones, catecholamines and corticosteroids. The secondary response is a physiological response resulting from the action and effect of stress hormones. Tertiary responses are manifest in somatic growth and obtain population level.

The evaluation of haematological parameters has provided a tool for facilitating fish health management (Chen *et al.,* 2004). Moreover, blood chemistry parameters are used as indicators of physiological stress response in fish (Lerman *et al*., 2004; Koeypudsa *et al.,* 2007). Classical stress

* Corresponding author.
Email address: kweena@chula.ac.th, kweena@hotmail.com

indicators are catecolamine, cortisol, haematocrit, haemoglobin, glucose, lactate, amino acid and liver glycogen (Guerriero *et al.,* 2002; Morales *et al.,* 2005; Koeypudsa *et al.,* 2006).

To reducing stress, sodium chloride (NaCl, salt) is recommended (Velasco-Santamaria and Cruz-Casallas, 2008; Koeypudsa and Kitkamthorn, 2009). Anti-stress accompanied with salt maintains stable blood electrolyte levels by reducing the osmoregulation and decreasing ionic imbalances (Tsuzuki *et al.,* 2001; Harpaz *et al.*, 2005). Sun *et al.* (1995) stated that fresh-water tilapia, cultured at 24°C, was unaffected by chill coma at 12°C when 1.5 % salt added. Andrews *et al.* (2002) reported that application salt at the level of up to 0.3% could be used to help reduce stress associated with physical damage and high nitrite level. However, the authors also revealed that concentrations above 1.0% salt were stressful to fish.

Ascorbic acid (vitamin C) also prevents fish from stress (Datta and Kaviraj, 2003; Dabrowski *et al.*, 2004). Barton (2000) additionally reported that stress can be reduced by overcrowded avoiding and by the administration of anaesthetic drugs or chemical agents to sedate fish, *e.g.,* ketamine - HCl, carbon dioxide, tricaine methane sulphonate (MS222), clove oil, benzocain, ether and quinaldine sulfate.

Low temperature, hypothermia, is a non-chemical technique to tranquilize fish and applies only to temperate fish (Ross and Ross, 1999). This method causes immobilization and a reduction in sensitivity, but is not included anaesthesia or analgesia (Sun *et al*., 1995; Ross and Ross, 1999). Overall, cooling has good calming properties and facilitates handling and transportation. Davis (2004) recommended that fish tolerate handling better when water temperature is low.

As salt and low temperatures are advantageous to fish, cooling in combination with slight raising of the salinity may have a synergistic benefit for aquaculture. This research focused on the physiological response in catfish when exposed to low temperature with and without salt, and high temperature with and without salt. Stress indicators were analyzed of primary, secondary and tertiary stress response levels. It was expected to identify conditions that favorable to reducing the stress response, which could be beneficial in fish health management and lead to improvements in aquaculture practice.

2. Materials and Methods

2.1 Experimental catfish

Hybrid catfish (*Clarias gariepinus* Burchell x *C. macrocephalus* Gunther) were purchased from a private fish farm in Suphanburi Province. This catfish, which has been artificially crossbred (Koeypudsa *et al.*, 2006). The fish had initial body weight of 7.54±1.82 g and a total length of 9.90± 0.96 cm. They were fed to satiation with floating commercial pellet feed (Win, Lee Feed Mill Public Company Limited, Bangkok), once, daily, in the afternoon. Water was 10% changed every day in the morning. All experimental catfish were acclimatized to laboratory environment and feed for 2 weeks, and then fasted for 1 day before the experiment began to allow complete gastrointestinal evacuation. Each experimental catfish was used only one time.

2.2 Experimental design

This research comprised 2 experiments, short-term (1 day) and long-term (30 days) exposure. At high temperature (Ht, 29.5±0.5°C), fish were raised in an open-air laboratory. Low temperature exposures (Lt, 19.5±0.5°C) were conducted in an air-conditioned room. Catfish were exposed to 4 conditions, *i.e.,* high temperature with 0.1% sodium chloride (HtWs), (Koeypudsa and Jongjareanjai, 2010), high temperature without 0.1% sodium chloride (HtW/s), low temperature with 0.1% sodium chloride (LtWs), and low temperature without 0.1% sodium chloride (LtW/s). Experimental catfish were exposed in water with supplemental aeration. Medium osmolarity from Ws groups (HtWs, LtWs) and W/s groups (HtW/s, LtW/s) were 140 and 7 mosmol/l respectively in number. Salinity from Ws groups (HtWs, LtWs) and W/s groups (HtW/s, LtW/s) corresponded to concentration of 1.0 and 0 g/l, respectively.

2.2.1 Short-term (1 day) experiment

Two hundred catfish were divided into 4 groups as follows: HtWs, HtW/s, LtWs and LtW/s. Each group was comprised of 10 fish / 60 l glass aquarium in 5 replicates. One fish from each replicate was randomly selected at 0 min, 15 min, 45 min, 2 h, 4 h, 6 h and 24 h. After tranquilization with 5 mg/l clove oil, blood was taken from the caudal vein by tuberculin syringe (Nipro, Osagka, Japan).

2.2.2 Long-term (30 days) experiment

The 200 catfish were separated to 4 groups (HtWs, HtW/s, LtWs and LtW/s) at a rate of 10 catfish / 60 l glass aquarium and had 5 replicates. After laboratory condition exposure, one fish from each replicate was randomly selected to investigate on 0 d, 1 d, 5 d, 10 d, 15 d, 20 d and 30 d. The elected catfish was sedated with 5 mg/l clove oil (Koeypudsa and Jongjareanjai, 2010). Individual fish weight and length were recorded before the withdrawal of blood. Caudal blood was drawn by tuberculin syringe (Nipro, Osagka, Japan) from anaesthetized fish.

2.3 Blood chemistry determination

Blood clotting time (s) was performed by the glass slide method (Koeypudsa *et al.*, 2006). Concentrations of serum cortisol (ng/ml) were investigated by radioimmunoassay (Coat-A-Count Cortisol®, Diagnostic Products Corporation, USA). Plasma metabolite, glucose (mg %), was obtained with Automated Clinical Chemistry Analyzer (Sapphire 350®, Audit Diagnostics Ltd., Carrigtwohil, Ireland).

Plasma osmolarity (mosmol/l) was measured using Cryoscopic Osmometer (Osmomat 030, Gonotec, Berlin). Plasma electrolytes: sodium (Na^+, mEq/l), potassium (K^+, mEq/l), and chloride (Cl^-, mEq/l), were evaluated with Vitros DTEII module (Johnson & Johnson Clinical Diagnostics, Careside Inc., CA). Monovalent ratios (Na^+/K^+, Na^+/Cl^-, $Na^+ + K^+/Cl^-$) were also calculated.

2.4 Statistical analysis

Data were expressed as mean of 5 replications ± s.d. (standard deviation). All statistical analyses were conducted using one-way ANOVA. Differences among groups were compared using Duncan's Multiple Range test. A *p*-value of less than 0.001 was taken to indicate statistical significance.

3. Results

3.1 Short-term (1 day) experiment

The values of haematology, *i.e.,* blood clotting time, cortisol, glucose, osmolarity, Na^+, K^+ and Cl^- were disturbed and statistical significance throughout the experimental period (Figure 1). Blood clotting time at 4 h (48.8±0.8 – 50.2± 1.3 s) and osmolarity at 2 h (240±2.0 – 242±2.0 mosmol/l) among 4 groups (HtWs, HtW/s, LtWs, LtW/s) were not statistically significant different.

3.2 Long-term (30 days) experiment

As shown in Figure 2, the direction of blood clotting time trend lines were decreased (HtWs, LtW/s, LtWs) but trend line direction of HtW/s group was increased. All 4 trend line directions (HtW/s, HtWs, LtW/s, LtWs) of Cl^- were decreased. Means of fish weight and length were not statistically significant throughout experiment (Figure 3). The beginning of fish weight and length were 7.54±1.82 – 11.50± 1.17 g and 9.90±0.96 – 11.30±0.97 cm, respectively. When the experiment stopped, fish weight and length were 12.22± 1.93 – 13.76±1.37 g and 11.20±0.57 – 12.60±0.89 cm, respectively. Ratios of Na^+/K^+ among 4 groups (HtW/s, HtWs, LtW/s, LtWs) were statistical significance differences (Table 1). Table 2 showed Na^+/Cl^- ratio and HtWs group had no statistically significant (1.21 ± 0.01 – 1.36 ± 0.03). Sum of Na^+ and K^+ to Cl^- ratio were not statistically significant at 10 d, 15 d, 20 d and 30 d (Figure 4).

4. Discussion

4.1 Short-term (1 day) experiment

Figure 1 shows stress indicators of catfish (blood clotting time, cortisol, glucose, osmolarlity and electrolytes) when exposed to laboratory condition for 1 day in the short-term experiment. There were considerable variations in stress indicators. This implies that catfish attempt to adjust their physiological mechanisms in order to restore the disturbed homeostasis.

Cortisol of stressed groups (HtWs, LtW/s, LtWs) were significantly elevated levels within 15 min in comparison to cortisol level of unstressed group (HtW/s). Similar result was reported that channel catfish (*Ictalurus punctatus*) under confinement stress had significant increases in cortisol within 15 min (Small, 2004).

Cortisol is primary stress response and is important in the restoration of internal homeostasis during and after stress (Pottinger, 1998; Goos and Consten, 2002; Ndong *et al.,* 2007). Moreover, cortisol is also involved in hydromineral balance and carbohydrate metabolism (Eckert *et al.,* 2001; Fiess *et al*., 2007). Thus, statistical significant levels of electrolytes and glucose are considered to be an effect of the stress hormone, cortisol.

There were no significant differences among 4 groups (HtW/s, HtWs, LtW/s, LtWs) in osmolarity and blood clotting time at 2 h and 4 h respectively. These may indicated that catfish successfully maintain their internal balances within 2 h – 4 h. Since catfish is warm water eurythermic species, it has been successfully cultured in brackish-water pond and can withstand of variety of environment condition (Carlson *et al.*, 1995; Eckert *et al.,* 2001; Fashina-Bombata and Busari, 2003; Souza-Bastos and Freire, 2009). These results suggested that the catfish was not stressed, would adapt and survive in changed situations (HtWs, LtW/s, LtWs) compared to natural condition (HtW/s).

It is difficult to obtain estimates of resting levels of blood clotting time, cortisol, glucose, osmolarity and electrolytes since, haematological levels vary considerably among species, within species and overtime, even within an individual (Maxime *et al.,* 1995). The variables in values of stress indicators could be due to different methods, genetic defects, sexual maturation, pathological status, sex and species (Jagadeeswaran and Liu, 1997; Jussila *et al.,* 2001).

4.2 Long-term (30 days) experiment

Blood clotting time is easily measured and has been claimed to be an indicator of stress in crustaceans (Jussila *et al.,* 2001). Coagulation of fish blood is a crucial defense response. Blood accelerates clotting, confines intrusion of pathogens and prevents blood losses after injuries (Martin *et al.,* 1991). The blood of stressed or infected animal shows reduced clotting ability or fails to clot (Durliat and Vranckx, 1983, Sarathi *et al.,* 2008). Harikrishnan *et al.* (2003) reported that the haemostatic process of *Aeromonas hydrophila* infected fish was extended beyond the normal. Koeypudsa *et al.* (2006) stated that anoxic catfish had an approximately 2-fold prolongation of blood clotting time compared to normoxic catfish.

In contrast to those, this research showed decreased of blood clotting time trend lines (HtWs, LtW/s, LtWs; Figure 2). These implied that stress in catfish was reduced resulting from 0.1% sodium chloride and low temperature.

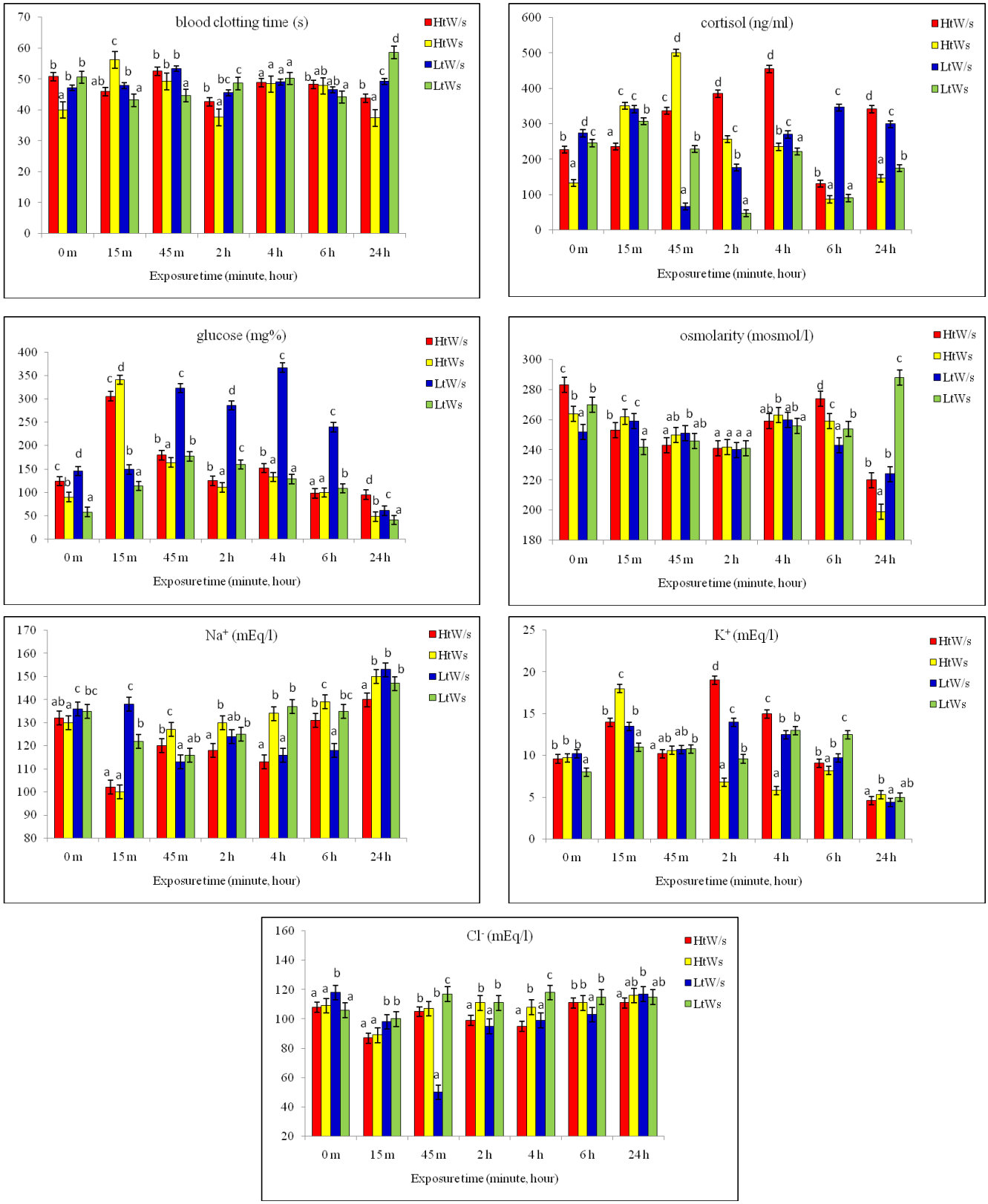

Figure 1. Fish haematology (means ± s.d.) among 4 experimental groups from short-term (1 day) experiment, blood clotting time (s), cortisol (ng/ml), glucose (mg%), osmolarity (mosmol/l), Na^+ (mEq/l), K^+ (mEq/l) and Cl^- (mEq/l).
Different lower cases letters indicate statistical significance ($p \leq 0.001$, ANOVA, Duncan).
HtWs = high temperature with 0.1% sodium chloride, HtW/s = high temperature without 0.1% sodium chloride, LtWs = low temperature with 0.1% sodium chloride, LtW/s = low temperature without 0.1% sodium chloride.

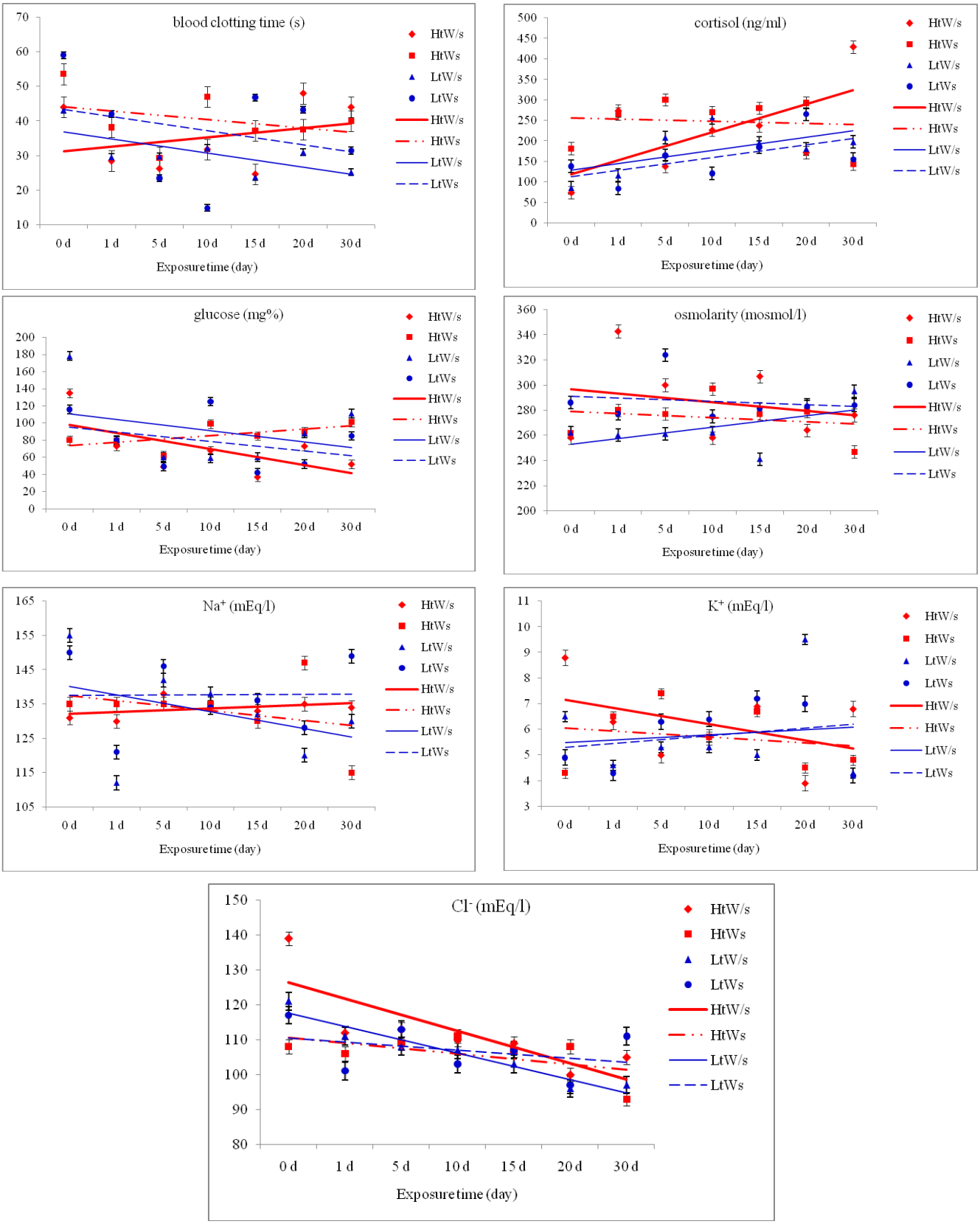

Figure 2. Trend lines direction of fish haematology (means ± s.d.) among 4 experimental groups from long-term (30 days) experiment: blood clotting time (s), cortisol (ng/ml), glucose (mg%), osmolarity (mosmol/l), Na^+ (mEq/l), K^+ (mEq/l) and Cl^- (mEq/l). HtWs = high temperature with 0.1% sodium chloride, HtW/s = high temperature without 0.1% sodium chloride, LtWs = low temperature with 0.1% sodium chloride, LtW/s = low temperature without 0.1% sodium chloride.

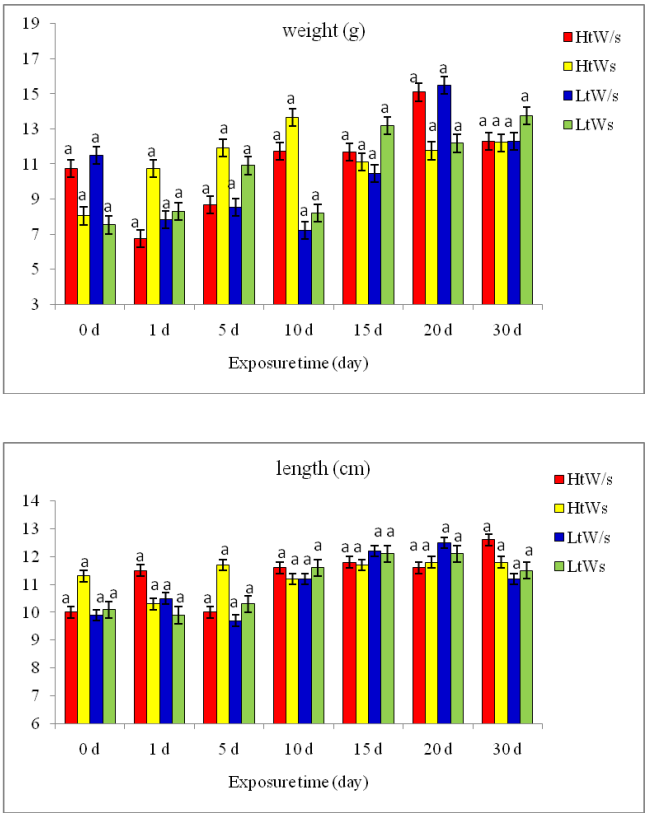

Figure 3. Fish weight and length (means ± s.d.) among 4 experimental groups from long-term (30 days) experiment.
Different lower case letters indicate statistical significance ($p \leq 0.001$, ANOVA, Duncan).
HtWs = high temperature with 0.1% sodium chloride,
HtW/s = high temperature without 0.1% sodium chloride,
LtWs = low temperature with 0.1% sodium chloride,
LtW/s = low temperature without 0.1% sodium chloride.

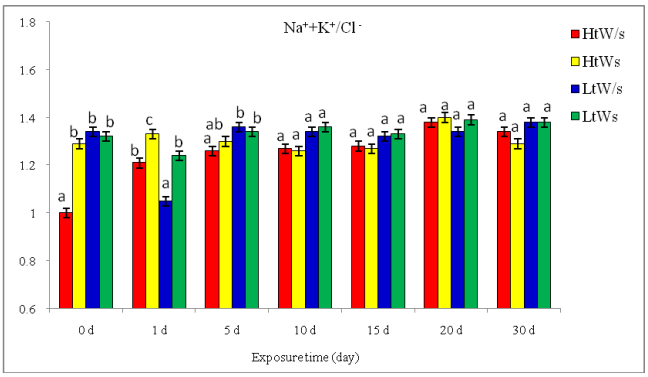

Figure 4. Sum of Na^+ and K^+ to Cl^- ratio (means ± s.d.) among 4 experimental groups from long-term (30 days) experiment.
Different lower case letters indicate statistical significance ($p \leq 0.001$, ANOVA, Duncan).
HtWs = high temperature with 0.1% sodium chloride,
HtW/s = high temperature without 0.1% sodium chloride,
LtWs = low temperature with 0.1% sodium chloride,
LtW/s = low temperature without 0.1% sodium chloride.

When subjected to low temperature, catfish become lethargic and lessen their sensitivities (Sun *et al*., 1995; Ross and Ross, 1999; Alcorn *et al.,* 2002). Lower temperature is a cause of decreased metabolic rate (Buentello *et al.,* 2000; Davis, 2004) which decrease the magnitude of stress responses, blood clotting time (LtW/s). Salt adding (HtWs, LtWs) could be a consequence of excessive water loss, dehydration, and increased viscosity of the blood (Taylor and Miller, 2001). The immobilized state and blood viscosity of catfish might accelerate blood clotting time.

The primary stress response of fish involves increasing the stress hormone, cortisol (Ndong *et al,* 2007). Trend lines direction of cortisol were increased (HtW/s, LtW/s, LtWs; Figure 2). Small (2004) stated that eugenal significantly reduced the cortisol response to confinement fish. In this research, catfish were sedated with 5 mg/l clove oil. The constituent of clove oil is 70-90% eugenol (Ross and Ross, 1999). Eugenol is recommended as a sedative for food aquatic animals and requires no withdrawal period. Ross and Ross (1999) stated that 25-100 mg/l eugenol gave effective anaesthesia in common carp (*Cyprinus carpio*). At low concentrations as used in this research (5 mg/l), clove oil is unable to block cortisol release in catfish. This could explain the elevated trend lines of cortisol (HtW/s, LtW/s, LtWs; Figure 2).

Cortisol direction line (HtWs, Figure 2) have trend to reduce. This is believed to indicate a decreasing stress level (Shrimpton and McCormick, 2003). In this research, the results show that glucose trend lines direction are dropped (HtW/s, LtW/s, LtWs; Figure 2). This may indicate that catfish are able to lower their metabolic rate. Cortisol is known to stimulate glucose production due to gluconeogenesis and glycogenolysis in the liver (Grutter and Pankhurst, 2000; Affonso *et al.*, 2002; Ishibashi *et al.,* 2002; Gollock *et al.*, 2005a). Fish enhanced blood glucose as an energy substrate for metabolically coping with the energy demand (Ruane *et al.,* 2001; Ishibashi *et al.,* 2002; Gollock *et al.,* 2005b). Although cortisol trend lines are increased (HtW/s, LtW/s, LtWs; Figure 2), blood sugar levels are unaffected and show a trend to hypoglycemia. It is suggested that glucose synthesis and energy requirement are reduced, which is related to stress reduction (Perez-Rostro *et al.,* 2004).

The present study shows that body fluid osmolarity of W/s and Ws groups (276.50±24.56 and 280.64±16.21 mosmol/l) are higher than their environment (7 and 140 mosmol/l). Fiess *et al*. (2007) reported that water temperatures, below and above the optimum levels, are known to impair osmoregulatory ability and to provoke disturbances in the maintenance of hydromineral balance. As shown in Figure 2, the direction of the Na^+ (LtWs) trend to be upward. This could be because the catfish is hyperosmotic to its environment and as a consequence must deal with constant osmotic influx of water and depletion of salt. To counteract these diffusive gains and losses, NaCl is replaced by active uptake across the gill (Murphy and Houston, 1974; Tsuzuki

Table 1. Na^+ to K^+ ratio (means ± s.d.) among 4 experiments from long-term (30 days) exposure.
Different lower case letters at the same column indicate statistical significance ($p \leq 0.001$, ANOVA, Duncan).

Day	HtW/s	HtWs	LtW/s	LtWs
0 d	14.95 ± 0.47^{a}	31.38 ± 1.24^{c}	23.88 ± 1.15^{b}	30.53 ± 0.44^{c}
1 d	20.73 ± 0.35^{b}	20.91 ± 0.67^{a}	24.40 ± 1.69^{b}	28.15 ± 2.17^{c}
5 d	27.68 ± 0.53^{d}	18.34 ± 0.92^{a}	26.85 ± 0.98^{b}	23.24 ± 0.59^{b}
10 d	23.72 ± 1.16^{c}	23.96 ± 1.54^{b}	26.10 ± 1.78^{b}	20.95 ± 0.57^{ab}
15 d	19.26 ± 0.79^{b}	19.35 ± 0.62^{a}	26.36 ± 1.01^{b}	18.84 ± 0.96^{a}
20 d	34.69 ± 1.21^{e}	32.62 ± 1.62^{c}	12.58 ± 0.39^{a}	18.27 ± 0.67^{a}
30 d	19.65 ± 0.43^{b}	24.06 ± 1.59^{b}	30.23 ± 1.41^{c}	35.36 ± 2.77^{d}

HtWs = high temperature with 0.1% sodium chloride, HtW/s = high temperature without 0.1% sodium chloride, LtWs = low temperature with 0.1% sodium chloride, LtW/s = low temperature without 0.1% sodium chloride.

Table 2. Na^+ to Cl^- ratio (means ± s.d.) among 4 experiments from long-term (30 days) exposure.
Different lower case letters at the same column indicate statistical significance ($p \leq 0.001$, ANOVA, Duncan).

Day	HtW/s	HtWs	LtW/s	LtWs
0 d	0.94 ± 0.02^{a}	1.25 ± 0.03^{a}	1.28 ± 0.02^{bc}	1.28 ± 0.05^{ab}
1 d	1.16 ± 0.02^{b}	1.27 ± 0.02^{a}	1.00 ± 0.19^{a}	1.20 ± 0.04^{a}
5 d	1.22 ± 0.01^{bc}	1.23 ± 0.02^{a}	1.31 ± 0.03^{bc}	1.29 ± 0.02^{ab}
10 d	1.22 ± 0.02^{bc}	1.21 ± 0.06^{a}	1.29 ± 0.03^{bc}	1.29 ± 0.03^{ab}
15 d	1.21 ± 0.03^{bc}	1.21 ± 0.01^{a}	1.28 ± 0.02^{bc}	1.26 ± 0.03^{ab}
20 d	1.34 ± 0.03^{d}	1.36 ± 0.03^{a}	1.24 ± 0.03^{b}	1.31 ± 0.01^{b}
30 d	1.27 ± 0.04^{cd}	1.23 ± 0.05^{a}	1.34 ± 0.03^{c}	1.34 ± 0.03^{b}

HtWs = high temperature with 0.1% sodium chloride, HtW/s = high temperature without 0.1% sodium chloride, LtWs = low temperature with 0.1% sodium chloride, LtW/s = low temperature without 0.1% sodium chloride.

et al., 2001). Therefore, the use of salt in water could reduce the plasma-water gradient and consequently the loss of ions from fish into the environment (Gomes *et al.,* 2006; Trumble *et al.,* 2006; Souza-Bastos and Freire, 2009).

All 4 lines of the Cl^- (HtW/s, HtWs, LtW/s, LtWs) had trend towards hypochloremia (Figure 2). This might be due to fish loss of Cl^- through the gills (Pillans *et al.,* 2006; Good *et al.,* 2009). However, catfish homeostasis for monovalent ions is active because $Na^+ + K^+ / Cl^-$ were not significantly different at 10 d, 15 d, 20 d and 30 d (Figure 4). This could be explained that hydromineral balance has actively and quickly been restored (Peruzzi *et al.,* 2005; Salm *et al.,* 2006). Otherwise, hypochloremia function might be cause of fish death.

In this research (Figure 2), mean concentrations of Na^+, Cl^- and K^+ in HtW/s group were corresponding to 133.71±2.69, 112.57±12.47, and 6.20±1.55 mEq/l. These results agree with those of Wells *et al.* (1986) and Lorenz *et al.* (2002). These authors reported that plasma K^+ level is much lower than Na^+ and Cl^- level. Plasma K^+ levels reflect the relatively low contribution to osmolarity but the important role in nerve, kidney, heart, digestive system and muscle function (Ham *et al.,* 2003; Pillans *et al.,* 2006). Furthermore, Wells *et al.* (1986) stated that plasma K^+ appeared to be a potential indicator of stress in fish.

Catfish body weight and total length are shown in Figure 3. All 4 groups (HtW/s, HtWs, LtW/s, LtWs), somatic growth was non-statistically significant different throughout experiment. Although low temperature influences feed consumption, metabolic rate and energy expenditure, the catfish were in the growth phase. Catfish have high ability to switch nutrient source for providing energy (Affonso *et al.,* 2002;

Lermen *et al.,* 2004; Perez-Rostro *et al.,* 2004). Carbohydrate is an important source of energy when catfish are exposed to low temperature. At high temperature, catfish utilize protein as an energy source. Most of all, catfish can adapt to changes in medium temperature as can other ectothermic vertebrates. Morvan-Rocher *et al.,* (1995) reported that body temperature of carp reached the environmental temperature within 19.50± 1.04 min and typically displayed a body temperature ± 1°C of the ambient water temperature.

In conclusion, catfish were fully adapted to their environments fluctuation (HtWs, LtW/s, LtWs) as compare to natural condition (HtW/s). Non-statistically significant means of monovalent ratio (Na^++K^+/Cl^-) indicated that adaptation process was complete and catfish were unstressed. Stress indicators were unaffected by low temperature (Lt; 19.5±0.5°C) and salinity (Ws; 0.1% sodium chloride) since catfish is strongly able to maintain homeostasis without suffering.

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