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Research

Impacts of Different Salinities on Growth Performance, Stress Response, and Feeding Activity of Shortfin Eel, *Anguilla bicolor*

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ABSTRACT

Due to the declining wild fishery stock of Shortfin eel (*Anguilla bicolor*), eel aquaculture has become increasingly important in Southeast Asian countries. Consequently, there is a rising urgency to cultivate *A. bicolor* as an export commodity due to its high demand and value. However, little is known about the optimum culture conditions to enhance the optimal growth performance of *A. bicolor* in captivity. Four different salinities (0, 10, 20, 30 ppt) were tested on *A. bicolor* for 28 days and its growth performance, stress level, and feeding activity were investigated. Findings revealed *A. bicolor* was able to survive in all salinities without any mortality recorded. Meanwhile, A. bicolor gained significantly higher body weight at 10 ppt (4.33±0.87) compared to those in 0, 20, and 30 ppt despite being insignificant different in the final total length. *A. bicolor* reared in 10 ppt also attained relatively higher feed intake and low feed conversion ratio indicating its excellent feeding utilization. No significant differences were also found in the stress level of *A. bicolor* in all salinities indicating its tolerance and adaptation in all salinities. The present study concludes 10 ppt as suggested salinity to further enhance the growth of *A. bicolor* as it promotes excellent feeding performance, low stress levels, and overall optimal growth.

Key words: Feeding performance, growth rate, shortfin eel, salinity, stress response

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INTRODUCTION

Anguilla species are highly valued and harvested at various life stages for large-scale global trade and consumption (Crook, 2013). The eel (*Anguilla* sp.) is a fishery product commanding high selling prices, commonly cultivated using either intensive or extensive systems, particularly in Asia (Altun, 2005). Countries such as China require an annual eel supply exceeding 70,000 tonnes for consumption, but their domestic production only reaches 20,000 tons, Japan demands 300,000 tons of eel per year, while Korea and Taiwan need 15,000 and 5,000 tons per year, respectively (Kementerian Kelautan & Perikanan, 2011). The FAO (2010), estimated the global production of eel to be 8,440 tons, valued at 36 million USD.

Commercial eel farming began around 1890-1900 (Shiraishi & Crook, 2015). The farming and consumption of eels in East Asia primarily revolve around the Japanese eel species, *Anguilla japonica* (Shiraishi & Crook, 2015). Despite Japan's significant eel production, it heavily relies on imports of live *A. japonica* fry from other countries to supplement its national supplies (Shiraishi & Crook, 2015). More than 70% of Japan's live eel fry imports since 2007 have been from Hong Kong (which has no glass eel population), most of these imports originating in Taiwan (Ringuet *et al*., 2002). With the substantial decline in wild eel harvests, Japanese eel farmers are exploring alternative species such as *A.*

bicolor, which has a similar texture and taste, to meet market demand (Arai *et al*., 2015). Consequently, *A. bicolor* is now regarded as a commercially valuable species. In 2019, Indonesia's eel production from capture fisheries and aquaculture was recorded at 500 tons, while global eel production from aquaculture was 269.000 tons in 2018 (Arai & Chino, 2022).

Anguilla spp. often referred to as freshwater eels, are catadromous fish capable of inhabiting a wide range of water bodies, including marine, brackish, and freshwater (Shiraishi & Crook, 2015). According to Arai *et al*. (2020), eels spend most of their life cycle in freshwater rivers, estuaries, and coastal waters and return to seawater to spawn and subsequently die.

Like other fish, eels depend heavily on environmental conditions for growth and survival (Boeuf & Payan, 2001). Among other factors, many studies have reported an influence of water salinity on fish development, physiology, behavior, and growth performance (Boeuf & Payan, 2001). At early larval stages of most fish species, egg fertilization and incubation, yolk sac resorption, early embryogenesis, swim bladder inflation, and larval growth are highly associated with salinity (Boeuf & Payan, 2001). For larger fish, salinity is a key factor in controlling growth, particularly with the energy budget (Boeuf & Payan, 2001). Fish utilize 20 to 50% of the total energy budget solely for osmoregulation (Boeuf & Payan, 2001).

Given that Anguillid eels are typically viewed as freshwater fishes, eel farming is predominantly performed in freshwater (Lamson *et al*., 2009). However, variations in growth depending on salinity have been documented in several species and have proven to enhance better growth performance in captivity (Boeuf & Payan, 2001). Studies have reported that marbled eel (*A. marmorata*) prefer salinity at 18.4 ppt, while southern shortfin eel (*A. australis*) prefer 17.5 ppt and New Zealand longfin eel (*A. dieffenbachia*) prefer 0 ppt (Traifalgar & Cadiz, 2020). Studies have shown that *A. japonica* grows better in estuarine than in freshwater environments due to the greater abundance of trophic resources and similar osmolality of the environment and internal medium of fish, which results in a lower cost of osmoregulation (Chino & Arai, 2010).

Since *A. bicolor* is a catadromous fish, it is important to understand the optimum salinity for its survival and growth performance. However, little is known about the effect of salinity on the growth performance, stress response, and feeding activity of *A. bicolor*. Therefore, the present study aims to evaluate the effect of salinity on growth performance, feeding activity, and stress response of *A. bicolor*.

MATERIALS AND METHODS

Experimental design

A total of twelve cylindrical fiber tanks were prepared with a capacity of 1.3 tonnes of water volume each. The current study was experimental research with a completely randomized design (CRD), consisting of four treatments which are 0, 10, 20, and 30 ppt with three replications for each treatment. This study was conducted at the JAPFA Aquaculture Research Center in Banyuwangi, East Java, Indonesia.

Healthy shortfin eels which had been cultured under intensive feeding management for at least three months at the JAPFA Aquaculture Research Centre were selected with an initial body weight of 355.13±2.58 g and a total length of 55.53±0.37 cm. A total of 120 selected fish were randomly divided into a group of 10 fish and carefully transferred in each of 12 units of 1.3-tonne fiber tanks.

Fish husbandry

Throughout the fish husbandry process, fish behavior was monitored as frequently as possible to guarantee they were suitable and fit for the experiment. To maximize the rearing system, each tank was secured with a net to prevent possible escape of fish and disturbance. A mesh size of 5 cm net was chosen to ease observation and feeding to be given.

Fish were exposed to salinities of 0, 10, 20, and 30 parts per thousand (ppt), and salinity was adjusted gradually. Shortfin eel was fed twice daily at 08:00 and 16:00. During these feedings, a feeding rate (FR) of 1% was fixed to ensure that the fish received an adequate and nutritionally balanced diet, similar to the previous culture system. High-quality pellets from KAE and JAPFA Comfeed were used for feeding, with a high crude protein content of 52%. To record the daily feed consumption of each tank, the amount of feed consumed at each feed was recorded for each tank. After a feeding period of one hr, the remaining wet pellets were carefully collected and weighed by both manual removals using the net bottom cleaning process. During the experiment, the tank bottom was cleaned every day. The discharged water is also replenished daily.

Parameter

At the beginning of the study, on day 0, the initial number, body weight, and average body length of each shortfin eel were recorded to compare the assessment of growth performance. To measure fish growth, 5 of the 10 fish in each tank were selected. Growth measurements included (1) body weight, where the weight of each fish was measured in grams (g) using a high-precision electronic scale, while total length was carefully determined in centimeters (cm) using a tape measure. Survival rate and other parameters related to fish growth were measured such as mean body weight gain, mean total length gain, specific growth rate (SGR), and condition factor. The condition factor (K) of a fish reflects physical and biological circumstances and fluctuations by interacting among feeding conditions, parasitic infection, and physiological factors. Condition factor is also an index to understand the lifecycle of a fish by referring to the coefficient value, derived from its length-weight relationship data. Condition factors were calculated by using this equation (Kurbah & Bhuyan, 2018). These parameters were calculated based on Equations 1-6:

$$\text{Survival (\%)} = \frac{\text{The number of fish that survived}}{\text{The number of fish stocked initially}} \times 100 \quad \text{Equation 1}$$

$$\text{Mean body weight gain (\%)} = \frac{\text{Final weight-initial weight}}{\text{time (days)}} \times 100 \quad \text{Equation 2}$$

$$\text{Mean total length gain (\%)} = \frac{\text{Final length-initial length}}{\text{time (days)}} \times 100 \quad \text{Equation 3}$$

$$\text{SGR (\%/day)} = \frac{\text{Ln final weight (g)-Ln initial mean weight (g)}}{\text{time (days)}} \times 100 \quad \text{Equation 4}$$

$$W = a \times L^{b} \quad \text{Equation 5}$$

$$\text{Condition factor (Kn)} = \frac{\text{Weight of a fish at a given length}}{\text{Weight of fish from the length-weight regression}} \times 100 \quad \text{Equation 6}$$

Feeding performance analysis

Feed intake was measured as the amount of feed consumed by a shortfin eel in each treatment for a specific period. In this case, the daily feed given was recorded and the amount was adjusted as the fish gained weight accordingly. The weight of unconsumed feed after every feeding was recorded and discarded. Feeding performances were analyzed by measuring total feed intake and feed conversion ratio. These parameters were calculated based on Equations 7 & 8:

$$\text{Total feed intake(g/fish)} = \frac{\text{Weight of the feed eaten by fish per tank}}{\text{Number of fish per tank}} \quad \text{Equation 7}$$

$$\text{Feed conversion ratio} = \frac{\text{Weight of feed fed to the fish}}{\text{Fish total weight gain}} \quad \text{Equation 8}$$

Stress response analysis

At the end of the experiment, fish from each tank were sacrificed for histological analysis. This analysis was used to evaluate the stress response of fish after being subjected to different salinity, focusing on the density of mucous cells and club cells as a stress indicator. According to the histological technique used to analyze the mucus integrity of Atlantic salmon conducted by Fernandez *et al*. (2015), the eel skin was then fixed in 10% neutral buffered formalin. The samples were subsequently processed routinely for paraffin embedding. They were then sectioned into 5 µm thickness and stained with hematoxylin and eosin. The density of the mucous cells was determined by counting them on the histological results of the eel skin at 200x magnification.

Statistical analysis

Quantitative data including survival, growth, feed conversion ratio, and stress response were subjected to rigorous statistical analysis. One-way ANOVA analysis of variance was performed to detect possible differences between multiple groups. In addition to ANOVA, the Duncan multiple range test, a post hoc analysis was chosen to analyze subtleties within specific groupings of data. The statistical analysis allowed this study to gain valuable insight into the complicated dynamics of fish growth while ensuring that the conclusions were both robust and deeply rooted in a thorough understanding of the relationships among the various variables.

RESULTS

Survival and growth

Over 28 experimental days, no mortality was found throughout the experimental period. The growth performance of eels reared in these different salinities demonstrated that *A. bicolor* achieved significantly higher (P<0.05) mean daily weight gain when reared in 10 ppt (4.33±0.87 g) compared to those reared at 0 ppt (2.02±0.79 g) (Table 1). Conversely, no significant difference was found in comparison to those reared at 20 ppt and 30 ppt. In contrast, in the mean body weight gained, significant differences were observed in shortfin eel reared in 10 ppt (4.33±0.87 g/day) compared to 0 ppt (2.02±0.79 g/day). However, no statistically significant differences were detected in the final body weight of shortfin eels that were reared in different salinities. Shortfin eel, when reared in a salinity of 10 ppt exhibited a considerably higher mean final body weight (478.60±44.15 g) however, like final body weight, there were no statistically significant differences when shortfin eels cultured reared in different salinity conditions. However, shortfin eel exhibited comparatively greater total length when reared in 10 ppt salinity (60.02±1.76 cm).

In the mean specific growth rate (SGR), significant differences were observed in shortfin eel reared in different salinities. The findings revealed the shortfin eel reared in 10 ppt exhibited a significantly higher specific growth rate of 0.52±0.17%. However, no significance was detected when comparing shortfin eel in 10 ppt and those in 30 ppt with a specific growth rate of 0.81±0.23% and those reared in 20 ppt with a specific growth rate of 0.60±0.36%. Meanwhile, shortfin eel exhibited a significantly higher condition factor when grown in salinity of 30 ppt (1.109±0.120). Findings revealed the length-weight relationship was at the highest b value attained from shortfin eel reared in 30 ppt (3.40) (Figure 1). A value of b greater than 3 reflecting fish exhibits positive allometric where fish become heavier and a b value lower than 3 reflecting fish exhibits negative allometric where fish become slimmer with increasing length.

Table 1. Growth performance of shortfin eel reared in different salinities

Attributes	Salinity			
	0 ppt	10 ppt	20 ppt	30 ppt
Survival (%)	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
Mean final body weight (g)	413.97±39.13^{a}	478.60±44.15^{a}	418.05±43.04^{a}	444.27±39.85^{a}
Mean final total length (cm)	58.08±1.8^{2}a	60.02±1.76^{a}	58.21±1.56^{a}	57.05±1.41^{a}
Mean body weight gain (g)	2.02±0.79^{a}	4.33±0.87^{b}	2.33±1.44^{ab}	3.26±1.12^{ab}
Mean total length gain (cm)	0.11±0.02^{a}	0.15±0.05^{a}	0.09±0.02^{a}	0.08±0.05^{a}
SGR (%)	0.52±0.17^{a}	1.04±0.13^{b}	0.60±0.36^{ab}	0.81±0.23^{ab}
Condition factor	1.005±0.105^{a}	1.078±0.105^{b}	1.014±0.0.098^{a}	1.109±0.120^{b}

Feed utilization

This study revealed feed intake was not influenced by the salinity as there were no significant variations observed among the different treatment groups. However, the highest feed intake was achieved by shortfin eel reared in 10 ppt salinity at 139.41±29.98 g/day (Figure 2). Following closely were the shortfin eel reared in 0 ppt, which showed a daily feed intake of 101.64±18.63 g/day. Meanwhile, the most efficient feed conversion ratio was observed in the groups of shortfin eel reared at a salinity of 10 ppt, which was remarkably low at 1.147±0.030 (Figure 3). In contrast, less efficient feed conversion was observed in shortfin eel reared at 20 ppt, where the FCR was higher at 2.072±1.37 (Figure 3).

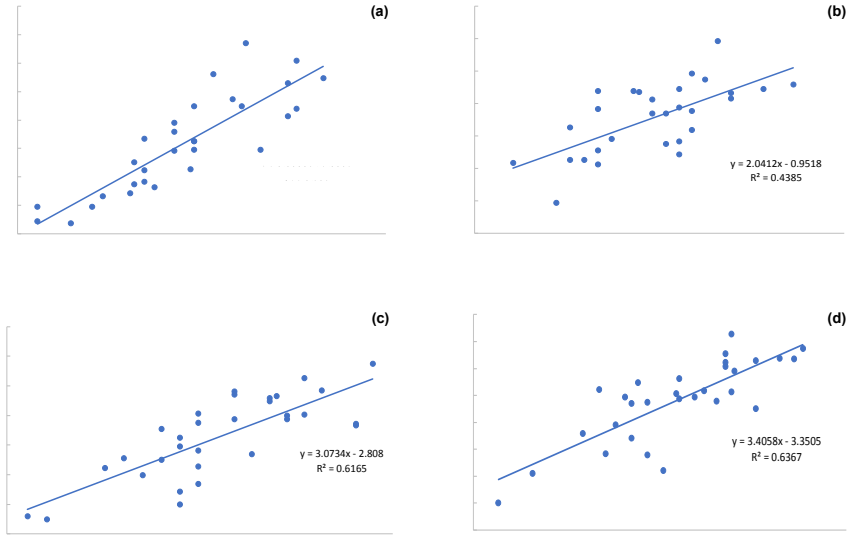

Fig. 1. Length-weight relationship of shortfin eel reared in different salinity levels. (a) 0 ppt, (b) 10 ppt, (c) 20 ppt and (d) 30 ppt.

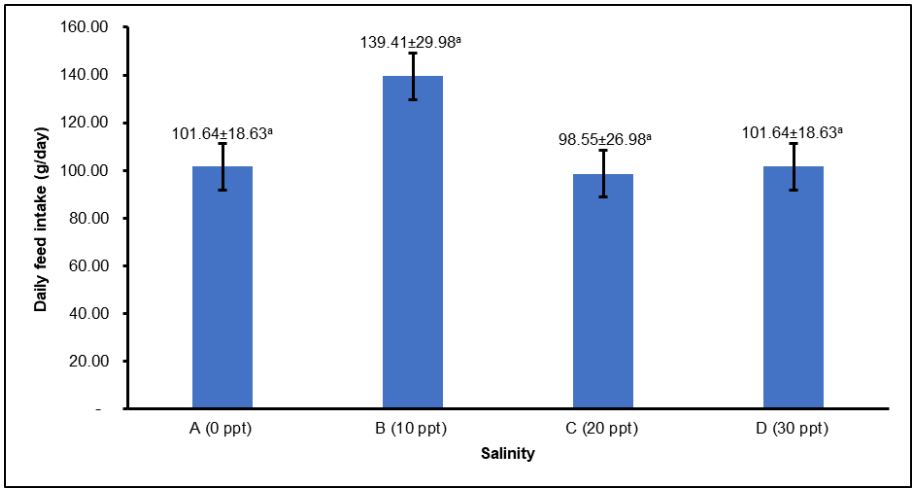

Fig. 2. Daily feed intake (g/day) of shortfin eel reared in different salinity.

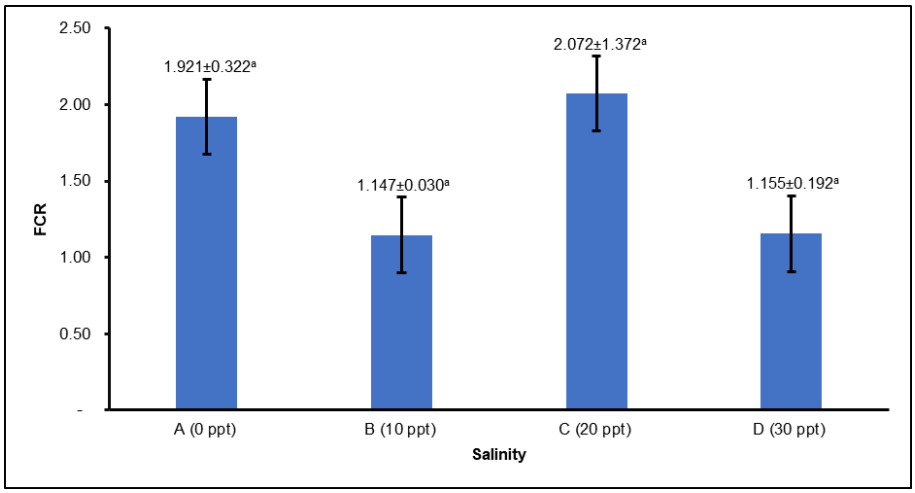

Fig. 3. Feed conversion ratio (FCR) of shortfin eel reared in different salinity

Stress response

In this study number of mucous cells exhibited no statistically significant differences among the groups, there were noticeable variations in mucous cell density. Notably, shortfin eel reared in 10 ppt salinity displayed the highest density of mucous cells, with an average of 24.00±7.94 cells/mm (Figure 4). In club cell density, shortfin eel reared in 0 ppt (19.93±3.08 cells/mm) exhibited significantly higher density of club cells compared to those reared in 30 ppt, 20 ppt, and 10 ppt (Figure 5). The mucous cells were frequently observed on the epidermis layer of all examined shortfin eel, regardless of the salinity conditions in which they were reared (Figure 6).

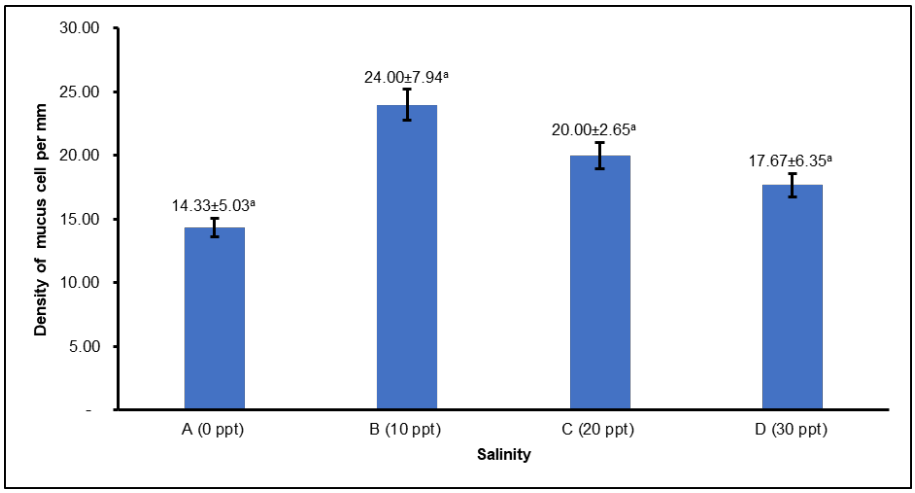

Fig. 4. Density of mucous cell (cell mm^{-1}) of shortfin eel reared in different salinity.

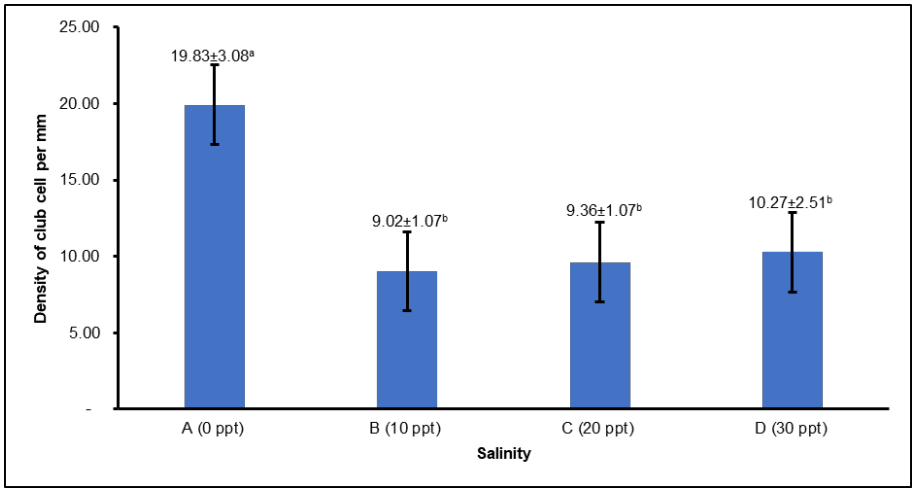

Fig. 5. Density of club cell (cell mm^{-1}) of shortfin eel reared in different salinity.

DISCUSSION

In the present study, the shortfin eel achieved a survival rate of 100% despite being reared in a range of salinities, from freshwater to brackish water to seawater. These results indicate that salinity does not affect the survival of the shortfin eel, and in fact, shortfin eels have been shown to have a high tolerance to changes in salinity. In current aquaculture, shortfin eels are exclusively cultured in freshwater, either in ponds or in tank systems (Putra *et al*., 2021; Sadi *et al*., 2022; Budiardi *et al*., 2022). The results of this study open the possibility of farming shortfin eels in different salinities. This will allow farmers near the coast and in estuaries to grow shortfin eels, which has not been practiced before.

The present study showed that no significant differences were observed in all growth parameters of shortfin eels reared in different salinities, except that those reared in 10 ppt achieved significantly higher body weight and specific growth rate and were also relatively higher in all growth parameters. Hence, salinities did not affect the growth performance of shortfin eel.

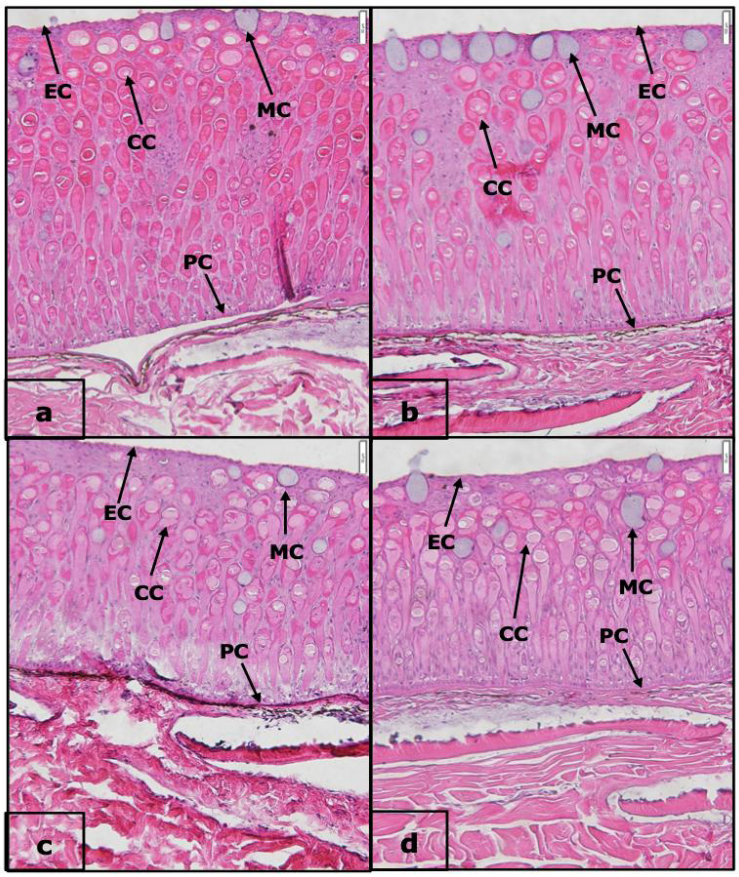

Fig. 6. Histological analysis of shortfin eel epidermis tissue. (a) Shortfin eel reared in 0 ppt (b) shortfin eel reared in 10 ppt, (c) shortfin eel reared in 20 ppt, (d) shortfin eel reared in 30 ppt. Microscopic insights (200x magnification under histological analysis unveil abundant club cells on shortfin eel mucus cells. Club cell (CC), mucous cell (MC), epithelial cell (EC), and pigment cell (PC).

Fish achieve better growth in different salinity levels when they reach the isotonic point of the fish body, in which the osmotic pressure of the external environment is close to the osmotic pressure of the fish body) the growth rate of fish is the fastest (Barman *et al*., 2005; Shi *et al*., 2010). In the present study, the shortfin eel is considered to have reached its isotonic point at 10 ppt, which could lead to several energy-saving advantages compared to the shortfin eel under hypotonic and hypertonic conditions at 0, 20, and 30 ppt, which requires more energy to adapt its body to reach the isotonic point instead of consuming energy for growth. Both juvenile steelhead trout and fall chinook salmon were found to have higher metabolic rates when reared at lower and higher salinities, which required more energy as part of the metabolic processes and resulted in slower growth (Morgan & Iwama, 1991).

Shortfin eels, like many other eel species, are known for their remarkable life cycle that involves migrations between freshwater and marine environments, which exposes them to varying salinity levels. When shortfin eels are reared in environments with different salinity levels (0, 20 & 30 ppt), they may indeed evolve several physiological mechanisms to maintain osmotic balance in their bodies which results in lower growth performance. Relatively lower growth was observed in shortfin eels in 0 ppt, which is not beneficial to current eel-rearing practices because they require more energy to compensate for the need to adapt and achieve isotonic conditions.

According to Kim *et al*. (2004), during fish acclimation to ambient water salinity, protein above the amount needed for growth can also be catabolized and used as an energy source. The present study firmly believed that the slow growth of shortfin eel as shown by those reared in 0, 20, and 30 ppt had a direct influence on its metabolism. When shortfin eel was initially reared in 0 ppt, they did not trigger to use of their metabolism to support better growth as similarly mentioned by Nordlie (2009) whereby higher salinity will trigger some physiology and metabolism processes in the body for growth. Energy metabolism for osmoregulation is a research hotspot in fish physiology, which extremely affects the energy allocation in fish growth and reproduction and then decreases the economic benefits (Tseng & Hwang, 2008).

The condition factor (K) of a fish is influenced by various physical and biological factors, including

feeding conditions, parasitic infections, and physiological factors (Le Cren, 1951). This observation also serves as an indication of fluctuations in food reserves, thereby serving as a potential indicator of the overall health and well-being of the fish population. Additionally, body condition offers a cost-effective alternative to the costly in vitro proximate analyses of tissues (Sutton *et al*., 2000). Hence, the acquisition of knowledge regarding the condition factor assumes significant importance in the management of culture systems, as it equips producers with specific information about the environmental conditions in which organisms are undergoing development (Araneda *et al*., 2008).

Although no significant differences in feed intake and FCR of shortfin eels at different salinities were observed in the present study, the highest feed intake with the lowest FCR was observed in shortfin eels at 10 ppt, indicating efficient feeding performance compared to those reared in 0, 20 and 30 ppt. Higher feed intake and low FCR seen in shortfin eel reared in 10 ppt might have contributed to better feed utilization, digestion, and absorption. Klein *et al*. (1998) stated if the fish are reared under optimal conditions, including appropriate salinity, there is no disturbance of the general physiological development of the fish. This condition, allows normal development to take place in the fish body, including the function of the overall activity of digestive enzymes, which allow it to digest the nutrients in the feed and efficiently convert them into growth (Ali *et al*., 2004).

In the present study, histological analysis revealed that mucus cells were present in the epidermis of all shortfin eels reared at different salinities. The density of mucous cells varied in each treatment. However, the number of mucus cells in each salinity did not show significant differences. Similar to other fish species, the shortfin eel produces mucus cells on the surface of its epidermis that contain innate immune components secreted by goblet cells, which form the primary defense against various pathogenic microbes in response to the environment (Dash *et al*., 2018). The density of mucus cells recorded in the present study was considered low (24.00 ±7.94 cells/mm) compared to those fish under stressful conditions recorded in seabass (200-900 cells/mm) by Vatsos *et al*. (2010) and Atlantic salmon (150-1000 cell/mm) recorded by Landeira-Dabarca *et al*. (2013).

CONCLUSION

Anguila bicolor exhibited 100% survival across various salinities. However, it displayed notable improvements in body weight growth when reared in 10 ppt, along with relatively better total length, feed intake, and FCR compared to other salinity levels. The fish also displayed non-stress rearing when reared in 10 ppt, indicating that this salinity level is optimal for their culture.

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ETHICAL STATEMENT

All experiment were conducted in accordance with the researcher guidelines of the code of practice for the care and use of animal for scientific purpose, Universiti Malaysia Sabah. Approval code was AEC0009/2024

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