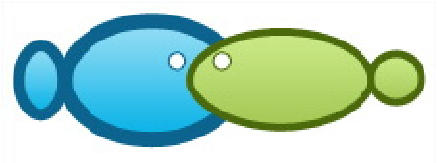

Optimization of salinity and calcium on Indonesian shortfin eel *Anguilla bicolor* maintenance

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Abstract. Salinity and calcium affect the osmoregulation activity. In the optimum salinity and calcium conditions, the energy for osmoregulation will be minimal thus allowing for more available energy portion for growth. This study aimed to determine the values of optimum salinity and the optimum calcium content range at optimum salinity which can improve the survival and growth of Indonesian shortfin eel *Anguilla bicolor.* This research was conducted in two stages: determination of optimum salinity which was done for seven days and determination of optimum calcium content range at optimum salinity which was carried out for 14 days. The research design used was complete randomized design (CRD). Each stage consisted of five treatments and three replications. This research was conducted from November to December 2016. An aquarium with size of 60 x 30 x 30 cm was used in this experiment which further was filled with 30 liters of water per aquarium, with stocking density of 2 g L^{-1}. The fish used were *A. bicolor* with weight of 0.13-0.20 g. During maintenance, A. bicolor were not fed (fasted). The results of the first stage showed that survival rate was not significantly different between treatments ($P>0.05$), while the second stage was $P<0.05$. The rate of decline in absolute biomass was significantly different between treatments ($P<0.05$) both in first and second stage. The optimum salinity was 8 ppt salinity and the optimum range of calcium level was 25-75 mg $CaCO_3$ L^{-1} (equivalent to 10-30 mg Ca L^{-1}).

Key Words: survival, growth, $CaCO_3$, osmoregulation.

Introduction. World eel demand is 268,342 tons/year (FAO 2014). Moreover, according to the Ministry of Maritime and Fisheries data, eels demand for Jakarta Capital City reached three tons/month (KKP 2011). High demand for eels in Asian and European markets encourages the development of eel cultivation (*Anguilla* spp) in the world (Liao 2000).

According to ICES (2005), the increase in consumer demand for eel seeds (especially the glass eel stadia) has resulted in overexploitation of the population in nature, resulting in declining population in nature and eel production in the world. The selling price of glass eel in Indonesia reaches 143 - up to 250 US$/kg (container price in Cilacap and Pelabuhan Ratu), this price may fluctuate depending on its abundance. The high selling price Indonesian shortfin eel *Anguilla bicolor* is also one of the causes of the increase in exploitation of *A. bicolor*.

The causes of high exploitation of *A. bicolor* from nature are breeding technology which has not been found and inadequately developed eel cultivation technique today; thus, the use of seeds in cultivation activity is not efficient. The development of better eel cultivation method is expected to increase the survival and growth so that impact on the efficiency improvement of seed utilization, which in the end can decrease the level of seed exploitation; hence, sustainability of *A. bicolor* in nature can be maintained.

Efforts to preserve eel populations in nature have been done Europe and Japan by issuing rules of utilization. The International Union for Conservation of Nature (IUCN) has placed *A. bicolor* into the near threatened category (www.traffic.org 2015). Indonesia has also issued Ministerial Decree No.19/MEN/2012 on the "Prohibition of Glass eel seed

(*Anguilla* spp.) Export from the Territory of the Republic of Indonesia with eel size of ≤150 g/eel".

Environmental manipulation in term of salinity and calcium is one method that can be used to improve the survival and growth of *A. bicolor*. According to Nordlie (2009), O'Neill et al (2011), Perez-Robles et al (2012) and Fazio et al (2013), salinity is one of the determinants of fish growth. Furthermore, Cairns et al (2008) stated that *Anguilla* in temperate climate area farmed in freshwater have slow growth. Sutrisno (2008) stated that the best salinity for *Anguilla bicolor* seeds was 5 mg L^{-1} with a survival rate of 100% and a specific growth rate of 2.33%. Affandi & Riani (1995) stated that a good salinity ranged from 0 to 7 ppt for survival and growth of eel seeds (glass eel and elver).

Calcium in vertebrate animals including eel not only effects on osmoregulation but also plays a role in the formation of skeletons. Cheng et al (2006) and Fontagne et al (2009) suggested that calcium deficiencies in fish might disrupt the formation of hard tissue structures, osmoregulation, and nerve transmission. Increased levels of calcium in freshwater can be done with an increase in salinity/or with the addition of calcium. In accordance with research results by Kaligis et al (2009), it is necessary to add calcium in rearing media of *Litopenaeus vannamei* acclimatized at salinity of 0 g L^{-1}. Salinity and calcium play a role in maintaining the balance of osmotic pressure in fish body and environment, hence the metabolism goes well. The addition of various calcium sources such as $Ca(OH)_2$, CaO and $CaCO_3$ to the media and through feeding can increase the growth of prawns *Macrobrachium rosenbergii* (Zaidy et al 2008; Hadie et al 2009), sutchi catfish *Pangasianodon hypophthalmus* (Hastuti et al 2012) and eel *A. bicolor* on elver stadia (Scabra et al 2016).

Therefore, this research was done to improve the survival and growth of *A. bicolor* through environmental manipulation, especially the addition of salinity and calcium to minimize the utilization of *A. bicolor* from nature. The objectives of this study were to determine the optimum salinity value and calcium content range in optimum salinity that maximize survival and suppress the absolute biomass reduction rate of *A. bicolor* in the fasted state.

Material and Method. This research was conducted in the Laboratory of Production Technology and Management of Aquaculture, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB, Darmaga Bogor from November to December 2016. This research consisted of two stages:

First stage. This study was conducted for seven days. During the maintenance, experimental fish were not fed so that the growth response indicated was in the form of weight reduction. The research design used was a complete randomized design (CRD) with five treatments: (a) salinity of 0 ppt, (B) salinity of 4 ppt, (C) salinity of 8 ppt, (D) salinity of 12 ppt and (E) salinity of 16 ppt, and each treatment had three replications. *A. bicolor* used originated from Cimandiri estuary, Pelabuhan Ratu, Sukabumi West Java, with weight ranged from 0.13 to 0.20 g. *A. bicolor* was transported from a catching area using water with a salinity of 10 ppt. After post-transport, *A. bicolor* were maintained in a tank with a salinity of 10 ppt for four days. Full aeration was performed on each experimental unit. The stocking density used was 2 g L^{-1}. Salinity adaptation was done gradually, namely by changing the salinity of 2 ppt every 6 hours. After reaching the highest salinity (16 ppt) and the lowest salinity (0 ppt), the *A. bicolor* were stocked into each experimental unit according to the treatment provided. The aquarium used was 60x30x30 cm filled with 30 L of water. The best treatment in the first stage of the study will be used in the second stage of the study.

Parameters measured include:

- Survival Rate (SR) was calculated using:

$$SR\ (\%) = (N_t / N_o) \times 100$$

Description: SR - Survival rate (%)
N_t - Final number of experimental fish (fish)

N_0 - Initial number of experimental fish (fish)

- Rate of decline in absolute biomass (RDAB) which was calculated based on the following equation: $RADB = (B_t - B_o) / t$

Description: RDAB - rate of decline in absolute biomass (g/day)
B_t - average biomass of fish at time t (g)
B_0 - average biomass of fish at initial time (g)
t - time of sampling (day)

- Oxygen consumption level (OC) in standard metabolism was calculated using the equation below:

$$OC = [V \times (DO_o - DO_t)] / w \times t$$

Description: OC - oxygen consumption level (mg O_2 g^{-1} $hour^{-1}$)
V - water volume in the tank (L)
DO_o - initial dissolved oxygen concentration (mg L^{-1})
DO_t - final dissolved oxygen concentration (mg L^{-1})
w - weight of experimental fish (g)
t - observation period (hour)

- Osmoregulation activity in the form of osmotic gradient (OG) was measured by calculating the difference between the osmotic pressure of the media and the osmotic pressure of the fish body fluid. The formula used is as follow:

$$OG = [ODI - OM]$$

Description: OG - Osmotic gradient (mOsm L^{-1} H_2O)
ODI - Osmolarity of fish body fluid (mOsm L^{-1} H_2O)
OM - Osmolarity of media (mOsm L^{-1} H_2O)

- Physic and chemical parameters of water were temperature measured using thermometer, pH measured using pH-meter, DO measured using DO-meter, NH_3 measured using spectrophotometer, while alkalinity was calculated through titration method (APHA 2012).

- Second stage

This study was conducted for 14 days. During the maintenance, experimental fish were not fed so that the growth response indicated was in the form of weight reduction. The salinity used was the optimum salinity value obtained from the first stage of the research. This study used a complete randomized design (CRD) with five treatments: (A_2) $CaCO_3$ of 0 mg L^{-1} (Ca of 47.6 mg L^{-1}), (B_2) $CaCO_3$ of 25 mg L^{-1} (equivalent to 10 mg Ca L^{-1}), (C_2) $CaCO_3$ of 50 mg L^{-1} (equivalent to 20 mg Ca L^{-1}), (D_2) $CaCO_3$ of 75 mg L^{-1} (equivalent to 30 mg Ca L^{-1}), and (E_2) $CaCO_3$ of 100 mg L^{-1} (equivalent to 40 mg Ca L^{-1}) and each treatment had three replications. *A. bicolor* used originated from the estuary Cimandiri, Pelabuhan Ratu, Sukabumi West Java, with weight ranged from 0.13 to 0.20 g. *A. bicolor* were transported from a catching area using water with salinity of 10 ppt. After post-transport, *A. bicolor* was adapted to a tank with a salinity of 8 ppt for four days. Full aeration was performed on each experimental unit. The stocking density used was 2 g L^{-1}. Adaptation was carried out for four days then fish were stocked into each experimental unit according to the treatment provided. A stock solution was made for each treatment which will be used to replace the water loss in the maintenance medium during the experiment. The aquarium used was 60x30x30 cm^3 with a volume of 30 L of water.

Parameters measured include:

1). Survival Rate (SR) was calculated using:

$$SR (\%) = (N_t / N_o) \times 100$$

Description: SR - Survival rate (%)
N_t - Final number of experimental fish (fish)
N_0 - Initial number of experimental fish (fish)

- Growth responses include:
- Rate of decline in absolute biomass (RDAB) which was calculated based on the following equation: $RDAB = (B_t - B_0) / t$

Description: RDAB - rate of decline in absolute biomass (g/day)
B_t - final average biomass of fish (g)
B_0 - initial average biomass of fish (g)
t - sampling time (day)

- Calcium body (CaB) used Takeuchi method
- The use of protein during the fasting (UP) was calculated using the equation below:

$$UP = [(Pt_o - Pt_t) / P_{to}] \times 100$$

Description: UP - the use of protein during the fasting (%)
Pt_o - initial total body protein of fish (g)
Pt_t - final total body protein of fish (g)

- Energy expenditure during fasting (UE) was calculated using the equation below:

$$UE = [(Et_o - Et_t) / Et_o] \times 100$$

Description: UE - the use of energy during fasting (%)
Et_o - initial total body energy of fish (kkal)
Et_t - final total body energy of fish (kkal)

3) Oxygen consumption level (OC) in standard metabolism was calculated using the equation below:

$$OC = [V \times (DO_o - DO_t)] / w \times t$$

Description: OC - oxygen consumption level (mg O_2 g^{-1} $hour^{-1}$)
V - water volume in the tank (L)
DO_o - initial dissolved oxygen concentration (mg L^{-1})
DO_t - final dissolved oxygen concentration (mg L^{-1})
w - weight of expermental fish (g)
t - observation period (hour)

4) Osmoregulation activity in the form of osmotic gradient (OG) was measured by calculating the difference between the osmotic pressure of the media and the osmotic pressure of the fish body fluid. The formula used is as follow:

$$OG = [ODI - OM]$$

Description: OG - Osmotic gradient (mOsm L^{-1} H_2O)
ODI - Osmolarity of fish body fluid (mOsm L^{-1} H_2O)
OM - Osmolarity of media (mOsm L^{-1} H_2O)

5) Physic and chemical parameters of water were temperature measured using thermometer, pH measured using pH-meter, DO measured using DO-meter, NH_3 measured using spectrophotometer, while alkalinity was calculated through titration method (APHA 2012).

The data obtained in the first and second stages will be analyzed using Analysis of Variance (ANOVA) with F test at 95% of confidence level. If treatment was significantly different, Duncan test was performed. The data of physicochemical of water were analyzed descriptively and presented in tables.

Results

First stage. Data on survival rate, the rate of absolute biomass reduction, gradient osmotic, and oxygen consumption level of glass eel in each treatment in the first stage of the study are presented in Table 1.

Table 1

Survival rate (SR), rate of decline in absolute biomass (RDAB), Osmotic gradient (OG) and oxygen consumption (OC) in each treatment

Parameter	Salinity treatment (ppt)				
	0 (A)	4 (B)	8 (C)	12 (D)	16 (E)
SR (%)	100±0.00[a]	100±0.00[a]	100±0.00[a]	100±0.00[a]	100±0.00[a]
RDAB (g day^{-1})	-0.67±0.010[a]	-0.59±0.020[c]	-0.35±0.012[e]	-0.56±0.007[d]	-0.64±0.014[b]
OG (mOsm L^{-1})	0.016±0.000[b]	0.024±0.001[c]	0.010±0.001[a]	0.014±0.001[b]	0.052±0.002[d]
OC (mgO_2 $hour^{-1}$ g^{-1})	0.500±0.005[a]	0.249±0.004[c]	0.053±0.005[a]	0.074±0.001[b]	0.505±0.011[a]

Statistical analysis showed that survival rate was not significantly different ($P>0.05$) between treatments, whereas the rate of absolute biomass reduction was significantly different ($P<0.05$) between treatments. The results showed that the lowest osmotic gradient and the lowest oxygen consumption level occurred at salinity of 8 ppt resulted in the lowest absolute biomass reduction rate. In addition, based on polynomial regression analysis, the optimal condition for survival and growth of *A. bicolor* was at salinity of 8.3 ppt.

Physicochemical parameters of water in the form of temperature, pH, DO, ammonia (NH_3), and alkalinity are presented in Table 2.

Table 2

The values of physic and chemical parameters of water at each treatment during maintenance

Parameter	Treatment (ppt)				
	0 (A_2)	4 (B_2)	8 (C_2)	12 (D_2)	16 (E_2)
Temperature (ºC)	26.0-26.5	26.0-26.3	26.0-26.4	26.0-26.4	26.0-26.5
DO (mg L^{-1})	6.1-6.7	5.6-6.3	6.0-6.3	6.0-6.4	5.8-6.1
pH	7.12-7.69	7.38-7.96	7.44-7.83	7.56-7.96	7.34-8.04
Alkalinity (mg L^{-1} $CaCO_3$)	29.90-44.85	44.85-59.80	59.80-74.75	74.75-89.70	89.70-104.65
NH_3 (mg L^{-1})	0.0017-0.0094	0.0001-0.0003	0.0001-0.0001	0.0001-0.0004	0.0001-0.0007

Physic and chemical parameters of water during the study were within the tolerance range for survival and growth of *A. bicolor*. However, there was an increase in pH and alkalinity along with the increasing salinity value.

Second stage

Data on survival rate, rate of decline in absolute biomass, protein consumption, energy consumption, osmotic gradient, oxygen consumption level, and body calcium of *A. bicolor* at each treatment in the first stage of the study are presented in Table 3.

Statistical analysis showed that the survival rate and rate of absolute biomass reduction were significantly different ($P<0.05$) between treatments. The results showed that the lowest osmotic gradient occurred in the addition of 50 mg L^{-1} $CaCO_3$ so that oxygen consumption level was also low which was indicated by low protein consumption and energy expenditure resulted in the lowest rate of absolute biomass reduction. While the level of body calcium increased with increasing levels of calcium which was equivalent to $CaCO_3$ added to the water.

Table 3

Survival rate (SR), rate of decline in absolute biomass (RDAB), body protein consumption (UP), body energy consumption (UE), osmotic gradient (OG), oxygen consumption (OC) and body calcium (CaB) of *Anguilla bicolor* in each treatment

Parameter	Treatment (mg L^{-1})				
	A_2 (0)	B_2 (25)	C_2 (50)	D_2 (75)	E_2 (100)
SR (%)	97.15±0.212^{a}	98.21±0.191^{b}	99.40±0.424^{c}	98.21±0.191^{b}	97.45±0.212^{a}
RDAB (g day^{-1})	-1.96±0.018^{b}	-1.85±0.005^{c}	-1.81±0.016^{c}	-1.84±0.005^{c}	-2.01±0.024^{a}
UP (%)	54.41±0.00^{d}	50.53±0.16^{c}	48.72±0.45^{b}	547.97±0.14^{a}	59.05±0.39^{e}
UE (%)	50.26±1.69^{bc}	45.68±2.02^{ab}	42.79±2.46^{a}	44.36±2.05^{a}	52.27±2.07^{c}
OG (mOsm L^{-1})	0.09±0.006^{c}	0.08±0.001^{b}	0.05±0.003^{a}	0.09±0.004^{c}	0.10±0.001^{c}
OC (mgO_2 $hour^{-1}$ g^{-1})	0.28±0.003^{d}	0.19±0.012^{b}	0.15±0.002^{a}	0.19±0.001^{b}	0.28±0.004^{c}
CaB (%)	0.39±0.006^{a}	0.60±0.010^{b}	0.64±0.009^{c}	0.71±0.009^{d}	0.78±0.010^{e}

The physic and chemical parameters of water in the form of temperature, pH, dissolved oxygen (DO), alkalinity, ammonia (NH_3), and nitrite (NO_2) are presented in Table 4.

Table 4

The values of physic and chemical parameters of water in each treatment during maintenance

Parameter	Treatment (mg L^{-1})				
	A_2 (0)	B_2 (25)	C_2 (50)	D_2 (75)	E_2 (100)
Temperature (ºC)	28.9-32.2	28.5 - 31.7	29.3-31.8	29.1-32.1	29.6-32.3
pH	6.31-7.88	6.35-7.85	6.44-7.94	6.53-8.13	6.71-8.25
DO (mg L^{-1})	5.4–6.1	5.3–6.2	5.4–6.2	5.2–5.9	5.2–6.3
Alkalinity (mg L^{-1} $CaCO_3$)	34.35-45.80	48.80-57.25	57.25-68.70	68.70-80.15	91.60-103.05
Ammonia (mg L^{-1})	$0.7x10^{-4}$–$20x10^{-4}$	$2.9x10^{-4}$–$30x10^{-4}$	$4.4x10^{-4}$ – $30x10^{-4}$	$4.3x10^{-4}$–$50x10^{-4}$	$1.7x10^{-4}$–$170x10^{-4}$
Nitrite (mg L^{-1})	0.18–0.53	0.06–0.48	0.14–0.22	0.13–0.44	0.11–0.43

The physic and chemical parameters of water during the study were within the tolerable limits for survival and growth of *A. bicolor*. However, there was an increase in pH and alkalinity by increasing $CaCO_3$ levels into the culture medium. While the highest ammonia content was in the treatment of 0 mg L^{-1} $CaCO_3$ and the lowest was in the treatment of 100 mg L^{-1} $CaCO_3$.

Discussion

First stage. The ranges of physic and chemical values of water, namely temperature, DO, pH, NH_3, and alkalinity during maintenance which are shown in Table 2 were still within the tolerable range of *A. bicolor*. This is in accordance with the opinion of Heriati (2005) that states the feasibility of DO for fish was >3 mg L^{-1} and alkalinity were 50-300 mg L^{-1}. Ritonga (2014) states that the feasibility of pH was 6-8 and the temperature 23-32ºC. Wahyudi et al (2015) found that the feasibility ammonia for eel must be <0.01 mg L^{-1}. The suitability of physic and chemical value of water during the study led to the survival rate of *A. bicolor* which reached 100% (Table 1).

Weight loss is caused by the body's material components (proteins, fats, carbohydrates) that are converted into the energy used by the *A. bicolor* during fasting.

The lower weight loss during fasting indicates the less energy used for metabolic activity and other biological activities. According to Boeuf & Payan (2001), salinity affects fish growth which is associated with energy utilization for osmoregulation. Tseng & Hwang (2008) stated that fish maintained in salinity which is closer to the concentration of ions in the blood, use less energy for osmoregulation and more energy for growth.

The results of the study (Table 1) showed that the lowest absolute biomass reduction rate was found in the salinity of 8 ppt because it was closer to ion concentration in the blood; thus, less energy was used than in other treatments. This is in accordance with Boeuf & Payan (2001) which stated that fish approaching isotonic condition use less energy for metabolism than fish in hypotonic and hypertonic conditions.

Several studies have shown that American eel *A. rostrata* originated from different habitats (freshwater and brackish water) have different growth patterns because they are genetically different (Cote et al 2009). *A. Anguilla* and *A. rostrata* maintained in seawater show significantly higher growth compared with freshwater experiments (Edeline et al 2005; Lamson et al 2009).

Salinity of 4 ppt, 12 ppt and 16 ppt showed greater weight loss compared to that of 8 ppt. According to Boeuf & Payan (2001), salinity change can affect osmotic pressure of *A. bicolor* body fluid; thus, body component was more used as energy for osmoregulation activity. This statement is reinforced by the results of the study that osmotic gradient measurements continued to increase with a change in salinity value which was farther from the optimum salinity (Table 1). The high osmotic pressure of the media at high salinity is due to increased concentration of dissolved ions such as sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), chloride (Cl), sulfate (SO_4^{2-}), and bicarbonate (HCO^{3-}). Furthermore, Boeuf & Payan (2001) stated that salinity affects hormone secretion, standard metabolism, feeding and feed conversion.

Environmental condition of hypoosmotic and hyperosmotic caused an increase in body metabolism activity of *A. bicolor* so that affected energy utilization of the body, oxygen consumption, osmoregulation activity and excretion rate. *A. bicolor* maintained at nearly isoosmotic conditions (8 ppt), were less in using body energy for osmoregulation and respiration which resulted in lower biomass degradation and lower ammonia excretion. This is consistent with Boeuf & Payan (2001) which stated that fish in isotonic conditions have lower metabolic rates than those in hypotonic and hypertonic conditions. Moreover, (Boeuf & Payan, 2001) stated that osmoregulation activity also uses a high proportion of energy ranges from 20 to 50% of total energy expenditure depending on the environmental salinity. In hyperosmotic or hypoosmotic conditions, the energy used for the osmoregulation process is so large that the energy portion for growth will be smaller (Kucuk 2013; Lisboa et al 2015).

Second stage. The values of physic and chemical parameters of water shown in Table 4 such as temperature, DO, pH, alkalinity, NH_3 and NO_2 for 14 days of fasting were still in the tolerance range for survival and growth of *A. bicolor*. This is in accordance with the opinion of Heriati (2005) that the feasibility of DO for fish is >3 mg L^{-1} and alkalinity 50-300 mg L^{-1}. Ritonga (2014) stated that the feasibility of pH is 6-8 and the temperature 23-32ºC. Wahyudi et al (2015) found that the feasibility of ammonia for eel must be <0.01 mg L^{-1}. Knosche (1994) stated that the feasibility nitrate must be <0.5 mg L^{-1}. The suitability of water quality values during this study resulted in a survival rate of *A. bicolor* ranged from 97.15-99.40 (Table 3).

Different amount of calcium in the optimal cultivation medium caused increase in osmotic pressure in the media. The results showed that the increase of calcium which was equivalent to $CaCO_3$ added to medium with salinity of 8 ppt resulted in an increase of osmotic pressure of maintenance media namely A_2 treatment of 0.02 mOsm L^{-1} H_2O, B_2 of 0.05 mOsm L^{-1} H_2O, C_2 of 0.08 mOsm L^{-1} H_2O, D_2 of 0.10 mOsm L^{-1} H_2O and E_2 of 0.13 mOsm L^{-1} H_2O. This is in accordance with Tsuzuki et al (2007) which stated that teleostei fish adaptation including eel in sea water is performed by entering more water into the body to replace water loss due to osmotic pressure; thus, this process led to inclusion of 30-70% of calcium and then absorbed by the digestive tract (Satoh et al 2000).

Calcium absorption is started in the intestine, if there is a lot of available calcium

in aquaculture media, then calcium will enter the body and is directly circulated to the blood vessels through a diffusion process which does not require energy. However, if a small amount of calcium is available then calcium metabolism will be performed through an active transport process that requires vitamin D to induce Ca-binding protein and activate ATPase to transport Ca to the blood vessels and then stored it in the bone (Jones & de Luca 1988). This active transport process requires energy thus affecting growth.

The addition of $CaCO_3$ as much as 50 ppm in the optimum (8 ppt) medium provided the isoosmotic condition for *A. bicolor* so that the osmotic gradient value was low, whereas without the addition of $CaCO_3$ and with the addition of 25 mg L^{-1}, 75 mg L^{-1} and 100 mg L^{-1} $CaCO_3$, either hyperosmotic or hypoosmotic was obtained which led to an increase in osmotic gradient values (Table 3). Increased osmotic gradient caused an increase in osmotic load so that it affected oxygen consumption level (Table 3).

Changes in calcium levels in water lead to hypercalcemia or hypocalcemia. When the body is unable to tolerate the changes, the osmotic load will increase so that the fish are not able to adapt and die. When the calcium content in the water is optimal or close to isoosmotic conditions, the osmotic load will be low so that the catabolism of protein, fat and carbohydrates will produce energy that will be used for osmotic regulation which leads to less energy consumption for metabolism while growth will increase. In addition, the osmotic load will affect the level of feed consumption by the fish and affect the process of anabolism resulting in changes in growth.

The growth response shown in this study was a negative growth response in the form of weight loss due to fasting performed during the study. The smaller the weight loss indicated the more comfortable living environment for *A. bicolor*. The results showed that the increase in body energy utilization occurred when *A. bicolor* had a lack or excess of calcium in the body (Table 3). Increased use of body energy in the treatment of A_2, B_2, D_2 and E_2 for metabolic activity led to an increase in the rate of absolute biomass reduction (Table 3).

Optimal salinity and calcium conditions in aquaculture media can maintain a balance of osmotic pressure between the fish body and its environment which causes the metabolism of the body performs well, thus increasing the growth. Based on data on protein consumption (Table 3), it is seen that treatment D_3 used the least protein compared with other treatments, while the lowest body energy consumption occurred in treatment C_3. This shows that treatment D_3 used more energy derived from fat.

Based on the data, treatment E_2 showed the highest level of calcium. This is likely to cause hyperosmotic conditions in eel (Satoh et al 2000), thus increasing the use of protein and energy compared with other treatments resulted in the highest biomass weight loss (Table 3). When Ca^{2+} is high in plasma, it will activate protease enzymes that play a role in breaking down proteins. If protease is active, it will disrupt the permeability of cell membranes due to the differences in gradient concentrations (Verbost et al 1989); therefore, more energy will be used.

Based on the research results, the optimum calcium content range that can improve survival and growth of *A. bicolor* ranged from 25 to 75 mg L^{-1} $CaCO_3$ or equivalent to 10-30 mg Ca L^{-1}. While the results of polynomial regression analysis showed that the calcium content range of 46.6-60.0 mg L^{-1} $CaCO_3$ or equivalent to 18.64-24.00 mg Ca L^{-1} was the optimal condition for the survival and growth of *Anguilla bicolor*.

Conclusions. The optimum condition for survival and growth of *A. bicolor* was in the media with a salinity of 8.3 ppt, a calcium content ranged from 46.6 to 60.0 mg L^{-1} $CaCO_3$ or equivalent to 18.64-24.00 mg Ca L^{-1}.

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References

Affandi R., Riani, 1995 Effect of salinity on survival rate and growth of eel *Anguilla bicolor*. Journal of Aquatic Sciences and Fisheries 3(1):39-48.

Boeuf G., Payan P., 2001 How should salinity influence fish growth? Comparative Biochemistry and Physiology Part C Toxicology and Pharmacology 130(4):411-423.

Cairns D., Tremblay V., Caron F., Casselman J., Verreault G., Jessop B., de Lafontaine Y., Bradford R., Verdon R., Dumont P., Mailhot Y., Zhu J., Mathers A., Oliveira K., Benhalima K., Dietrich J., Hallet J., Lagace M., 2008 American eel abundance indicators in Canada. Canadian Data Report of Fisheries and Aquatic Sciences No. 1207, 78 pp.

Cheng W. C., Liu H., Kuo C. M., 2006 Effect of dissolved oxygen on hemolymph parameters of freshwater giant prawn *Marcobrachium rosenbergii* de Man. Aquaculture 220:843–856.

Cote C. L., Castonguay M., Verreault G., Bernatches L., 2009 Differential effects of origin and salinity rearing conditions on growth of glass eel of the American eel *Anguilla rostrata*: implications for stocking programmes. Journal of Fish Biology 74:1934-1948.

Edeline E., Dufour S., Elie P., 2005 Role of glass eel salinity preference in the control of habitat selection and growth plasticity in *Anguilla anguilla*. Marine Ecology Progress Series 304:191-199.

Fazio F., Marafioti S., Arfuso F., Piccione G., Faggio C., 2013 Influence of different salinity on haematological and biochemical parameters of the widely cultured mullet, *Mugil cephalus*. Marine and Freshwater Behaviour and Physiology 46:211-218.

Fontagné S., Silva N., Bazin D., Ramos A., Aguirre P., Surget A., Abrantes A., Kaushik J. S., Power M. B., 2009 Effects of dietary phosphorus and calcium level on growth and skeletal development in rainbow trout *Oncorhynchus mykiss* fry. Aquaculture 297:141–150.

Hadie L. E., Hadie W., Prihadi T. H., 2009 The effectiveness of calcium minerals on yuwana giant prawn growth *Macrobrachium rosenbergii*. Journal of Aquaculture Research 4:65-72.

Hastuti Y. P., Djokosetiyanto D., Permatasari I., 2012 Giving of bone flour extract and calcium oxide (CaO) to seed fish growth (*Pangasius* sp.) on salinity media. Indonesian Aquaculture Journal 11:168-178.

Herianti I., 2005 Environmental engineering to spur the development of *Anguilla bicolor bicolor* eels. Oceanography and Limnology of Indonesia 37:25–41.

Jones G. P., de Luca H. F., 1988 HPLC of vitamin D and its metabolites. In: High performance liquid chromatography and its application to endocrinology. Makin H. L. J., Newton R. (eds), Monographs on Endocrinology. Berlin. Springer-Verlag 30:95-139.

Kaligis E., Djokosetiyanlo D., Affandi R., 2009 The effect of calcium addition and salinity on acclimatization on the improvement of postlarvae passage of vaname shrimp *Litopenaeus vannamei* Boone. National Oceanic Journal 2:101–108.

Knosche R., 1994 An effective biofilter type for eel culture in recirculation system. Aquaculture Engineering 13:71-82.

Kucuk S., 2013 The effects of salinity on growth of goldfish, *Carassius auratus* and crucian carp, *Carassius carassius*. African Journal of Biotechnology 12:2082–2087.

Lamson H. M., Cairns D. K., Shiao C. J., Iisuka Y., Tzeng W. N., 2009 American eel, *Anguilla rostrata*, growth in fresh and salt water: implications for conservation and aquaculture. Fisheries Management and Ecology 16:306-314.

Liao I. C., 2000 Encyclopedia of food science and technology. Francis F. J. ed., 2nd edition, Vol. 1, New York: John Wiley and Sons, pp. 584–592.

Lisboa V., Barcarolli I. F., Sampaio L. A., Bianchini A., 2015 Effect of salinity on survival, growth and biochemical parameters in juvenile Lebranch mullet *Mugil liza* (Perciformes: Mugilidae). Neotropical Icthyology 13:447–452.

Nordlie F. G., 2009 Environmental influences on regulation of blood plasma serum

components in teleost fish: a review. Review in fish Biology and Fisheries 19:481-564.
Perez-Robles J., Re A. D., Giffard-Mena I., Diaz F., 2012 Interactive effects of salinity on oxygen consumption ammonium excretion, osmoregulation and Na^+/K^+-ATPase expression in the bullseye puffer (*Sphoeroides annulatus*, Jenyns 1842). Aquaculture Research 43(9):1372-1383.
O'Neill B., De Raedemaecker F., McGranth D., Brophy D., 2011 An experimental investigation of salinity effects on growth, development and condition in the European flounder (*Platichthys flesus*. L.). Journal of Experimental Marine Biology and Ecology 410:39-44.
Ritonga T., 2014 Respon benih ikan sidat (*Anguilla bicolor bicolor*) terhadap derajat keasaman (pH) [skripsi]. Bogor: Institut Pertanian Bogor.
Satoh H., Suzuki K., Yoshijima S., 2000 Present status and aspect on eel aquaculture in Japan. In: Abstracts of The 3rd East Asian Symposium on Eel Research—Sustainability of Resources and Aquaculture of Eels. Keelung: Taiwan Fisheries Research Institute.
Scabra A. R., Budiardi T., Djokosetiyanto D., 2016 Production performance of *Anguilla bicolor bicolor* with the addition of $CaCO_3$ into culture media. Indonesian Aquaculture Journal 15:1-7.
Sutrisno, 2008 Determination of water salinity and proper type of natural feed in the maintenance of eel seeds *Anguilla bicolor*. Jurnal Akuakultur Indonesia 7:71–77.
Tseng Y. C., Hwang P. P., 2008 Some insights into energy metabolism for osmoregulation in fish. Comparative Biochemistry and Physiology, Part C 148:419–429.
Tsuzuki M. Y., Sugai J. K., Maciel J. C., Francisco C. J., Cerqueira V. R., 2007 Survival, growth and digestive enzyme activity of juveniles of the fat snook *Centropomus parallelus* reared at different salinities. Aquaculture 271:319–325.
Verbost P. M., Van J. R., Flik G., Lock R. A. C., Wendelaar B., 1989 The movement of cadmium through freshwater trout branchial epithelium and its interference with calcium transport. Journal of Experiment Biology 145:185–197.
Wahyudi H., Affandi R., Hariyadi S., 2015 Response of sidat *Anguilla bicolor bicolor* against ammonia (NH_3) maintenance media [Essay]. Bogor: Bogor Agriculture Institute.
Zaidy A. B., Affandi R., Kiranadi B., Praptokardiyo K., Manalu W., 2008 Utilization of aquatic media calcium in the moulting process and its consequences for the growth of giant prawns *Macrobrachium rosenbergii* de Man. Journal of Water Sciences and Fisheries Indonesia 15:117–125.
*** APHA (American Public Health Association), 2012 Standard methods for the examination of water and wastewater. Washington DC (US): American Public Health Association.
*** FAO (Food and Agriculture Organization), 2014 Globefish Research Programme, Eel Anguilla spp.: Production and Trade. Rome, Italia: FAO Fishstat Plus.
*** ICES (International Council for the Exploration of the Sea), 2005 Report of the ICES/EIFAC working group on eels. ICES CM 2005/ACFM: 01, Ireland, 71 pp.
*** KKP (Marine and Fisheries Ministry), 2011 Sidat fish farming guide. Jakarta, Indonesia: Center for marine and fisheries extension, KKP RI.
*** Ministerial Decree No.19/MEN/2012, Ministry of Marine and Fisheries of the Republic of Indonesia, 2012 Prohibitions of expirations (*Anguilla* spp) from the territory of the Republic of Indonesia outside the country area Republic of Indonesia. Jakarta.
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