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Effect of Stocking Density on Growth, Biochemical Composition, and Blood Parameters in the Pacific Shortfin Eel (*Anguilla bicolor pacifica*) Elvers

Frolan A. Aya[1,*], John Carlo L. Unida[1], Luis Maria B. Garcia[2], and Ma. Rowena Romana-Eguia[1,3]

[1]Aquaculture Department, Southeast Asian Fisheries Development Center, Binangonan Freshwater Station, Binangonan, Rizal 1940, Philippines
[2]Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City 1101, Philippines
[3]Biology Department, College of Science, De La Salle University, 2401 Taft Avenue, Malate, Manila 0922, Philippines

*Author for correspondence; Email: faya@seafdec.org.ph; Tel./Fax: (63-2) 927 7825

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This study examined the effect of stocking density on growth, biochemical composition, and blood parameters of the Pacific shortfin eel *Anguilla bicolor pacifica*. Elvers (1.95 ± 0.14 g body weight) were randomly stocked in indoor tanks and reared over 186 d at three stocking densities (0.3, 0.6, and 0.9 kg m^{-3}) set up in triplicates. Except for survival and biometric indices, elvers maintained at 0.3 and 0.6 kg m^{-3} densities exhibited higher growth and feed utilization than those held at 0.9 kg m^{-3}. Yield increased with stocking density, which were significantly higher at 0.6 and 0.9 kg m^{-3}. RNA/DNA ratio did not reflect growth rate, but trends in survival and RNA/DNA ratio with stocking density were positively related. In contrast to body proximate composition, increasing stocking density resulted in significantly higher erucic acid (22:1n-9) and total saturated fatty acid levels at 0.6 and 0.9 kg m^{-3} densities, respectively. Serum glutamic pyruvic transaminase activity was significantly elevated at 0.6 kg m^{-3}, while total protein, glucose, and triglycerides slightly decreased with increasing stocking density. Results suggest that Pacific shortfin eel elvers can be reared in indoor tanks at a stocking density of 0.3 – 0.6 kg m^{-3} to achieve acceptable growth, feed performance, and health condition.

Keywords: *Anguilla bicolor pacifica*, fatty acids, growth, RNA/DNA ratio, stocking density

INTRODUCTION

Tropical anguillid eels, such as the Pacific shortfin eel *Anguilla bicolor pacifica*, are widely distributed in southeast Asian countries such as Indonesia, Viet Nam, and the Philippines (Aoyama et al. 2018; Marini et al. 2021). *A. bicolor pacifica* fetches a higher price in the market (Cuvin-Aralar et al. 2019) and grows faster during culture (Aya and Garcia 2022) compared to the giant mottled eel *A. marmorata*, which is considered as the dominant eel species in the Philippines and other Asian countries (Jamandre et al. 2007; Aoyama et al. 2015). Unlike in temperate eel species (Degani et al. 1988; Chiu Liao et al. 2002), published information on the production performance of these anguillid eels is scarce (Aya and Garcia 2022; Aya et al. 2023). Therefore, studies on the development of production techniques appropriate for tropical anguillid eels are needed.

Developing aquaculture protocols necessitates that optimal culture conditions such as stocking density be established especially that eel species exhibit heterogeneous growth, both in terms of length and weight (Hirt-Chabbert et al. 2014). Webb et al. (2007) reported that stocking density is a crucial factor during culture as it can directly influence fish growth, survival, and welfare. At high stocking density, fish may manifest chronic stress such as elevated levels of plasma cortisol (Li et al. 2012) and glucose (Odhiambo et al. 2020), reduced growth (Żarski et al. 2008), high mortality rates (Szkudlarek and Zakes 2007), and fin damage (Jones et al. 2011). It can also enhance cannibalism (Chiu Liao and Chang 2002) due to food competition and decrease water quality because of high fish wastes (Hosfeld et al. 2009). In

comparison, low stocking density ensures better fish growth but is always associated with high costs of production (Majhi et al. 2023). Stocking density can also affect RNA/DNA ratio (Huertas and Cerdà 2006; Swain et al. 2022), body composition (Montero et al. 1999; Tan et al. 2018), and serum biochemical parameters (Kumar and Engle 2016; Tan et al. 2018). Previous studies on eels reported an optimum density of 2 up to 5 kg m^{-2} for the European glass eel *A. anguilla* (Degani et al. 1988; Huertas and Cerdà 2006) and 4 g L^{-1} for *A. bicolor bicolor* elvers (Harianto et al. 2014).

Proper stocking density should be established to optimize fish production and maximize profitability. In comparison to the giant marbled eel, density effects on the performance of the Pacific shortfin eel are poorly studied. Therefore, this study examined whether stocking density affects growth, biochemical composition, and blood parameters of Pacific shortfin eel *A. bicolor pacifica* elvers.

MATERIALS AND METHODS

Source of Glass Eels and Pre-rearing Conditions

Pre-sorted *A. bicolor pacifica* glass eels were procured from eel consolidators in Mindanao, Philippines. Rearing of glass eels to elver size was already described by Aya and Garcia (2022). Briefly, glass eels, at an initial density of 5 fish L^{-1}, were maintained in indoor tanks where they were given bloodworms (*Tubifex* sp.) for 10 wk and co-fed formulated eel diet (49.77% crude protein; 10.21% crude lipid) until 24 wk. After this period, elvers were randomly assigned into 3 stocking density groups. Trials were carried out under natural (12 h light:12 h dark) photoperiod.

Experimental Design

About 720 elvers (mean initial body weight of 1.95 ± 0.14 g) were randomly allocated to one of 9500-L capacity fiberglass tanks assigned to 3 stocking densities (0.3, 0.6, and 0.9 kg m^{-3} corresponding to 40, 80, and 120 fish $tank^{-1}$) set up in triplicates. Deep well water was supplied to the tanks at an effective water volume of 0.25 m^3. Tanks were provided with five 35.0 cm × 5.5 cm PVC pipes as shelter. Elvers were fed at 10% of estimated body weight with formulated eel diet prepared in paste form twice daily (0800 and 1600 h) for 186 d. To estimate the actual feed consumed, uneaten feeds were collected 2 h after feeding in each replicate tank, oven-dried at 60°C for 10 h, and weighed. The formulation and proximate and amino acid composition of the formulated eel diet were already reported by Aya and Garcia (2022). The total lipids and fatty acid composition of the eel powder diet are shown in Table 1.

Table 1. Total lipids (g 100 g^{-1}) and fatty acid composition (% of total FA) of formulated eel powder diet.

Total lipids	8.61 ± 1.02
Fatty acids	
8:00	0.70 ± 0.09
9:00	0.37 ± 0.04
10:00	0.58 ± 0.05
11:00	0.79 ± 0.66
12:00	2.55 ± 0.61
13:00	0.50 ± 0.03
14:00	12.24 ± 3.59
15:00	4.20 ± 0.24
16:00	9.14 ± 1.71
17:00	3.47 ± 0.28
18:00	0.01 ± 0.02
19:00	1.51 ± 0.12
20:00	4.04 ± 0.34
14:01	1.10 ± 0.10
16:01	7.36 ± 0.80
18:01	0.95 ± 0.68
18:1*n*-9	4.46 ± 0.26
20:1*n*-9	11.50 ± 1.51
22:1*n*-9	9.79 ± 0.80
18:2*n*-6 (LA)	3.07 ± 0.50
20:4*n*-6 (ARA)	4.73 ± 0.41
20:3*n*-3	0.12 ± 0.03
20:5*n*-3 (EPA)	2.79 ± 0.62
22:6*n*-3 (DHA)	7.83 ± 1.14
∑SFA	36.06 ± 3.72
∑MUFA	35.16 ± 2.29
∑*n*-6	15.33 ± 1.34
∑*n*-3	10.74 ± 1.64
∑PUFA	26.08 ± 2.71
n-3/*n*-6	0.70 ± 0.08

Data expressed as mean ± SD (*n* = 3). LA, linoleic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids.

Water Quality Monitoring

Cleaning of tanks and total replacement of water were done during monthly sampling. Temperature and dissolved oxygen (DO) levels of the rearing water were measured once daily (0900 h) using a multi-probe parameter. pH and Total Ammonia Nitrogen (TAN) were recorded using a pH meter (Beckman Model Phi 50) and an API Ammonia Water Test Kit (Chalfont, Pennsylvania, USA), respectively once a week

during the entire trial. Temperature varied from 25.2 to 29.7°C, DO between 3.84 and 5.68 mg L^{-1}, pH from 7.88 to 8.75, and TAN between 0 and 2.0 mg L^{-1}.

Sampling and Estimation of Growth Parameters

Every 30 d, the number of surviving eels per replicate tank was counted and total biomass was measured to examine the increment in body weight and to adjust the daily feed ration. After 186 d, elvers in each replicate tank were starved for 24 h before measurements for individual length and weight were performed. Blood samples were taken from the caudal vein of three randomly collected and sedated (2 mL of 2-phenoxyethanol L^{-1} of water) elvers per tank using heparinized syringes, centrifuged (5 000 g) for 10 min, and serum samples collected and stored at -20°C for analysis of blood parameters. The same eel specimens were dissected to separate the liver and viscera to estimate viscerosomatic (VSI) and hepatosomatic (HSI) indices. Growth, feed utilization parameters, and biometric indices were calculated as follows:

Coefficient of variation for final body weight (CV_{BW})(%) = standard deviation/mean × 100)

Weight gain (%) = 100 (final weight (g) - initial weight (g)) / initial weight (g)

Specific growth rate (% d^{-1}) = ($\log_e$ final weight - $\log_e$ initial weight) × 100 / days

Yield (g m^{-3}) = total weight at harvest (g) / volume of rearing water (m^3)

Feed intake (g $fish^{-1}$) = feed consumption (g) / number of fish at harvest

Feed conversion ratio (FCR) = dry feed intake (g) / wet weight gain (g)

Protein efficiency ratio (PER) = wet weight gain (g) / protein intake (g)

Viscerosomatic index (VSI, %) = (viscera weight (g) / body weight (g)) × 100

Hepatosomatic index (HSI, %) = (liver weight (g) / body weight (g)) × 100

Condition factor (CF) = (wet body weight (g) / total length^3 (cm)) × 100 (Froese 2006)

RNA/DNA Ratio

About 50 mg of muscle tissue from the dorsal part of the fish was immediately dissected from each specimen and weighed using an analytical balance (Sartorius BSA 822) for determination of RNA and DNA content. RNA extraction was done using Trizol reagent (20 µL RNA resuspension) while DNA was extracted with DNAzol reagent (Genomic DNA Isolation Reagent; Molecular Research Center; 200 µL DNA resuspension). The quantities of RNA and DNA (3 µL volume) were measured using Implen GmBH Nanophotometer (München, Germany) and expressed as µg mg^{-1} of each sample. RNA/DNA ratio was calculated by dividing the concentrations of RNA and DNA in each sample.

Whole Body Proximate, Total Lipids, and Fatty Acid Analysis

The whole body proximate composition (moisture, crude protein, crude lipid, and ash) of five elvers per replicate tank was analyzed following standard methods of AOAC (2016).

Formulated eel powder diet and freeze-dried whole fish body were analyzed for total lipids (Bligh and Dyer 1959) and fatty acid composition using the AOAC Official Method 996.06 (AOAC 2001). Extracted lipids were methylated with 14% boron trifluoride (BF_3) in methanol and toluene to convert the lipids into fatty acid methyl esters (FAMEs). The final derivatized FAMEs were stored in 1 mL hexane added with C:11 ISTD and analyzed using gas chromatography (Clarus 600 Gas Chromatograph) mass spectrometry (Clarus 600T Mass Spectrometer) (GC/MS). The GC/MS is equipped with a flame ionization detection and Elite-5ms capillary column (0.25 internal diameter, 60 m length, 0.25 µm film thickness). Identification of fatty acids (% of total fatty acids) was done by analyzing their retention times with that of known standard (cod liver oil) and their concentrations expressed as a percentage of total area.

Blood Parameters

Serum samples collected at the end of the 186-d trial were measured for glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), total protein (TP), total cholesterol (TC), and glucose levels. Measurements were done using an ACCENT 200 automated chemistry analyzer (PZ Cormay S.A., Warszawa, Poland).

Statistical Analysis

Data are presented as mean ± standard deviation. The Anderson-Darling and Barlett tests, respectively were used for normality and homogeneity of variance. Data were analyzed using one-way analysis of variance (ANOVA). Tukey's post hoc test was used when significant differences were observed at $P < 0.05$ level of significance. Arcsine transformation of percent data were performed before analysis.

RESULTS AND DISCUSSION

Survival, Growth Performance and Feed Utilization

Stocking density, a critical factor for fish performance and welfare in aquaculture, should be established to optimize production and maximize the economic benefits of farming. In this study, after 186 d, mean survival rates of elvers at harvest were independent of stocking density (Table 2). The highest mean survival rate was observed in elvers reared at 0.9 kg m^{-3} (66.11 ± 5.55%), followed by those reared at 0.3 kg m^{-3} (50.83 ± 7.22%), and lowest at 0.6 kg m^{-3} (47.92 ± 15.73%). Similar studies have reported no significant effects of density on final survival (Sukardi et al. 2018). In contrast, density-dependent survival was observed for silver perch *Bidyanus bidyanus* (Rowland et al. 2006). However, it appears that the mortalities observed from day 92 onwards in all treatments may be triggered by an increase of stocking density (Fig. 1). After 92 d of the experiment and up to day 186, density increased to 1.8 – 4.9, 3.9 – 7.2, and 3.4 – 8.0 kg m^{-3} for the low, medium, and high densities, respectively, suggesting that crowding stress and social interactions may have led to

reduced survival rates (47.92 – 66.11%), which were lower than those determined for other studies. Aya and Garcia (2022), in a study with *A. bicolor pacifica* elvers stocked at 30 fish m^{-3} in cages, reported a survival rate of 80.0 ± 16.7% after 210 d. However, Sukardi et al. (2018), in a 60-d study on *A. bicolor* glass eels reared in biofloc-based system at varying densities of 54.95 – 164.84 fish m^{-3}, obtained a lower survival of 50.3–51.3%. In the case of *A. marmorata* yellow eels, survival was 100% when cultured at densities of 12–28 kg m^{-3} in a recirculating aquaculture system (RAS) for 71 d (Tan et al. 2018). Such differences in survival rates in the present and earlier studies were therefore related to the specific developmental stage of eel species, fish husbandry, and experimental conditions. Indeed, earlier studies (De Leo and Gatto 1995; Lobòn-Cerviá and Iglesias 2008) reported improved natural survival rates of *A. anguilla* with increasing size or age.

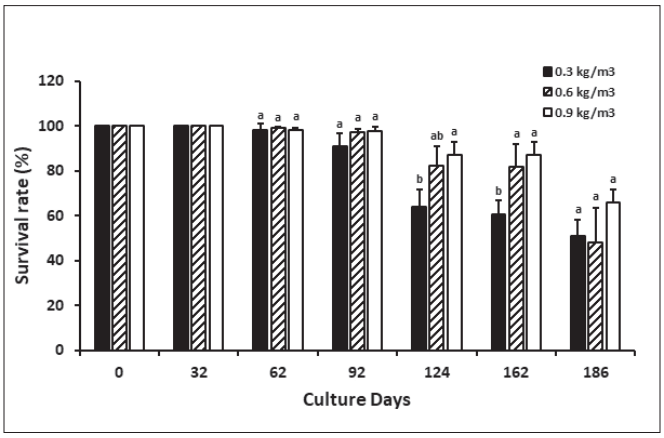

Fig. 1. Mean survival rate (%) of the Pacific shortfin eel *Anguilla bicolor pacifica* elvers reared in indoor tanks at different stocking densities for 186 d. Means with different superscript letters are significantly different (*P* < 0.05).

Although the highest survival rates were achieved at 0.9 kg m^{-3} density, *A. bicolor pacifica* elvers maintained at 0.3 – 0.6 kg m^{-3} resulted in significantly better growth rates. Elvers maintained at 0.3 kg m^{-3} were larger (62.41 ± 20.58 g) than those held at 0.9 kg m^{-3} (25.39 ± 1.76 g) possibly due to low survival in this group but were not significantly different from that of 0.6 kg m^{-3} (49.12 ± 10.21 g) (Table 2). Although mean body weight was lower at 0.9 kg m^{-3} density on day 92 until the end of the density trial (Fig. 2), high stocking density resulted in significantly lower CV (6.95 ± 0.48%) or more uniformly sized elvers (Table 2). Earlier studies (Knights 1987; Wickins 1987) on European eel (*A. anguilla*) reported lower growth variability at higher stocking density, as observed in this study. Similarly, Tan et al. (2018) reported better growth performance of *A. marmorata* yellow eels at a lower density of 12 kg m^{-3}. In addition, percent weight gain (WG) and specific growth rate (SGR) were significantly elevated in elvers maintained at 0.3 (2998 ± 637% and 2.67 ± 0.16% d^{-1}) and 0.6 kg m^{-3} (2375 ± 528%; 2.49 ± 0.17% d^{-1}) than those reared at 0.9 kg m^{-3} (1243 ± 76%; 2.03 ± 0.04% d^{-1}) (Table 2). Harianto et al. (2014) also showed that *A. bicolor bicolor* elvers maintained at 4 g L^{-1} in a recirculating system for 60 d had the highest SGR and biomass. In contrast, Sukardi et al. (2018) did not find any significant influence of stocking density on the final weight of *A. bicolor* glass eels in biofloc condition. In other fish species such as juvenile turbot *Scophthalmus maximus*, high-density rearing resulted in poor growth rate caused by increased social interactions (Irwin et al. 1999). Factors such as food and space availability, water quality, and physiological status may contribute to poor growth at high stocking density (Riar et al. 2021). Final yield, however, differed among stocking density groups, with significantly higher values at 0.6 and 0.9 kg m^{-3} density groups (Table 2).

Table 2. Growth performance, feed utilization efficiency and biometric indices of the Pacific shortfin eel *Anguilla bicolor pacifica* elvers reared in indoor tanks at different stocking densities after 186 d of culture.

Parameters	Stocking density (kg m^{-3})			*P* value
	0.3	0.6	0.9	
Survival rate (%)	50.83 ± 7.22	47.92 ± 15.73	66.11 ± 5.55	0.154
Final body weight (g)	62.41 ± 20.58^{a}	49.12 ± 10.21^{ab}	25.39 ± 1.76^{b}	0.038
CV_{BW} (%)[1]	35.25 ± 10.45^{a}	21.48 ± 5.00^{b}	6.95 ± 0.48^{c}	0.006
Weight gain (%)	2998 ± 637^{a}	2375 ± 528^{a}	1243 ± 76^{b}	0.011
Specific growth rate (% day^{-1})	2.67 ± 0.16^{a}	2.49 ± 0.17^{a}	2.03 ± 0.04^{b}	0.003
Yield (g m^{-3})	4922 ± 884^{a}	7224 ± 1427^{b}	8047 ± 755^{b}	0.027
Feed intake (g $fish^{-1}$)	171.70 ± 58.36^{a}	151.89 ± 36.11^{ab}	84.83 ± 10.29^{b}	0.083
Feed conversion ratio	2.95 ± 0.24^{a}	3.40 ± 0.31^{ab}	3.77 ± 0.42^{b}	0.062
Protein efficiency ratio	0.17 ± 0.01^{a}	0.15 ± 0.01^{ab}	0.14 ± 0.01^{b}	0.037
Viscerosomatic index	2.12 ± 0.42	3.04 ± 0.93	2.95 ± 0.86	0.336
Hepatosomatic index	1.42 ± 0.40	1.60 ± 0.18	1.28 ± 0.29	0.452
Condition factor	0.23 ± 0.05	0.23 ± 0.03	0.20 ± 0.01	0.428

Data expressed as mean ± SD (*n* = 3). Different superscript letters within the same row indicate a significant difference (*P* < 0.05).
[1]CV_{BW} = coefficient of variation for body weight.

Feed intake (FI) followed a similar trend as protein efficiency ratio (PER) decreased with increasing stocking density, supporting the lower growth observed at high stocking density. FI was significantly higher in elvers stocked at 0.3 (171.70 ± 58.36 g fish^{-1}) than those held at 0.9 kg m^{-3}, resulting in better PER (0.17 ± 0.01) and feed conversion ratio (FCR: 2.95 ± 0.24) not significantly different from those maintained at 0.6 kg m^{-3} density (PER: 0.15 ± 0.01; FCR: 3.40 ± 0.31) (Table 2). Similar results were reported in *A. marmorata* yellow eels reared in RAS (Tan et al. 2018). In addition, high FCR (3.77 ± 0.42) was noted at the highest stocking density, suggesting lower feed consumption which has subsequent impact on water quality (Montero et al. 1999; Hosfeld et al. 2009). These findings confirm the negative effects of overcrowding on the feed use efficiency of fish (Aliabad et al. 2022). However, a slight but not significant increase in FCR from 1.52 to 1.80 with stocking density was also reported in *A. marmorata* yellow eels (Tan et al. 2018), whereas no particular trend in FCR was noted in *A. bicolor* glass eels (Sukardi et al. 2018). An opposite trend between FCR and stocking density was found in *A. bicolor bicolor* elvers, with the lowest FCR of 1.22 at 4 g L^{-1} and highest FCR of 2.75 at 2 g L^{-1} (Harianto et al. 2014). In addition, stocking density did not influence the hepatosomatic (HSI) and viscerosomatic (VSI) indices (Table 2) at the end of the density trial. Similarly, condition factor showed that density had no significant effect, although slightly higher values at 0.3 and 0.6 kg m^{-3} densities were observed (Table 2), suggesting better physiological condition than in the highest stocking density.

RNA/DNA Ratio

The RNA/DNA ratio is considered an indicator of fish growth since RNA functions in protein synthesis (Clemmesen 1994; Ferron and Leggett 1994). However, the muscle RNA/DNA ratio of the Pacific shortfin eel in the three stocking densities were independent of their growth rates after 186 d (Table 2; Fig. 3). This finding is in contrast with previous studies on the silver sea bream *Sparus sarba* (Deane et al. 2003), the Senegalese sole *Solea senegalensis* (Sánchez et al. 2010), and the three-spined stickleback *Gasterosteus aculeatus* (Pottinger et al. 2011), showing a positive correlation between RNA/DNA ratio and growth rate. It has been shown that low RNA/DNA suggests loss in the capacity for protein synthesis (Ferron and Legget 1994). Interestingly, low RNA/DNA at the 0.6 kg m^{-3} density group resulted in lower survival rates (Fig. 3), suggesting that nucleic acid ratio seemed to respond to a change in survival (Table 2). In fact, there are pieces of evidence showing that reduced RNA/DNA ratios are linked with decreased survival (Canino et al. 1991; Canino and Calderone 1994; Clemmesen et al. 1997; Pepin et al. 1999), findings that corroborate the present study.

Body Proximate, Total Lipids and Fatty Acid Composition

Although stocking density did not affect whole body composition at the end of the density trial (Table 3), the higher body moisture and crude lipid contents at the highest stocking density suggest that the changes were not related to body size (Aya and Garcia 2022). This result may indicate that lipids are preferentially used for energy during poor or crowding conditions. Conversely, the higher body protein contents at the 0.3 kg m^{-3} density group might be associated with the highest growth rate due to increased protein synthesis in this treatment. However, results are contradictory with that of Tan et al. (2018), who reported that stocking density significantly influenced body composition of *A. marmorata*, with higher body moisture and protein levels and lower lipid and ash contents at high density.

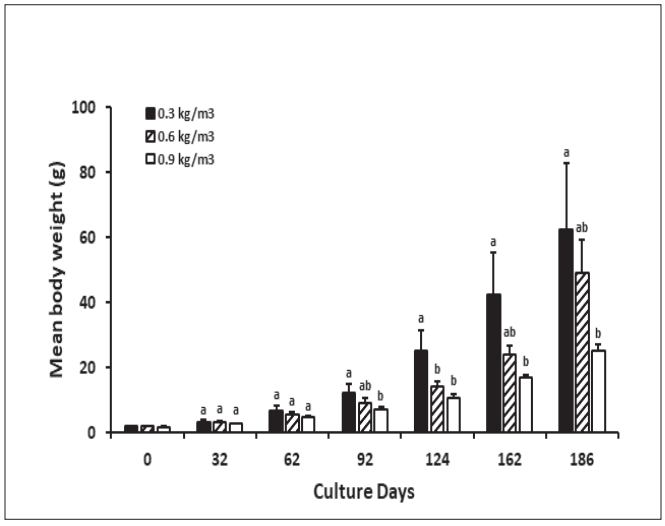

Fig. 2. Mean body weight (g) of the Pacific shortfin eel *Anguilla bicolor pacifica* elvers reared in indoor tanks at different stocking densities for 186 d. Means with different superscript letters are significantly different (*P* < 0.05).

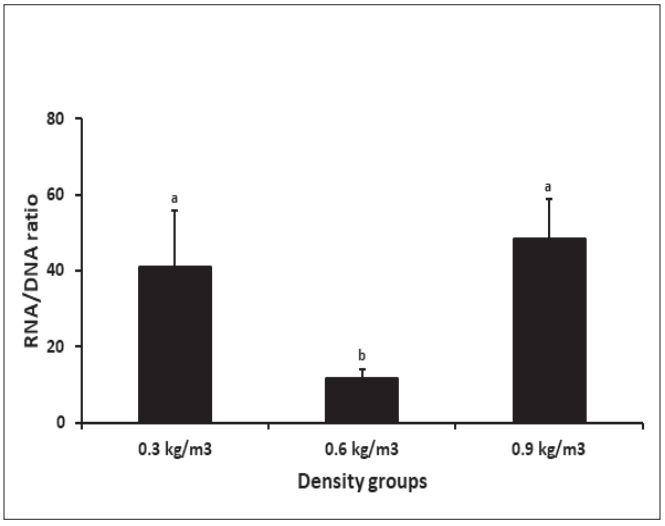

Fig. 3. RNA/DNA ratio in muscle tissues of the Pacific shortfin eel *Anguilla bicolor pacifica* elvers reared in indoor tanks at different stocking densities for 186 d. Means with different superscript letters are significantly different (*P* < 0.05).

Table 3. Proximate body composition (% dry matter) of the Pacific shortfin eel *Anguilla bicolor pacifica* elvers reared in indoor tanks at different stocking densities after 186 d of culture.

	Stocking density (kg m^{-3})			
	0.3	0.6	0.9	*P* value
Moisture	2.72 ± 0.88	4.74 ± 2.67	5.15 ± 0.21	0.320
Crude protein	63.97 ± 2.59	62.48 ± 2.18	59.31 ± 2.48	0.131
Crude fat	19.51 ± 0.82	21.08 ± 2.75	25.81 ± 9.90	0.511
Ash	8.01 ± 0.59	8.12 ± 0.20	7.94 ± 1.63	0.974

Data expressed as mean ± SD (*n* = 3).

Table 4 shows the total lipids and fatty acid profiles in muscle of elvers maintained at different stocking densities. High total lipids, ranging from 9.76 to 20.02 g 100 g^{-1} dry sample, were not statistically different—an effect that may have arisen due to high lipid content in formulated eel diet. The fatty acid composition in the muscle was not influenced by stocking density, except for erucic acid (22:1n-9) and total saturated fatty acid (SFA) contents which differed between stocking densities and were present at higher levels in the diet. Higher deposition or absorption of erucic acid (13.29%) in the 0.6 kg m^{-3} density group may have resulted in elevated HSI and amount of total lipids in this group. Total SFAs were significantly higher at the highest stocking density, with palmitic acid (16:0) being the most abundant SFA (11.43%). Total n-6 and n-3 polyunsaturated fatty acids (PUFAs) were slightly but not significantly higher at 0.6 kg m^{-3}, resulting in a lower n-3/n-6 ratio than the other density groups.

Table 4. Total lipids (g 100 g^{-1}) and fatty acid composition (% of total FA) of the Pacific shortfin eel *Anguilla bicolor pacifica* elvers reared in indoor tanks at different stocking densities after 186 d of culture.

	Stocking density (kg m^{-3})			
	0.3	0.6	0.9	*P* value
Total lipids	9.76 ± 3.84	20.02 ± 6.21	18.68 ± 7.06	0.145
Fatty acids				
8:00	1.00 ± 0.25	0.53 ± 0.57	1.48 ± 2.07	0.669
9:00	0.58 ± 0.18	0.36 ± 0.34	0.94 ± 1.18	0.630
10:00	0.54 ± 0.06	0.45 ± 0.04	0.65 ± 0.56	0.765
11:00	0.35 ± 0.08	0.51 ± 0.11	3.28 ± 2.45	0.077
12:00	3.81 ± 0.71	2.67 ± 0.04	3.83 ± 2.25	0.525
13:00	1.44 ± 0.20	1.15 ± 0.14	1.25 ± 0.52	0.610
14:00	7.65 ± 3.55	8.16 ± 0.79	5.39 ± 3.63	0.516
15:00	5.07 ± 2.19	4.97 ± 0.52	6.23 ± 1.39	0.563
16:00	7.15 ± 2.53	8.40 ± 2.22	11.43 ± 3.10	0.206
17:00	4.40 ± 0.68	3.52 ± 0.39	4.27 ± 0.67	0.227
18:00	0.66 ± 1.08	0.01 ± 0.02	0.05 ± 0.04	0.413
19:00	1.28 ± 2.21	2.65 ± 0.49	4.42 ± 0.86	0.086
20:00	0.06 ± 0.06	1.92 ± 1.69	0.12 ±0.02	0.099
14:01	1.85 ± 0.23	1.43 ± 0.17	1.70 ± 0.44	0.294
16:01	8.01 ± 1.39	6.09 ± 1.30	8.78 ± 2.39	0.236
18:01	2.20 ± 3.28	5.30 ± 4.56	4.63 ± 3.97	0.627
18:1*n*-9	13.95 ± 5.57	5.28 ± 6.69	4.57 ± 6.85	0.216
20:1*n*-9	9.66 ± 2.01	8.46 ± 0.93	9.24 ± 1.01	0.597
22:1*n*-9	7.60 ± 1.24^{b}	13.29 ± 1.49^{a}	6.90 ± 0.41^{b}	0.001
18:2*n*-6 (LA)	3.92 ± 0.71	4.43 ± 2.99	1.76 ± 1.55	0.289
20:2*n*-6	3.57 ± 1.95	6.19 ± 5.28	0.08 ± 0.07	0.078
20:4*n*-6 (ARA)	1.75 ± 1.22	3.22 ± 2.10	7.53 ± 4.33	0.226
20:3*n*-3	6.09 ± 3.39	1.93 ± 2.36	2.01 ± 2.31	0.988
20:5*n*-3 (EPA)	4.11 ± 4.53	4.10 ± 3.61	4.01 ± 3.02	0.707
22:6*n*-3 (DHA)	3.26 ± 5.64	0.10 ± 0.03	2.63 ± 4.47	0.638
∑SFA	33.91 ± 3.79^{b}	33.39 ± 1.13^{b}	43.24 ± 6.04^{a}	0.047
∑MUFA	43.26 ± 7.96	39.85 ± 10.61	35.82 ± 6.10	0.585
∑*n*-6	7.55 ± 1.95	13.85 ± 4.46	9.38 ± 5.95	0.278
∑*n*-3	11.95 ± 5.08	10.90 ± 8.86	8.81 ± 3.73	0.828
∑PUFA	19.50 ± 6.56	24.74 ± 13.03	18.19 ± 9.62	0.715
n-3/*n*-6	1.56 ± 0.55	0.70 ± 0.45	1.14 ± 0.48	0.187

Data expressed as mean ± SD (*n* = 3). Different superscript letters within the same row indicate a significant difference ($P < 0.05$). LA, linoleic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids.

Blood Chemistry

Blood biochemical parameters are useful indices to examine the health status of fish. GPT and GOT enzymes are sensitive indices to examine hepatic health condition (Fırat and Kargın 2010). In this study, after 186 d, an elevated serum GPT at the 0.6 kg m^{-3} density group (Table 5) suggests that stocking density could lead to liver damage, which was supported by an increased although not significant HSI values. High stocking density produced a chronic stress situation with a slight reduction in HSI as observed in this study. Similarly, an increase in serum GOT at the 0.6 kg m^{-3} treatment group was also in response to crowding stress, supporting the previous report of Hao et al. (2014) for the loach *Paramisgurnus dabryanus*. In addition, while stocking density did not cause a significant effect on total protein, a slight decrease may be due to the inhibition of cell formation following crowding-related stress (Tan et al. 2018). In this study, a decrease in serum glucose, a transient index of high stress levels (Hsieh et al. 2003), with stocking density may be attributed to a chronic stress effect leading to the depletion of glycogen stores (Santos et al. 2010). Likewise, Tan et al. (2018) observed that *A. marmorata* stocked at higher densities had lower serum glucose levels than the lowest density group at the end of the 71-d rearing period. On the contrary, glucose levels were elevated in the Nile tilapia *Oreochromis niloticus* when held at high stocking densities (Odhiambo et al. 2020). In addition, Montero et al. (1999) reported a significant increase in total plasma cortisol, glucose, and protein levels after 15 wk in gilthead seabream *Sparus aurata* juveniles reared at high

Table 5. Blood parameters of the Pacific shortfin eel *Anguilla bicolor pacifica* elvers reared in indoor tanks at different stocking densities after 186 d of culture.

	Stocking density (kg m^{-3})			
	0.3	0.6	0.9	*P* value
GOT[1]	71.00 ± 23.91	85.30 ± 9.26	76.76 ± 26.59	0.724
GPT[2]	40.16 ± 16.38[b]	78.66 ± 13.90[a]	41.05 ± 12.73[b]	0.027
Total protein	7.57 ± 1.86	5.90 ± 0.87	5.33 ± 1.62	0.248
Glucose	198.00 ± 63.53	172.33 ± 76.40	122.33 ± 18.48	0.338
Cholesterol	453.10 ± 138.81	393.37 ± 118.89	398.30 ± 151.54	0.843
Triglycerides	520.17 ± 83.85	430.17 ± 110.14	429.33 ± 88.70	0.453

Data expressed as mean ± SD (n = 3). Different superscript letters within the same row indicate a significant difference (P < 0.05).
[1] GOT, glutamic oxaloacetic transaminase; [2]GPT, glutamic pyruvic transaminase.

stocking density. Similarly, in cage-cultured Indian major carp *Labeo rohita* fingerlings, elevated levels of serum cortisol and glucose measured after 240 d of culture were detected at high stocking density (Swain et al. 2022). In this study, the higher cholesterol and triglyceride levels in the lowest density group suggest lipid utilization to cope with a higher energy demand (Tan et al. 2018). In contrast, except for leukocyte parameters, cortisol and blood glucose levels in *A. bicolor bicolor* elvers were not significantly affected by stocking density (Harianto et al. 2014).

CONCLUSION

This study provides relevant information on the culture conditions of anguillid eels in the tropics. Results show that the Pacific shortfin eel *A. bicolor pacifica* elvers can be reared at stocking densities of 0.3 to 0.6 kg m^{-3} in indoor conditions to achieve better growth, feed performance, and health condition. Further studies focusing on high density culture of glass eels in outdoor conditions and the economics of culture operation are necessary.

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