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Channa striata juvenile on various salinity levels  
of acid sulfate water**

by

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\_ Survival and growth response of snakehead fish *Channa striata* juvenile on various salinity levels of acid sulfate water 1Purnamawati, 2Kukuh Nirmala, 3Ridwan Affandi, 4Eko Dewantoro, 5Diah A. S. Utami 1Study Program of Aquaculture, Department of Marine Science and Fisheries, Pontianak State Polytechnic, Pontianak, West Kalimantan, Indonesia; 2Department of Aquaculture, Faculty of Fisheries and Marine Science, Dramaga Campus, Bogor Agricultural University, Bogor, West Java, Indonesia; 3Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Dramaga Campus, Bogor Agricultural University, Bogor, West Java, Indonesia.

4Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Muhammadiyah University of Pontianak, Pontianak, West Kalimantan, Indonesia; 5Study Program of Aquaculture, Marine and Fisheries Polytechnic of Jembrana, Jembrana, Bali, Indonesia. Corresponding author: Purnamawati, pur\_polnep@yahoo.com Abstract. The aim of this study was to analyze the effect of salinity levels on biometric and physiological responses of snakehead fish (*Channa striata*) juvenile reared in acid sulfate water medium.

The experiment was conducted through completely randomized design (CRD) with salinity levels of 0, 3, 6, and 9 ppt as treatments, and each treatment had six replications. The snakehead fish juvenile with an average length of  $2.4 \pm 0.2$  cm and an average weight of  $0.21 \pm 0.04$  g reared in the aquarium sizing 30 x 25 x 35 cm with a stocking density of 2 fish L<sup>-1</sup>, for 40 days.

The fish were fed commercial feed with a protein content of 40% two times a day (morning and afternoon) to apparent satiation. The water was continuously aerated and water replacement was done every 2 days about 10% of the total water volume in the

aquarium. The results showed that salinity significantly affected the biometric and physiological responses of snakehead fish juvenile.

The medium with a salinity level of 3 ppt gave the best results shown by the highest value of survival (77%), growth rate (5.62% day<sup>-1</sup>), feed efficiency (87.5%), protein retention (38.32%), energy retention (25.50%) and albumin content (4.52 g 100 mL<sup>-1</sup>), and had the lowest value of osmotic gradient (0.097 osmoL kg<sup>-1</sup> H<sub>2</sub>O), oxygen consumption rate (1.99 mg O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>), and blood glucose (25.05 mg 100 mL<sup>-1</sup>).

Key Words: air-breathing fish, biometric responses, freshwater fish, osmolarity, physiological responses. Introduction. Most of aquaculture practices have been applied in tidal lands, but in general, the aquaculture productivity in these areas has still been relatively low. This is due to various problems, such as the low pH at a range of 2.53-3.39, the sulfate range at 6.91-8.7 mg L<sup>-1</sup>, and the iron (Fe) range at 0.72-2.83 mg L<sup>-1</sup>, the low dissolved oxygen level (<5 mg L<sup>-1</sup>), and a high range of salinity shock, that can be problems for the stenohalin fish.

The entrance of seawater causes the high difference in salinity levels in the tidal area between the rainy season and the dry season reaching 0-28 ppt (Purnamawati et al 2017). In this suboptimal medium, not all fishery commodities can be reared in the tidal waters, especially in acid sulfate mediums. In aquaculture business, a high production rate is the most important target that has to be achieved. Production is determined by the growth rate and survival.

Salinity is one of the important environmental factors that affect the growth performance in many fish species (Altinok & Grizzle 2001; Kang'ombe & Brown 2008; Luz et al 2008; Dayal et al 2011; Sarma et al 2013; Ma et al 2016). The effects of salinity have been studied in several fish species reared in ponds, tanks, and floating cages (Cruz et al 1990; Watanabe et al 1990; Sarma et al 2013).

Salinity can directly affect the physiological activity of an organism, either on its osmoregulation or bioenergetically (Dutil et al 1997; Alava 1998; Mommsen et al 1999; Morgan & Iwama 1999; Kammerer et al 2010). The higher the salinity, the higher the osmotic pressure and vice versa. The body fluid of the freshwater fish tends to become hyperosmotic in such an environment (Wedemeyer 1996), so it requires energy for osmotic regulation in order to keep the fish alive.

The way to reduce the use of energy for osmoregulation is by lowering the osmotic gradient between the fish and the environment by setting the salinity of the medium (Evans & Claiborne 2005), so the expenditure of the energy for adaptation can be

replaced to maximize the growth of the fish. The decline of the energy for adaptation caused by the increase of the salinity level in the medium has been demonstrated in several species of freshwater fish (Peterson & Maedor 1994). The maximum growth of several freshwater fish species occurs at a salinity of 3-5 ppt (James et al 2003).

However, this statement is debatable (Boeuf & Payan 2001; Sarma et al 2013). Several previous studies revealed that the air-breathing fish can survive in brackish water. *Monopterus albus* can grow at a salinity level of 10 ppt (Pedersen et al 2014). *Anabas testudineus* can tolerate a salinity range up to 30 ppt (Chang et al 2007).

*Clarias batrachus* can survive a salinity range up to 8 ppt. However, the growth and survival of those species are low than compared to the fish that live in freshwater (Sarma et al 2013). The salinity tolerance of the Channidae family has still been unknown (Nakkrasae et al 2015).

A study that conducts the measurement of the salinity level in tidal land, especially in acid sulfate medium, has never been done. Snakehead fish (*Channa striata*) is a freshwater fish classified in Perciformes order and Channidae family (Nakkrasae et al 2015). This fish is a potential and important species to be developed as aquaculture commodity (Mollah 1985; Marimuthu et al 2009; Mollah et al 2009; Rahman et al 2013) and has a high economic value.

Moreover, the flesh of this fish is used as a treatment of post-surgical therapy and can increase body endurance (Gam et al 2006; Marimuthu et al 2009). This species commonly lives in ponds, fields and rivers, preferring the stagnant water and muddy medium. It can survive in the dry season by digging mud when its skin and breathing apparatus remains humid. The natural habitat of *C.*

*striata* is spread from freshwater to brackish water (Nakkrasae et al 2015). Based on these facts, it is necessary to conduct a study to analyze the response of *C. striata* juvenile on various salinity levels of acid sulfate water medium. The aim of this study was to analyze the effect of salinity levels on biometric and physiological responses of *C. striata* juvenile reared in acid sulfate water medium.

**Material and Method** The study was conducted at Fish Seed Center, Department of Agriculture, Animal Husbandry and Fisheries, Pontianak, West Kalimantan. The measurement of albumin levels, the proximate analysis of the fish feed and the proximate analysis of the experimental fish were conducted at Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, West Java, Indonesia.

The measurement of plasma glucose levels was conducted at the Environmental and Food Technology Laboratory, Tanjungpura University, Pontianak, West Kalimantan, Indonesia. Experimental design. The experiment was conducted in the laboratory through a completely randomized design (CRD). The treatments used in this experiment consisted of four treatments, namely 0, 3, 6, and 9 ppt. Each treatment had 6 replications. Experimental fish.

The experimental fish used in this study were *C. striata* juveniles with an average initial length of  $2.4 \pm 0.2$  cm and an average initial weight of  $0.21 \pm 0.05$  g. The fish were reared in mediums equipped with aeration equipment.

Experimental tanks and mediums. The containers used were 24 units of glass aquarium sizing 30 x 25 x 35 cm.

Those containers were filled with acid sulfate water from tidal land in Kubu Raya Regency, West Kalimantan, Indonesia. The water had been prepared in a reservoir tank and deposited for 3 days. To get a suitable experimental medium according to salinity levels applied for the treatments in this study, there was an addition of salt (1 gram per liter) into acid sulfate water medium to increase salinity level becoming 1 ppt higher than the initial salinity level.

The top of the aquarium was closed with a net to avoid the fish jump out of the container. The rearing of the fish. The experimental fish were adapted to a laboratory environment, with the aim to adjust the fish to new environmental conditions. The adaptation was performed in 4 units of glass aquariums sizing 30 x 25 x 35 cm for 7 days.

Furthermore, the fish were acclimatized to salinity by increasing salinity in the medium gradually until it reached salinity levels applied as the treatments in this study (0, 3, 6, and 9 ppt). The *C. striata* that had been acclimatized were reared in an aquarium with a stocking density of 2 fish L<sup>-1</sup> (Vivekanandan 1977). The experiment was conducted for 40 days.

The fish in all treatments were fed by commercial feed with a protein content of  $\pm 40\%$ , and with a feeding frequency of 2 times a day to apparent satiation. During the experiment, the mediums were continuously aerated and water exchange was conducted as much as 10% of the total volume of medium every two weeks. Substitute water was provided in other aquariums with the same salinity level of the replaced water.

The observation of the number of living fish was conducted every day until the end of the experiment to obtain the data of fish survival. The measurements of the length and the weight of the fish were done every 10 days to get the growth data. The amount of feed consumed was known from the sum of daily feed consumed during the experiment.

The physiological responses (osmotic gradients, oxygen consumption and blood glucose) were measured at the end of the experiment. The observations and measurements of temperature, pH and dissolved oxygen in the medium were carried out 2- every day, while the measurements of SO<sub>4</sub>, H<sub>2</sub>S, alkalinity, hardness, and NH<sub>3</sub> were performed at the beginning and at end of the experiment. Experimental parameters.

The measurements of water quality parameters were performed following the procedures described by APHA (1989). The measurement of albumin levels followed the method described by Infusino & Panteghini (2013), while the proximate analysis of the fish feed and the experimental fish were carried out according to the procedure by Takeuchi (1988).

The measurement of plasma glucose levels was performed using a liquicolor glucose commercial COD-PAP kit with the calorimetric method and the results of the measurement were read with a spectrophotometer at a wavelength of 500 nm following the procedure by Wedemeyer and Yasutake (1977). The osmotic gradient was analyzed according to Clark et al (1983), while the oxygen consumption rate was analyzed according to Liao & Huang (1975).

Survival is the percentage of the final number of the living fish at the end of the study with the initial number of the fish. Survival was calculated using a formula described by Kang'ombe & Brown (2008) as follows:

Where:

$$SR = \text{Survival (\%)} \quad SR = (N_t \times N_0^{-1}) \times 100$$

N<sub>t</sub> = The number of fish at the end of the study (individual)

N<sub>0</sub> = The number of fish at the beginning of the study (individual)

Specific growth rate (SGR) was calculated using a formula stated by Weatherley & Gill (1989) as follows:

$$SGR = [(\ln W_2 - \ln W_1) / (t_2 - t_1)] \times 100$$

Where: SGR = Specific growth rate (% day<sup>-1</sup>)

W<sub>1</sub> = The average weight of the fish at the beginning of the study (g)

W<sub>2</sub> = The average weight of the fish at time t<sub>2</sub> (g)

$t_2 - t_1$  = Experimental duration

The feed efficiency of the *C. striata* juvenile was calculated using a formula constructed by Kang'ombe & Brown (2008) as follows:

$$e = \frac{((W_t + D) - W_o)}{F} \times 100$$

Where: e = Feed efficiency

$W_o$  = The weight of the fish at the beginning of the study (g)

$W_t$  = The weight of the fish at the end of the study (g)

D = The weight of the dead fish during the study (g)

F = The weight of total feed given during the study (g dry weight) Statistical analysis.

Survival, SGR, albumin level, feed efficiency, protein retention, energy retention, osmotic gradient, oxygen consumption rate and blood glucose level were analyzed through analysis of variance (ANOVA) with a confidence level of 95%. The Least Significant Difference (LSD) test was applied, where significant effects were found. Physical and chemical parameters of the water were interpreted descriptively. Results and Discussion Results.

The average values of water physical-chemical parameters (temperature, pH,  $SO_4$ ,  $H_2S$ , hardness, alkalinity,  $NH_3$ , and dissolved oxygen) for 40 days of the present experiment can be seen in Table 1. The water quality parameters of acid sulfate water medium with various salinity levels used for the rearing of *C. striata* juveniles is in the tolerance range. From all parameters observed, only the water temperature reached the optimum range.

Most of water physical-chemical parameters values observed in this study could still support the life of *C. striata*, except hardness in acid sulfate water medium with salinity levels of 6 and 9 ppt that reached 395.83 mg L<sup>-1</sup> and 589.33 mg L<sup>-1</sup>, respectively, and alkalinity in acid sulfate water medium with a salinity level of 0 ppt. Those values were below the tolerance value.

Table 1 Water physical-chemical parameters of acid sulfate water medium with various salinity levels in the 40-day-rearing of *Channa striata* juveniles

Parameters Salinities Tolerance and optimum 0 ppt 3 ppt 6 ppt 9 ppt \_ranges pH  
 4.97±1.37 5.65±1.02 5.88±1.12 6.26±1.05 4.25–9.4\*1) 2-) Sulfate (SO<sub>4</sub> ^\*2) (mg L-1)  
 33.33±19.66 23.33±19.66 15.00±5.48 33.33±29.44 5–100 Sulfide (H<sub>2</sub>S) (mg L-1)  
 0.00±0.00 0.00±0.00 0.02±0.00 0.00±0.00 <0.1\*2) Total hardness (mg L-1 CaCO<sub>3</sub>) Total  
 alkalinity (mg L-1 CaCO<sub>3</sub>) \_122.33±19.28 266.67±16.32 395.83±27.76 589.33±22.45  
 20–300\*4) 18.20±1.65 22.77±1.80 32.73±4.64 30.97±2.81 20–150\*3) NH<sub>3</sub> (mg L-1)  
 0.02±0.01 0.03±0.01 0.01±0.01 0.01±0.00 <1.57\*4) Dissolved oxygen (mg L-1) 5.43±0.40  
 5.92±0.39 5.72±0.31 5.70±0.30 >5\*\*1) Temperature (oC) 29.76±0.83 30.41±0.64  
 30.16±0.69 29.65±0.96 26–32\*1) 1)Courtenay Jr.

& Williams (2004), 2)Boyd (1998), 3)Wedemeyer (1996), and 4)Qin et al (1997), \*) tolerance range and \*\*) optimum. Physiological responses (osmotic gradients, oxygen consumption, and blood glucose) and biometric responses (survival, growth, and feed efficiency) of *C. striata juveniles* reared at mediums with different salinity levels can be seen in Table 2 and Table 3.

Water salinity affects metabolic rate and stress level of the fish. This could be seen in oxygen consumption rates and blood glucose levels. The medium with a salinity level of 3 ppt resulted in the lowest oxygen consumption rate (P<0.05) with a value of 1.99 mg O<sub>2</sub> g<sup>-1</sup> hour<sup>-1</sup>, followed by mediums with salinity levels of 6 ppt, 9 ppt and 0 ppt, respectively. The lowest blood glucose level (P<0.05) with a value of 25.05 mg 100 mL<sup>-1</sup> was also found in the medium with a salinity level of 3 ppt (Table 2).

Table 2 Osmotic gradient (osmotic), oxygen consumption rate (O<sub>2</sub> cons) and blood glucose (glucose) of *Channa striata juveniles* reared in acid sulfate water medium with various salinity levels for 40 days Parameters \_ 0 ppt \_Sali 3 ppt \_nities 6 ppt \_ 9 ppt \_  
 \_Osmotic (OsmoL kg<sup>-1</sup> H<sub>2</sub>O) O<sub>2</sub> cons (mg O<sub>2</sub> g<sup>-1</sup> hour<sup>-1</sup>) Glucose (mg 100 mL<sup>-1</sup>)  
 \_0.121±0.006b 6.20±0.49c 28.56±1.00b \_0.097±0.005a 1.99±0.08a 25.05±1.19a  
 \_0.098±0.005a 3.99±0.43b 28.00±1.15b \_0.105±0.011a 4.36±0.41b 30.40±0.82c \_  
 \_Different superscript letters in the same row indicate significantly different results (P < 0.05). The survival of *C.*

*striata juveniles* during the study in the medium with a salinity level of 3 ppt showed the highest value (77%) and it was significantly different (P<0.05) from other salinity levels. Salinity also affected growth. The highest growth of *C. striata juveniles* (P<0.05) was found in *C. striata juveniles* reared in the medium with a salinity level of 3 ppt, which was 5.62% day<sup>-1</sup>.

The albumin content in the fish flesh was also influenced by the salinity of the rearing medium. The highest albumin content (P<0.05) was found in *C. striata juveniles* reared



in the medium with a salinity level of 3 ppt (4.52 g 100 mL<sup>-1</sup>). Feed efficiency, protein retention and energy retention relatively had the same pattern.

The highest values in feed efficiency, protein retention, and energy retention were found in *C. striata* juveniles reared in acid sulfate water medium with salinity levels of 3 ppt and 6 ppt which were significantly different ( $P < 0.05$ ) from *C. striata* juveniles reared in other salinity levels (Table 3).

Table 3 Survival, specific growth rate, albumin content, feed efficiency, protein retention and energy retention of *Channa striata* juveniles reared in acid sulfate water medium with various salinity levels for 40 days

Parameters	0 ppt	3 ppt	6 ppt	9 ppt
Survival (%)	58±6.12b	77±7.00d	67±6.53c	36±7.94a
Specific growth rate (% day <sup>-1</sup> )	3.47±0.26b	5.62±0.78c	3.80±0.51b	2.75±0.68a
Albumin content (g 100 mL <sup>-1</sup> )	4.15±0.06a	4.52±0.02d	4.46±0.04c	4.40±0.03b
Feed efficiency (%)	67.5±5.66a	87.5±7.80b	80.6±15.34b	60.8±6.96a
Protein retention (%)	17.06±4.44a	38.32±5.53b	30.89±5.82b	5.74±1.34a
Energy retention (%)	11.87±2.54a	25.50±3.26b	19.88±3.271b	3.45±1.29a

Different superscript letters in the same row indicate significantly different results ( $P < 0.05$ ). Discussion. The high survival and the growth rate of *C.*

*striata* juveniles reared in a medium with a salinity level of 3 ppt are related to the water quality conditions (Wedemeyer 1996). Water quality is one of the important factors that affects the growth of *C. striata*. If water quality parameters (dissolved oxygen, temperature, ammonia, alkalinity, hardness, sulfide, sulfate and pH) pass the optimum and the tolerance ranges, the fish growth will be hampered and those can cause death in the fish. The *C. striata* is classified as a strong fish, but it is sensitive to environmental changes.

Aquatic organisms require oxygen for the combustion process to produce energy for several activities such as swimming, growth and reproduction. The dissolved oxygen range during the rearing period was 5.43–5.92 mg L<sup>-1</sup>. The dissolved oxygen content in the study still met the optimum dissolved oxygen requirements and was not harmful to *C. striata* juveniles.

According to Boyd (1998), if the dissolved oxygen level is below 5 mg L<sup>-1</sup>, this condition will not cause death in the fish, if it does not occur for a long time. The *C. striata* have an additional respiratory organ called diverticula so they are able to live in a medium with minimum oxygen level and take oxygen from the air. The temperature range obtained during the rearing period was 29.65–30.41°C. This temperature range is still considered optimum and it can be tolerated by *C.*

striata (Courtenay Jr. & Williams 2004). The level of  $\text{NH}_3$  measured in this study was about 0.01–0.03 mg L<sup>-1</sup>. This level was relatively low and can be tolerated by *C. striata* juveniles, because this species can grow in a medium with an ammonia concentration of 1.57 mg L<sup>-1</sup> (Qin et al 1997). The sulfate content in the experimental medium was 15.00–33.33 mg L<sup>-1</sup>.

The value decreased with the increase of salinity levels from 0 to 6 ppt, but then it increased again in a medium with a salinity level of 9 ppt. Although this sulfate content was relatively high, it was still within the tolerance range for the life of the fish. On the other hand, the sulfide content in this study showed the opposite phenomenon. A low sulfate content was followed by the increase of sulfide content.

This is caused by the reduction of sulfate into sulfides. In all treatments, sulfides contained in the rearing medium were at a low level and it was still within the tolerance range for the life of the fish (Boyd 1998). The pH range during the rearing period was in the tolerance range to promote the growth of *C. striata* juveniles.

In the experimental medium with a salinity level of 3 ppt, the pH range observed supported the physiological activity of the fish. For the life of *C. striata*, the tolerance range of pH is between 4.25–9.4 (Courtenay Jr. & Williams 2004). At this range, the ability of fish gills to bind oxygen is more optimal. During the rearing period, the alkalinity in the experimental medium with a salinity level of 0 ppt was 18.20 mg L<sup>-1</sup> CaCO<sub>3</sub>.

This alkalinity was below the optimum range for the fish growth (Wedemeyer 1996; Cavalcante et al 2012). The alkalinity in the rearing medium with salinity range 3 ppt to 9 ppt showed an increase with the rise of medium salinity levels, but it was still in the optimum range (20–150 mg L<sup>-1</sup> CaCO<sub>3</sub>). In this alkalinity range, the ability of alkalinity to support water pH is also getting better.

The hardness in the experimental mediums with salinity levels of 0 and 3 ppt were 122.33 and 266.67 mg L<sup>-1</sup> CaCO<sub>3</sub>, respectively. These values were still within the tolerance limit for the life of the fish. In contrast, the hardness values in the experimental medium with salinity levels of 6 ppt and 9 ppt were 395.83 mg L<sup>-1</sup> CaCO<sub>3</sub> and 589.33 mg L<sup>-1</sup> CaCO<sub>3</sub>, respectively.

Those values were far above the tolerance range for the life of the fish. This phenomenon can occur because the excess supply of calcium affects water quality and ultimately affects growth. In the optimum range, the increase of hardness is useful in

relation to the calcium supply that is important for bone formation and exoskeleton, osmoregulation and reduces the toxicity of hydrogen ions, ammonia and metal ions.

However, if hardness is above the tolerance limit, it can certainly have a negative impact on the gill's ability to bind ions and homeostatic equilibrium in the fish body. The high value of alkalinity and hardness along with the increase in salinity is caused by the increase of cations and saline anions. In addition, the presence of sulfate in acid sulfate medium also increases the number of anions that play a role to bind magnesium as a cation affecting hardness (Boyd 1988). Salinity expressed in the form of osmolarity is one of the water quality parameters that can affect the survival and the growth of the fish.

Changes in medium salinity will affect the osmolarity of the medium and the fish body fluids (plasma). The high gradient between the dissolved substances concentration in the blood and the environment caused the water entrance to the freshwater fish body by osmosis through a semipermeable membrane.

If this process is not controlled, then the condition can cause the blood of the freshwater fish to contain excessive water (hemodilution) that is fatal for the fish life (Wedemeyer 1996). To prevent hemodilution, the freshwater fish must maintain a dynamic balance (steady-state) through osmotic regulation (osmoregulation) (Perry et al 2003; Evans & Claiborne 2005). The C.

striata is classified as stenohalin fish which is resistant to marginal water conditions, but it is sensitive to extreme environmental changes, especially salinity shocks. The results showed that the experimental medium with a salinity level of 3 ppt was the optimum condition for the ongoing physiological process of C. striata. Martínez- Porchas et al (2009) suggested that in order to adapt to the environment, the fish have a certain salinity tolerance range.

The organism's ability to adapt to the environment is influenced by several factors, such as the fish species, the age or the size of the fish, stocking density and the water quality condition in the medium where the fish lives. This is indicated by the survival of C. striata juveniles during the study, demonstrating that C.

striata juveniles reared in acid sulfate water medium with a salinity level of 3 ppt resulting survival with a value of 77% that showed the highest value when compared to those of 6 ppt (67%), 0 ppt (58%), and 9 ppt (36%). The high survival indicates that C. striata could adapt well even though that condition was a different environmental condition from C. striata natural habitat.

It was due to conducive conditions in the experimental medium with a salinity level of 3 ppt. This condition was indicated by a low osmotic gradient value (0.097 OsmoL kg<sup>-1</sup> H<sub>2</sub>O), low oxygen consumption level (1.99 mg O<sub>2</sub> g<sup>-1</sup> hour<sup>-1</sup>) and blood plasma glucose level (25.05 mg 100 mL<sup>-1</sup>) showed by *C. striata* juvenile reared in the medium with a salinity level of 3 ppt. The low osmotic gradient can be used to determine the osmoregulation of *C. striata* juvenile.

In the low osmotic gradient, the osmotic load will also be low and vice versa. The highest osmotic gradient was found at the salinity treatments of 0 ppt (0.121 OsmoL kg<sup>-1</sup> H<sub>2</sub>O) and 9 ppt (0.105 OsmoL kg<sup>-1</sup> H<sub>2</sub>O) proving that *C. striata* juveniles were osmoregulatory animals with hyperosmotic strategies like most of the freshwater fish.

Arjona et al (2009) state that more osmotic gradient can cause a higher use of energy for osmoregulation. The lower osmotic gradient will cause the energy used for osmoregulation becoming low, so the portion of energy for the growth will be higher. However, if the salinity is increased to a higher level it causes dehydration in the muscles, a significant increase in cortisol circulation, and adverse effects on feed consumption, feed conversion and growth (Luz et al 2008). Nakkrasae et al (2015) reported that *C. striata* with a weight of 120±1.24 g could live at a salinity range = 10 ppt, but the osmoregulation mechanism underlying this ability is still unknown.

Cortisol increases rapidly with the increase of plasma glucose and lactate. A different phenomenon occurred in *C. striata* juveniles reared in the medium with a salinity level of 0 ppt, they carried out an osmoregulation process to maintain their homeostatic condition. This condition was expressed from the highest oxygen consumption rate in the treatment of 0 ppt (6.20 mg O<sub>2</sub> g<sup>-1</sup> hour<sup>-1</sup>) causing energy for the growth process to be reduced. When *C.*

*striata* lived in salinity levels of 9 ppt and 6 ppt, the fish did a high body osmoregulation to meet the salt content in the body to be balanced, so that the fish would consume a lot of water from the environment in order to balance the osmotic pressure between the body and the environment. When the fish is in a high salinity level, the body has a lot of salt from the environment, this makes the kidney and gills that play a direct role in the osmoregulation process to be able to receive and remove excess salt in the body.

However, this condition certainly requires more energy resulting in increased oxygen consumption (higher metabolism). At a higher salinity level, the chloride cells of gills experience the increase in the transfer activity of entered sodium ions to be released in order to make the fish body's osmotic pressure being stable (Perry et al 2003; Evans &

Claiborne 2005).

The increase in energy requirements is also indicated by the increase of **the oxygen consumption rate** (Kirschner 1995; Morgan & Iwama 1999; Kidder III et al 2006a, b). At lower salinity levels, *C. striata* juvenile conducts fewer active transport to excrete excess sodium ions from gills, and the fish secretes more urine to balance the osmotic pressure between the **environment and the body** so the fish requires lower energy.

In optimum environmental conditions, the allocation of the energy used in standard metabolic processes (osmoregulation) becomes minimum and the energy portion for the growth will increase (Perry et al 2003; Evans & Claiborne 2005). This condition was indicated by the lowest blood glucose level of *C. striata* **reared in acid sulfate water medium with a salinity level of** 3 ppt (25.05 mg 100 mL<sup>-1</sup> or 1.39 mmol L<sup>-1</sup>).

Iwama et al (1999) and Martínez-Porchas et al (2009) explained that an increase in blood glucose indicates stress response of an organism **as a result of** cortisol release in the hypothalamus, through the bloodstream to the chromaffin tissue in the kidney, as a secondary stress continuation that increases glucose level through gluconeogenesis and glycogenolysis.

Furthermore, Reid et al (1998) and Mommsen et al (1999) also stated that under suboptimal conditions or stress conditions (internal or external) chromaffin cells release catecholamine, adrenaline and noradrenaline into blood circulation. This is in line with **the results of this study** demonstrating that the high blood glucose levels were produced by *C.*

*striata* juveniles in treatments with large osmotic gradients (0 ppt, 6 ppt and 9 ppt). That condition causes sub-optimal nutrient absorption from the consumed food and it is ultimately expressed by slow growth rate. The ability to absorb salt from this environment is influenced by corticosteroid that plays a role in osmoregulation, metabolism control, hydromineral balance and overall stress response (McCormick & Bradshaw 2006). Salinity level at a value of 3 ppt was the treatment that produced the smallest osmotic gradient.

This environmental condition caused the best **growth rate, albumin content** and feed efficiency ratio **of *C. striata* juveniles** among other treatments. It is similar to a statement affirmed by Partridge & Jenkins (2002) that the efficiency of feed utilization will run optimally if the osmotic gradient is in normal conditions, so the digestion process will be more efficient. Therefore, high or low feed efficiency is influenced by the magnitude of the osmotic load.

In isotonic condition, the body cells of *C. striata* are in an ideal condition, so the physiological processes in the fish body will run normally. In this condition, the feed digestion and absorption processes will take place quickly, so the stomach emptying rate will run quickly as well. This will cause hunger followed by the increase of feed consumption, so there will be more feed consumed (Arjona et al 2009). The *C.*

*striata* juveniles reared in the medium with a salinity level of 3 ppt consumed the feed of an amount of 79.76 g. This was the highest amount when compared to other treatments (0 ppt = 33.87 g, 6 ppt = 35.63 g and 9 ppt = 23.86 g). This showed that optimum salinity caused an increase in appetite and followed the amount of feed consumed.

According to Brett (1971), the amount of feed consumed by fish every day is one of the factors that influences the potential of the fish to grow optimally and the daily consumption rate is closely related to gastric capacity and emptiness. This was demonstrated by higher growth of *C. striata* juveniles reared in acid sulfate water medium with a salinity level of 3 ppt (5.62%). The difference in growth rate indicated that *C.*

*striata* reared in the medium with a salinity of 3 ppt was better in utilizing energy source derived from the feed. Feed converted to the fish flesh is related to feed consumption and energy used for osmoregulation. However, the higher the amount of feed consumed and the lower the energy used for osmoregulation will result in more amount of feed being converted to the fish flesh (Kang'ombe & Brown 2008).

In the medium with a salinity level of 3 ppt, the osmotic pressure conditions of the medium tend to approach the osmotic pressure or isosmotic of *C. striata* juveniles. According to Arjona et al (2009), isoosmotic conditions can increase growth, because the energy for osmoregulation is smaller or absent, so the energy for the growth is available in larger quantities.

This condition is reflected in the protein retention value of the treatment with the lowest osmotic value that was demonstrated by *C. striata* juveniles reared in the medium with a salinity level of 3 ppt (38.32%). This showed that the feed given could be utilized by the fish body properly, so the nutrient content of the feed could be retained in the body efficiently.

According to Ballestrazzi et al (1994) protein retention shows the large contribution of protein consumed in the feed to the increase of body protein. It is clear that an increase in salinity plays a role in the utilization of energy from the feed, because more protein is

stored and only a little amount of protein decomposes or is used for energy to maintain salt balance in the body (homeostatic). An increase in salinity also affects energy retention value.

A low osmotic gradient will reduce the workload of the Na<sup>+</sup>, -K<sup>+</sup>, -ATPase enzyme and the active transport of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup>, so energy (ATP) used for the osmoregulation process can be minimized and there will be more energy used for growth (Marshall 2002; Partridge & Jenkins 2002; Evans & Claiborne 2005; Tang & Lee 2007; Ern et al 2014). According to Cui et al (1992), energy retention shows the contribution of the energy from the feed consumed to the increase of the energy in the fish body. The feed given is an energy source that can be used for body maintenance, metabolic activity and growth. Furthermore, Jobling et al (1991) stated that high energy for body activities would reduce the energy budget for growth.

According to Nelson & Chabot (2011), energy in the feed is physiologically used for maintenance and metabolism, if there is energy residue, it will be deposited as body tissue in the process of growth and the synthesis of reproductive tissue. In hypotonic condition, *C. striata* will experience a large osmotic workload, as a result of high osmotic gradients compared to ideal condition.

This causes the energy used for the osmoregulation process to become greater (Arjona et al 2009). This phenomenon can be seen from the energy retention of *C. striata* reared the medium with a salinity level of 0 ppt that has an energy retention value of 11.87% and it was lower than the energy retention of *C. striata* juveniles reared at a salinity level of 3 ppt (25.50%).

This causes the energy excess used for osmoregulation to be used for other physiological processes, such as growth or production. Fats are usually stored as energy reserves for long-term energy requirements during full activity period or during periods without food and energy. In the medium with salinity levels of 0 ppt and 9 ppt, it was suspected that fat played a role as an energy source (protein sparing effect), so energy retention decreased while protein from the feed was more efficiently used to increase body weight. The results of the study showed that the feed efficiency of *C.*

*striata* juveniles reared in the medium with a salinity level of 3 ppt had the highest efficiency of the feed utilization (87.5%). The low osmotic loads cause the consumed feed to be used more for the fish growth (Tang & Lee 2007; Ern et al 2014). At the treatment of 3 ppt, the fish were more efficient in using energy for the osmoregulation process, so more energy could be used for growth.

Likewise, the efficiency of the feed utilization had decreased with the increasing of medium salinity from the optimum condition. The albumin content reached the highest value in the treatment of 3 ppt (4.52 g 100 mL<sup>-1</sup>). This was due to the hypertonic state of the body cells of *C. striata* juveniles which was an ideal condition, so the physiological processes in the body of the fish would run normally.

By maintaining albumin in the blood plasma, the fish can also maintain blood volume. According to Infusino & Panteghini (2013) albumin is one of the blood plasma proteins synthesized in the liver. Albumin plays a very important role in maintaining plasma osmotic pressure, transporting small molecules through the plasma and extracellular fluid (Suprayitno 2014).

Conclusions. According to our study, acid sulfate water at a salinity level of 3 ppt was the best rearing medium for *C. striata* juveniles. It was shown by minimum levels of osmotic gradient, oxygen consumption and blood glucose. At this salinity level, albumin content, protein and energy retention in *C. striata* juvenile's body reached the maximum levels.

It was also followed by the best physiological responses shown by a high growth performance, thus, in this environmental condition, *C. striata* juveniles had a survival of 77%, a specific growth rate of 5,62% day<sup>-1</sup> and a feed efficiency of 87.5%.

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