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Optimization of salinity range for rearing glass eel *Anguilla bicolor bicolor*

Optimasi kisaran salinitas pada pendederan glass eel *Anguilla bicolor bicolor*

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ABSTRACT

Fasting is one of a method that used for measured growth of fish in a shorter period of time. This study was aimed to determine the optimum range of salinity for improve the survival and growth of glass eel *Anguilla bicolor bicolor*. It used a completely randomized design (CRD) with four salinity treatments and three replications, namely (A) 0 g/L, (B) 10 g/L, (C) 20 g/L, and (D) 30 g/L. The fish used were of glass eel *A. bicolor bicolor* with 0.15–0.23 g of weight. The experiment was conducted in an aquarium of 60×30×30 cm^3 with a volume of 30 Liters and at a stocking density of 2 g/L for 14 days. During the maintenance, glass eels were fasted for have a significantly of biomass decline. Data collection was done at the start and the end of maintenance. Parameters measured included survival (%) and the rate of decline in absolute biomass (g). Physical and chemical parameters included temperature, dissolved oxygen, and pH which were measured daily, while ammonia and alkalinity were measured every seven days. Result showed that survival was not significantly different between treatments ($P>0.05$), while the rate of decline in absolute biomass was significantly different between treatments ($P<0.05$). Treatments of 0 g/L salinity was the lowest survival than the others. While treatment of 10 g/L salinity was the lowest rate of decline in absolute biomass. According to research, the optimum salinity was 10 g/L, and after analysis with quadratic regression analysis, the optimum range of salinity were 5.00–13.40 g/L.

Keywords: optimum salinity, survival, growth, glass eel, *Anguilla bicolor bicolor*

ABSTRAK

Pemuasaan merupakan salah satu metode pengukuran perubahan bobot ikan yang dipelihara dalam waktu singkat. Penelitian ini bertujuan untuk menentukan kisaran salinitas optimum untuk meningkatkan kelangsungan hidup dan pertumbuhan glass eel *Anguilla bicolor bicolor*. Rancangan penelitian yang digunakan adalah rancangan acak lengkap (RAL), dengan empat perlakuan salinitas dan tiga ulangan, yaitu (A) 0 g/L, (B) 10 g/L, (C) 20 g/L, dan (D) 30 g/L. Penelitian dilakukan selama 14 hari. Ikan yang digunakan adalah glass eel *A. bicolor bicolor* dengan bobot 0,15–0,23 g dengan padat tebar 2 g/L. Pemeliharaan dilakukan di akuarium berukuran 60×30×30 cm^3 dengan volume air 30 Liter/akuarium. Selama pemeliharaan glass eel dipuasakan sehingga diperoleh penurunan biomassa yang signifikan. Pengambilan sampel data dilakukan setiap tujuh hari berupa kelangsungan hidup (%) dan laju penurunan biomassa mutlak (g). Parameter fisika kimia air berupa ammonia dan alkalinitas dilakukan setiap tujuh hari, sedangkan suhu, oksigen terlarut (DO), dan pH dilakukan setiap hari. Hasil penelitian menunjukkan bahwa kelangsungan hidup tidak berbeda nyata antar perlakuan ($P>0,05$) sedangkan laju penurunan biomassa mutlak berbeda nyata antar perlakuan ($P<0,05$). Berdasarkan hasil penelitian, salinitas 10 g/L, 20 g/L, dan 30 g/L menunjukkan kelangsungan hidup 100%, sedangkan salinitas 0 g/L memberikan kelangsungan hidup terendah. Salinitas 10 g/L menunjukkan pemakaian energi terendah untuk metabolisme tubuh sehingga memberikan penurunan bobot biomassa terendah dibandingkan dengan perlakuan lainnya. Hasil penelitian menunjukkan salinitas optimum adalah 10 g/L, dan setelah dihitung menggunakan analisis regresi kuadratik, maka kisaran salinitas optimum adalah 5,00–13,40 g/L.

Kata kunci: salinitas optimum, kelangsungan hidup, pertumbuhan, glass eel, *Anguilla bicolor bicolor*

INTRODUCTION

A stadium of glass eel is the most vulnerable stage, which contributes to the high mortality if compared with stadia of elver and yellow eel (Durif & Elie, 2008; Okamoto *et al*., 2009; Okamura *et al*., 2009a,b; Chow *et al*., 2010; Clevestam *et al*., 2011). Its characteristic that migrates in large group from seawater to brackishwater causes the glass eel to be easily caught in large number in the estuary. Until now, technology of appropriate eel breeding has not yet been found that seed for eel culture still largely depends on nature, while its availability in nature highly depends on the season. Moreover, the growth is also very slow. Development of glass eel into yellow eel stadia in nature takes three to nine years, that is when the sex differentiation occurs and reaches the adult stage (silver eel) three to six years later (Aoyama, 2009). Other constraints include the seed that is not uniform in size, high feed conversion and vulnerable to disease. Development of eel culture method is expected to improve the survival and growth which will have impact on the efficiency of utilization of seed from nature. According Affandi *et al*. (2013) to optimize the usual environmental conditions with added ingredients (e.g: NaCl and $CaCO_3$) into the medium.

Fasting is one of a method that used for measured growth of fish in a shorter period of time. Glass eel in fasting condition shows negative growth response in term of decrease in body weight. Minimum weight loss occurs when eel is placed in media with optimal salinity for life; thus, the growth of eel will be maximal when eel is fed in this condition. Salinity relates to osmoregulation, that is ion exchange between environment and the body. The stability of acid-base regulation in fish body is also maintained due to ion exchange between the fish blood and water with salinity plays role as facilitato. Boeuf and Payan (2001) stated that fish reared in salinity close to ion concentration in blood (isoosmotic) will provide more energy for growth and spend less energy for osmoregulation. Salinity affects secretion of hormone, standard metabolism, appetite and feed conversion. Moreover, Boeuf and Payan (2001) described that hypoosmotic condition due to high salinity can increase the secretion of growth hormone, appetite, and aggressive behavior that leads to cannibalism.

Salinity is a determinant factor for growth of fishes, including eel (Nordlie, 2009; O´Neill *et al*., 2011; Perez-Robles *et al.,* 2012; Fazio *et al*., 2013). Several studies have shown the role of salinity on survival and growth of glass eel. Sutrisno (2008) suggested that salinity of 5 mg/L was the best salinity for eel seed *Anguilla bicolor* with survival rate of 100% and specific growth rate of 2.33%. Edeline *et al*. (2005) said that compared to freshwater, salinity of 34 g/L and temperature of 18 ºC resulted in the highest growth of glass eel *A. anguilla*. Lamson *et al*. (2009) stated that eel *A. rostrata* increased 2.2 times of length and 5.3 times of weight at salinity >28 g/L compared to freshwater. Furthermore, Kearney *et al*. (2008) said that salinity of 17.5 g/L and temperature of 17.5 ºC produced the highest survival on glass eel *A. australis* and *A. dieffenbachia* compared to salinity of 35 g/L and 0 g/L. Based on those former study result, it can be concluded that glass eel from each eel species has different salinity preferences, so it must be finding the optimum range of salinity that improve the survival rate and the growth rate of glass eel *A. bicolor bicolor*. Based on this finding, research to find the optimum salinity range for the survival and growth of glass eel *A. bicolor bicolor* is required to improve the efficient use of eel seed from nature. The purpose of this study was to determine the optimum salinity range which can improve the survival and growth of g glass eel *A. bicolor bicolor.*

MATERIAL AND METHODS

Fish treatment preparation

Glass eels *A. bicolor bicolor* used in this research were collected from estuary of Cimandiri, Pelabuhan Ratu, Sukabumi, West Java with 0.12−0.20 g of weight. Glass eels were transported from the fishing area and placed in water with salinity of 10 g/L. After being transported, glass eels were adapted to salinity of 10 g/L for four days before adapted to treatment salinity. Adaptation according to treatment salinity was done gradually by changing the salinity of 2 g/L for every 6 h. After reaching the highest (30 g/L) and the lowest (0 g/L) salinity, glass eels were stocked into each experimental unit according to the treatment given. Water stock of salinity makes with added sea water into fresh water.

Treatment design

This study used a completely randomized design (CRD) with four treatments namely (A) salinity of 0 g/L, (B) salinity of 10 g/L, (C)

salinity of 20 g/L, and (D) salinity of 30 g/L with three replications of each.

Fish maintenance

Glass eels were maintained for 14 days and were not given feed (fasted), which aims to obtain changes in fish weight that is maintained in the short term. Aquarium of 60×30×30 cm^3 with 30 Liters of volume was used. Stocking density used was 2 g/L. Full aeration was applied on each experimental unit and water changes was 20% every day.

Parameters measured

Parameters measured at the start and the end of maintenance included survival rate and physiology responses (rate of decline in absolute biomass (RDAB), calcium of the body (CaT), protein use during fasting (PU), energy use during fasting (EU), oxygen consumption rate (OC), osmotic gradient (GO). Physical and chemical parameters included temperature, dissolved oxygen, and pH which were measured daily, while ammonia and alkalinity were measured every seven days.

Survival rate (SR)

Survival rate (SR) was calculated based on:

$$SR = Nt / No \times 100$$

Note:
SR = survival (%)
Nt = the number of the alive fish at the final observation (individuals)
No = the number of the alive fish at the initial observation (individuals)

Rate of decline in absolute biomass (RDAB

Rate of decline in absolute biomass (RDAB) which was calculated based on the following equation:

$$RDBA = Bt - Bo / t$$

Note:
RDAB= rate of decline in absolute biomass (g/day)
B = average biomass of fish at time t (g)
Bo = average biomass of fish at initial time (g)
t = time of sampling (day)

Calcium of the body (CaT)

Calcium of the body (CaT) (mg/Kg) based on SNI 06–6989 12–2005, using atomic absorption spectrophotometer (AAS) method:

$$CaT = ((a-b) \times V \times DF \times 1000) / w$$

Note:
CaT = calcium of the body (mg/Kg)
a = concentration of sample solution (mg/L)
b = concentration of blanko solution (mg/L)
V = volume excract
DF = dilution factor
W = weight of sample (g)

Protein use during fasting (PU)

Protein use during fasting (PU), calculated using equation as follows:

$$PU = (Pbo – Pbt) / Pbo \times 100$$

Note:
PU = use of body protein during fasting (%)
Pbo = total of initial body protein (g)
Pbt = total of final body protein (g)

Energy use during fasting (EU)

Energy use during fasting (EU), calculated using equation below:

$$EU = (Ebo – Ebt) / Ebo \times 100$$

Note:
EU = use of body's energy during fasting (%)
Eb_o = total of initial body's energy (kcal)
Eb_t = total of final body's energy (kcal)

Oxygen consumption rate (OC)

Oxygen consumption rate (OC) on standard metabolism which was calculated with:

$$OC = V \times (DOo - DOt) / (w \times t)$$

Note:
OC = oxygen consumption rate (mg O_2/g/hour)
V = volume of water in culture tank (L)
W = weight of sample (g)
DOo = concentration of dissolved oxygen at initial observation (mg/L)
DOt = concentration of dissolved oxygen at time t (mg/L)
w = weight of experimental fish (g)
t = observation period (hour)

Osmotic gradient (GO)

Osmotic load is in the form of osmotic gradient (GO) which was determined by calculating the difference between osmotic pressure of the media

Table 1. Survival rate (SR), specific growth rate (SGR) and feed conversion ratio (FCR)

Parameter	Treatment (g/L)			
	0 (A)	10 (B)	20 (C)	30 (D)
Survival rate (%)	99.43±0.00a	100±0.00a	100±0.00a	100±0.00a
Rate of decline in absolute biomass (g/day)	-2.91±0.026a	-2.79±0.040b	-2.89±0.020a	-2.95±0.051a
Use of body protein (%)	70.69±3.49b	62.78±0.71a	70.00±0.85b	72.50±0.79b
Use of body energy (%)	68.17±2.39b	62.13±0.10a	69.39±0.42b	70.38±0.07b
Osmotic gradient (mOsm/L)	0.071±0.0007a	0.069±0.0014a	0.085±0.0007b	0.089±0.0007c
Oxygen consumption (mgO_2/hour/g)	0.428±0.0021d	0.120±0.0049a	0.277±0.0124b	0.309±0.0047c
Body calcium (mg/Kg)	0.482±0.005c	0.564±0.001d	0.226±0.006a	0.338±0.005b

Note: Different letter in the same row showed the significant different between the treatment (P<0.05).

and osmotic pressure of fish body fluids. The equation used is:

$$OG = [ODI - OM]$$

Description:
OG = osmotic gradient (mOsm/L H_2O)
ODI = osmolarity of fish body fluids (mOsm/L H_2O)
OM = osmolarity of the media (mOsm/L H_2O)

Physical and chemical water parameters

Physical and chemical water parameters included temperature which was measured using thermometer, pH was measured using pH-meter, dissolved oxygen was measured using DO-meter, NH_3 was measured using spectrophotometer, and alkalinity was calculated using titration method (UNESCO/WHO/UNEP, 1996).

Data analysis

Data obtained were analyzed using Microsoft Excel 2007 and SPSS ver. 21.1 on a 95% confidence interval. If significant difference was found, further test of Duncan was performed. Water quality data were analyzed descriptively and presented in table.

RESULTS AND DISCUSSION

Results

Data on survival, rate of decline in absolute biomass, protein use, energy use, calcium of the body, osmotic gradient, and oxygen consumption rate of glass eel in each treatment are presented in Table 1. Result of statistical analysis showed that survival was not significantly different (P>0.05) between treatments, while the rate of decline in absolute biomass was significantly different (P<0.05) between treatments.

The result showed that the lowest osmotic gradient was obtained at salinity of 10 g/L because closest the iso-osmotic conditions. Thus, affected the level of oxygen consumption concerning the low protein use and energy use. Further, this led to the decline rate in absolute biomass at the lowest salinity of 10 g/L. So are the ability to store the highest calcium level of the body was found at salinity of 10 g/L.

Quadratic regression analysis on the relationship between salinity and several growth parameters, namely rate of decline in absolute biomass, protein use, energy use, osmotic gradient, and oxygen consumption are presented in Table 2.

Based on the quadratic regression analysis, closest of a relationships is indicated by the value of r^2. The greater the value of r^2 the closer the causal relationship. The highest value of r^2 was found in RDAB and OG; thus, it was concluded that the optimum salinity affected the survival and growth of eel seed *A. bicolor bicolor* ranged between 5.00−13.40 g/L.

Physical and chemical water parameters (temperature, pH, DO, alkalinity, and NH_3) are presented in Table 3. Physical and chemical parameter of water in this research was still within the tolerable limit for the survival and growth of glass eel *A. bicolor bicolor*.

However, despite the physical and chemical water parameter of eel maintenance media was still within the suitability limit, there was increase in the value of pH, alkalinity, and NH_3 during the research along with the increase in the value of salinity.

Discussion

The range of physical and chemical value of water parameter shown in Table 3 including temperature, DO, pH, NH_3, and alkalinity in each treatment during maintenance was still in the range of tolerance for the survival and growth of eel seed. This finding is in accordance with the opinion of Ritonga (2014), Herianti (2005), and Wahyudi *et al*. (2015). Suitability of physical and chemical value of water parameter during this research resulted in high survival rate of eel seed,that was 99.43−100% (Table 1).

Different salinity in cultivation media led to different value of osmotic gradient. In teleosts, the plasma isosmotic was found in the water salinity of 12 g/L (Boeuf & Payan, 2001; Tsuzuki *et al.,* 2007; Herrera *et al.,* 2009; Nordlie, 2009). Teleost fish, including eel are able to maintain the ionic and osmotic homeostasis by using osmoregulatory mechanisms, which are energy demanding processes (Tseng & Hwang, 2008). Optimum salinity is a condition close to isosmotic thus produces the lowest value of osmotic gradient. In accordance with Lisboa *et al*. (2015) was stated that growth would be maximized when fish is reared in water of salinity near the isosmotic conditions. Otherwise, value of osmotic gradient is higher than the optimum salinity in hyperosmotic or hypoosmotic condition. Increase in osmotic gradient leads to increase in osmotic load thus affecting the rate of oxygen consumption. When the body cannot tolerate changes in salinity, osmotic load within the body will increase and later fish will not be able to adapt and die. When salinity level is close to isosmotic condition, osmotic load will be low so that catabolism of protein, fat and carbohydrates will also be low, resulting in less energy consumption for osmoregulation so that growth will increased.

Salinity also affects calcium content in the body of glass eel. According to Kucuk (2013), plasma osmolality and ionic (Na^+, Cl^-, K^+, and Ca^{2+}) concentrations slightly increased with salinity. Next, Kucuk *et al*. (2013); Scabra *et al*. (2016), was stated that salinity is described as the sum of all ions in water, likes sodium, chloride, calcium, magnesium, potassium, bicarbonate, and sulfate ions. In treatment of 10 g/L salinity which is close to optimum salinity, eel seeds were able to maintain the highest level of calcium in the body compared to other treatments (Table 1). Research results in Table 1 indicated that the lowest rate of decline in absolute biomass was found in treatment B (10 g/L) due to the reason that salinity of 10 g/L was closer to the ion concentration in the blood

Table 2. Value of the best growth parameter at optimum salinity

Parameter	Regression equation	r^2	Salinity (g/L)
Rate of decline in absolute biomass (g/day)	$y = -0.0005x^2 + 0.0134x - 2.9142$	**0.8877**	**13.40**
Use of body protein (%)	$y = 0.026x^2 - 0.6538x + 69.695$	0.6408	12.57
Use of body energy (%)	$y = 0.0176x^2 - 0.3877x + 67.186$	0.5348	11.01
Osmotic gradient (mOsm/L)	$y = 0.00002x^2 + 0.0002x + 0.0689$	**0.8266**	**5.00**
Oxygen consumption (mgO_2/hour/g)	$y = 0.0009x^2 - 0.0275x + 0.3983$	0.6364	17.21

Note: Number in bold shows the closer the causal relationships.

Table 3. Value of physical and chemical water parameter of each treatment during maintenance

Parameter	Treatment (g/L)				Suitability of physical and chemical of water
	A (0)	B (10)	C (20)	D (30)	
Temperature (°C)	28.9–30.5	29.6–30.8	29.1–30.5	30.2–30.6	23−32[1]
DO (mg/L)	5.7–6.1	5.4–6.3	5.4–6.6	5.1–5.9	>32
pH	7.18–7.24	8.20–8.34	8.24–8.45	8.45–8.52	6.0−8.0[1]
Alkalinity (mg/L CaCO3)	67.28−68.77	112.13–119.60	134.55–146.51	179.4–186.88	30−500[3]
NH3 (mg/L)	0.0001−0.0040	0.0013−0.0014	0.0015−0.0018	0.0020−0.0022	>0.01 mg/L[3]

Note: [1]Ritonga (2014), [2]Herianti (2005), [3]Wahyudi *et al*. (2015).

(iso-osmotic) that there will be less energy used for osmotic regulation in the body if compared to other treatments. Statistical analysis showed significant difference result between treatment A and treatment B, C, and D.

Salinity affects the osmoregulation activity. Boeuf and Payan (2001) stated that changes in salinity may affect the osmotic pressure of body fluids of glass eel so that more energy is used for osmoregulation. Results showed that there was increase in the value of osmotic gradient in salinity of 0 g/L, 20 g/L, and 30 g/L. Increase in the value of osmotic gradient affected the growth rate of eel seed which was reflected in the increasing decline in the weight of eel seed (Table 1). It explains that the higher the value of osmotic gradient, the greater the energy used for osmoregulation. The results showed that salinity of 10 g/L is close to isosmotic condition so that the use of body's energy for osmoregulation showed the lowest value. This result is confirmed by the lowest value of oxygen consumption compared to other salinity treatments (Table 1). Petersen *et al*. (2014), stated that eels survived prolonged exposure to 5 and 10 g/L, although plasma osmolality increased at 10 g/L

Boeuf and Payan (2001) suggested that salinity also affects the secretion of hormone, standard metabolism, appetite and feed conversion. Treatment of 10 g/L resulted in the lowest oxygen consumption rate of eel seeds during 14 days of fasting compared to other salinity treatments. This is in accordance with the opinion of Boeuf and Payan (2001) which stated that fish in a condition close to isotonic has lower metabolic rate than those at hypotonic and hypertonic condition. Hypoosmotic and hyperosmotic conditions cause metabolic activity of glass eel body increases which will affect oxygen consumption, osmoregulation activity, body's energy use, and excretion rate. Glass eels reared in condition close to isosmotic (10 g/L) used less energy for metabolism which impacted on lower ammonia excretion (Table 3).

Osmotic gradient value increased with salinity increases. The value osmotic gradient will be lower when it is closer to the isosmotic condition, consequently, oxygen consumption rate, protein use, and energy use by eel seed will also be low during fasting. As a consequence, eel used less energy for metabolism and more energy for growth. Result showed that the value of osmotic gradient, oxygen consumption rate, protein use, and energy use was found to be the lowest in treatment of 10 g/L salinity, thus resulted in the lowest rate of decline in absolute biomass.

Decrease in weight reflects the amount of energy spent by glass eel during fasting. The lower the weight loss during fasting indicates the less use of energy for metabolic activity and other biological activities. According to Boeuf and Payan (2001), salinity affects the growth of fish concerning the use of energy for osmoregulation.

Treatment A (0 g/L) showed the highest decrease in biomass and the lowest survival. This result was likely due to characteristics of glass eel which prefers brackishwater to freshwater. According to Wilson *et al*. (2007), glass eels will migrate from spawning area at the sea to the estuary to reach the elver stadia and grow in freshwater habitat until they reach the yellow eel stage. Edeline *et al*. (2005) also stated that glass eel can survive at acute salinities during migration from seawater into freshwater, yet glass eel will adapt to changes in salinity in the estuary area before reaching freshwater. Salinity treatment of 20 g/L and 30 g/L put the eel seeds in hyperosmotic conditions which esulted in higher decrease in weight than that in 10 g/L salinity. Fazio *et al*. (2013) stated that stress related to changes in water salinity can induce alterations in metabolic energy production and utilization.

Several studies found that glass eels *A. rostrata* from different habitats (freshwater and brackishwater) have different growth pattern as they are different in term of quantitative genetics (Cote *et al*., 2009). Glass eel *A. Anguilla* and *A. rostrata* reared in seawater showed significantly higher growth compared to those reared in freshwater (Edeline *et al*., 2005; Lamson *et al*., 2009).

Result of quadratic regression analysis (Table 2) concerning the relationship between salinity and growth showed causal relationship between salinity and the rate of decline in absolute biomass and also osmotic gradient of glass eel during maintenance. Changes in salinity affected the rate of decline in absolute biomass (r^2= 0.8877) and osmotic gradient (r^2 = 0.8266). Based on this result, optimum salinity range for the survival and growth of glass eel *A. bicolor bicolor* was 5.00−13.40 g/L.

CONCLUSION

Salinity of 10 g/L gave a good conditions for lived and growth of glass eel *A. bicolor bicolor*. But, the optimum range of salinity to improve

survival and growth of glass eel *A. bicolor bicolor* was 5.0–13.4 g/L. The increase of survival and growth rate of glass eel in culture will be impact to sustainability of it in natural waters.

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