

Alternate Day Feeding as a Cost-effective Strategy for Tank Culture of the Pacific Shortfin Eel *Anguilla bicolor pacifica*

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There is a growing interest in the aquaculture of tropical anguillid eels as an export commodity. However, studies on feeding strategies, and the present demand to reduce feed costs need to be addressed to ensure the economic viability of eel farming. In this study, the effects of daily (DF) and alternate day (ADF) feeding on growth, feed utilization, body composition, blood chemistry, liver and intestinal morphology, and economic viability in the Pacific shortfin eel *Anguilla bicolor pacifica* were examined. Each feeding group of 30 elvers (166.25 ± 16.23 g mean initial wt) were randomly stocked in triplicate 4 m³ outdoor concrete tanks. These were fed for 155 d with formulated eel powder diet (49.77% crude protein; 10.21% crude lipid) made into a paste. Growth and survival were not significantly different between the two feeding groups. However, feed efficiency was improved in the ADF group, with significantly higher protein efficiency ratio (0.63) than in the DF group (0.39). Feed conversion ratio (FCR) was lower in ADF (3.85) than in the DF group (6.44), resulting in a 40% reduction in total feed consumption. Biometric indices, body proximate composition, and blood chemistry were not significantly affected. Likewise, liver and intestinal morphology showed no apparent alterations between groups. Partial costs-and-returns analysis showed that ADF yielded higher net profit and profit index. These results suggest that feeding on alternate days promote compensatory growth, better feed utilization, and normal physiological condition of *A. bicolor pacifica*, and reduce feed cost in the rearing process. Therefore, alternate day feeding should be promoted as a sound feed management strategy in the tank culture of tropical anguillid eels.

Keywords: Blood chemistry; feeding regimes; growth metrics; proximate composition

Abbreviations: ADF—alternate day feeding, DF—daily feeding, DFI—daily feed intake, DO—dissolved oxygen, DTI—digestive tract index, FCR—feed conversion ratio, HSI—hepatosomatic index, L/LBM—lipid to lean body mass ratio, PER—protein efficiency ratio, RGL—relative gut length, SEAFDEC/AQD—Southeast Asian Fisheries Development Center, Aquaculture Department, VSI—Viscerosomatic index

INTRODUCTION

The freshwater eels of the genus *Anguilla* are composed of 19 species/subspecies (Froese and Pauly 2022), but only five temperate species (American eel *A. rostrata*, European eel *A. Anguilla*, Japanese eel *A. japonica*, Australian shortfin eel *A. australis*, and Australian longfin eel *A. dieffenbachii*) are known to be cultured. In the Philippines, seven different anguillid eel species (*A. japonica*, *A.*

celebesensis, *A. marmorata*, *A. interioris*, *A. bicolor bicolor*, *A. bicolor pacifica*, *A. luzonensis*) have been documented to date (Briones et al. 2007; Jamandre et al. 2007; Teng et al. 2009; Watanabe et al. 2009; Han et al. 2012; Aoyama et al. 2015). Anecdotal reports of the nascent farming of Philippine native eels are adopted mainly from the culture of Japanese and European eel species. Among the tropical eel species, the Pacific shortfin eel *A. bicolor pacifica* is the most preferred because of its tender and

delicious taste and higher market price (Cuvin-Aralar et al. 2019). In recent years, tropical anguillid eels are gaining popularity as an alternative species for culture due to the continuous decline of temperate eel species (Cuvin-Aralar et al. 2019). In fact, *A. bicolor pacifica* is being considered as an alternative to *A. japonica*, being the most favored eel species for consumption in East Asian countries (Cuvin-Aralar et al. 2019).

Eel nursery and grow-out production systems have experienced problems such as high cost of feed inputs. At present, most of the local eel farmers prefer to use imported eel feeds, although these are very expensive (Cuvin-Aralar et al. 2019). The present high demand to reduce feed costs needs to be addressed to ensure the economic viability of the culture of tropical anguillid eels. Since the prohibitive cost of eel feeds affects the further development of the eel industry in the Philippines, developing feed management strategies for anguillid eels can help abate production costs and increase income benefits for eel farmers. These strategies will also improve feed efficiency by reducing feed losses and rapid deterioration of rearing water quality.

Feeding management strategies such as skip or alternate day feeding have been studied in a number of species such as Nile tilapia *Oreochromis niloticus* (Bolivar et al. 2006; Cuvin-Aralar et al. 2012), milkfish *Chanos chanos* (de Jesus-Ayson and Borski 2010), and in Atlantic cod *Gadus morhua* (Bjørnevik et al. 2021). Such studies found that this feeding schedule resulted in comparable fish performance and lower feed conversion ratio compared to those fed daily. The effect of alternate day feeding on the production parameters of tropical anguillid eels is yet to be determined. To examine production and economic effects of feed management strategy for tropical anguillid eels, the Pacific shortfin eel *A. bicolor pacifica* was chosen due to its fast growth rate (Aya and Garcia 2022).

This study examined the effects of daily (DF) and alternate day feeding (ADF) strategies on growth performance, feed utilization, body composition, blood chemistry, liver and intestinal morphology, and economic viability of Pacific shortfin eel (*A. bicolor pacifica*). Information from this study is relevant to the efforts of Aya and Garcia (2022) and Aya et al. (2023) to develop an appropriate feed management strategy leading to compensatory growth for efficient culture of this tropical eel species.

MATERIALS AND METHODS

Experimental Fish and Growth Trial

The experiment was performed in 6 outdoor concrete tanks with a flow-through system measuring 2 × 2 × 1 m,

each containing 1.6 m³ of water. Each tank was filled with freshwater at 0.2 L per min flow rate. Tanks were covered with double black net to reduce light intensity (Hirt-Chabbert et al. 2014). A total of 180 elvers (mean initial weight: 166.25 ± 16.23 g; mean total length: 466.49 ± 5.27 mm) were randomly distributed in 6 tanks (30 eels per tank) in a completely randomized design on 03 March 2020. Ten pieces of PVC pipes (length: 50 cm, diameter: 5.5 cm) were placed in each rearing tank to provide refuge for elvers (Rodríguez et al. 2009). Two feeding strategies set up in triplicates were tested in the tank culture of the Pacific shortfin eel. One treatment group (control; DF) received feed daily while another treatment group received feed on alternate days or every other day (ADF; e.g., Mondays, Wednesdays, Fridays, and Sundays) for 155 d. Eels were fed twice daily (0800 and 1700 h) with a formulated eel powder diet (49.77% crude protein, 10.21% lipid) at 5% of biomass (percentage of dry weight of feed to wet weight of eels) from days 1 to 30, reduced to 3% of biomass from days 31 to 124, and at 1.5% of biomass for the remaining days of culture. The feeding rates were adjusted based on the feeding behavior of the elvers. Table 1 shows the proximate composition of the formulated eel powder diet (Aya and Garcia 2022).

Diet Preparation

Before the morning feeding period, the formulated eel powder diet was made into a paste by mixing 700 ml/kg dry feed with water in an electric mixer for 15 min. The paste diet was placed directly onto the feeding trays and the remaining feed was removed from the trays after 2 h, oven-dried (60°C for 10 h), and weighed to determine the amount of feed consumed.

Water Quality Management

All tanks were cleaned and water totally replaced during the monthly sampling. Water quality parameters such as temperature and dissolved oxygen (DO) were monitored twice a day (0900 and 1500 h) with a multi-probe parameter. pH and Total Ammonia Nitrogen (NH₃ and

Table 1. Proximate composition of formulated eel powder diet offered as paste to the Pacific shortfin eel *Anguilla bicolor pacifica* reared in outdoor tanks under two feeding strategies for 155 d¹.

	% Dry Matter
Moisture	4.72
Crude protein	49.77
Crude fat	10.21
Crude fiber	0.40
Nitrogen-free extract (NFE) ²	27.23
Ash	12.40

¹Source: Aya and Garcia (2022)

²Nitrogen-free extract, estimated by difference (NFE = 100 - (moisture + crude protein + crude lipid + crude fiber + ash)).

NH₄⁺) were analyzed once a week with a pH meter (Beckman Model Phi 50) and an Aquarium Pharmaceuticals API Ammonia Water Test Kit (Chalfont, Pennsylvania, USA), respectively. Aeration was provided in the rearing tanks to maintain the DO near saturation levels. The experiment was conducted under natural (12 h light: 12 h dark) photoperiod conditions.

Sampling

Eels from each replicate tank were weighed in bulk, counted, and then immediately returned to the same tank on April 3 (d 31), May 4 (d 62), June 4 (d 93), July 9 (d 128), and August 10, 2020 (d 155), and the amount of feed given was adjusted based on the elver's bulk weight data taken from each tank. At the end of the experiment, all surviving eels were starved for 24 h and anaesthetized with 2-phenoxyethanol (2 ml per L of water) (Aya and Garcia 2022) before they were immediately counted and measured for individual total length and body weight using a metal ruler and a mechanical hanging scale, respectively for the calculation of growth parameters. In addition, 3 eels per replicate tank were randomly collected, anaesthetized with 2-phenoxyethanol (2 ml per L of water), and dissected to remove the viscera, liver, digestive tract, and intestine for the calculation of the viscerosomatic (VSI), hepatosomatic (HSI), digestive tract (DTI), and relative gut length (RGL). Growth parameters, feed utilization and biometric indices were calculated (Cho et al. 2021; de Macêdo et al. 2021; Aya and Garcia 2022) using these equations:

$$\text{Weight gain (WG, \%)} = \frac{(\text{final weight (g)} - \text{initial weight (g)})}{\text{initial weight (g)}} \times 100$$

$$\text{Specific growth rate (SGR, \% / day)} = \frac{(\log_e \text{final weight} - \log_e \text{initial weight}) \times 100}{d}$$

$$\text{Survival rate (\%)} = \frac{(\text{total number of fish at harvest})}{\text{total number of fish stocked}} \times 100$$

$$\text{Yield (g/m}^3\text{)} = \frac{\text{Total weight at harvest (g)}}{\text{volume of rearing water (m}^3\text{)}}$$

$$\text{Daily feed intake (DFI, \% BW/day)} = \frac{\text{Feed consumption (g)}}{[(\text{Initial body weight (g)} + \text{Final body weight (g)}) / 2] / \text{days of culture}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Dry feed intake (g)}}{\text{Wet weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{wet weight gain (g)}}{\text{protein intake (g)}}$$

$$\text{Viscerosomatic index (VSI, \%)} = \frac{\text{viscera weight (g)}}{\text{body weight (g)}} \times 100$$

$$\text{Hepatosomatic index (HSI, \%)} = \frac{\text{liver weight (g)}}{\text{body weight (g)}} \times 100$$

$$\text{Condition factor} = \frac{(\text{wet body weight (g)} / \text{total length}^3 \text{ (cm)}) \times 100}{100}$$

$$\text{Digestive tract index (DTI, \%)} = \frac{[\text{wet weight of digestive tract (g)} / \text{wet body weight (g)}] \times 100}{100}$$

$$\text{Relative gut length (RGL)} = \frac{\text{digestive tract length (cm)}}{\text{total length (cm)}}$$

Proximate Composition Analysis

A total of 9 elvers per feeding group (3 individuals per replicate tank) were randomly collected and stored at -20°C for whole body proximate composition analysis (moisture, crude protein, crude fat, and crude ash) according to standard AOAC (2016) methods. Samples of elvers were dried at 105°C to constant weight to estimate the moisture content. Crude protein was determined using the Kjeldahl method (N × 6.25) after acid digestion. Crude lipid was measured by soxhlet extraction using the soxhlet system 1046 (Tacator AB, Hoganas, Sweden). Crude ash content was determined by incineration at 550°C for 3 h. Results were expressed on a dry matter basis. Proximate composition data were used to estimate the lipid to lean body mass ratio (L/LBM = [whole body lipid content (%) / (whole body protein content + whole body ash content)]) (de Macêdo et al. 2021).

Blood Chemistry Analysis

To examine the effect of feeding strategies on the physiological and health status of Pacific shortfin eel, 3 eels from each replicate tank were randomly collected and sedated with 2-phenoxyethanol (2 mL per L of water) before blood samples were withdrawn from the caudal vein using heparinized syringes, transferred into eppendorf tubes and centrifuged (5000 × g) for 10 min to obtain blood serum, which were then stored at -20°C for determination of haematological parameters (Damasaru et al. 2018). Serum total protein, cholesterol, glucose, triglycerides, and activities of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were analyzed using an ACCENT 200 automated chemistry analyzer (PZ Cormay S.A., Warszawa, Poland).

Liver and Intestinal Morphology

To assess the effect of feeding strategies on hepatic and intestinal morphologies, liver, and distal intestine, tissues from 3 representative elvers per replicate tank were dissected and fixed in 10% neutral buffered formaldehyde solution for 24 h and transferred to 70% ethanol. Fixed tissues were dehydrated in graded ethanol concentrations and embedded in paraffin blocks, which were then serially sectioned at 5 µm thickness using a microtome (Thermo Scientific HM 355S). Sectioned tissues were stained with hematoxylin and eosin (H & E). Five

histological sections of the liver and intestinal tissues of each specimen and 5 fields per section of each tissue were viewed under a digital microscope (Olympus CX43) at 40X and 400X magnifications and examined for abnormalities and lesions.

Economic Analysis

A partial costs-and-returns analysis was done to estimate the total revenue, total profit over feeds used, and profit index under the 2 feeding strategies, keeping other variable production costs – including labor and depreciation of materials – constant. The total revenue, total profit over feeds used, and profit index were calculated as follows:

$$\text{Total revenue (PhP)} = \text{total harvest (kg)} \times \text{selling price (PhP)}$$

$$\text{Total profit over feeds used} = \text{total revenue (PhP)} - \text{cost of feeds used (PhP)}$$

$$\text{Profit index} = \text{total profit over feeds used (PhP)} / \text{cost of feeds used (PhP)}$$

In the local market, table size eels between 200 and 300 g were sold at PhP 840/kg. The feed cost (PhP 124.95/kg) was computed based on the price of the feed ingredients at the time of purchase.

Statistical Analysis

Data are expressed as mean ± standard deviation of the different parameters from triplicate tanks and checked for normality and homogeneity of variance by Anderson-Darling and Barlett tests, respectively before analysis. Data on the water quality parameters, growth performance, feed utilization, biometric indices, proximate composition, and blood chemistry were compared by independent *t*-test with a 95% significance level (*p* < 0.05). All percent data were arcsine transformed prior to analysis.

RESULTS AND DISCUSSION

Fish Performance and Feed Utilization

The two feeding strategies did not significantly affect the growth parameters of eels after 155 d of culture (Table 2). The DF group was slightly heavier, with mean BW of 294.21 g after 93 d and 399.19 g after 155 d; however, mean BW was not significantly different from those in the ADF group, weighing 265.26 and 336.89 g, respectively at the same period (Fig. 1a). In addition, mean percent weight gain and specific growth rate (%/d) were comparable between the DF (141 ± 38% and 0.56 ± 0.10%/d) and ADF (106 ± 28% and 0.46 ± 0.09%/d) groups. Similarly, yield between DF (6158 ± 774 g/m³) and ADF (5389 ± 160 g/m³) groups were not statistically different.

This study demonstrates that Pacific shortfin eel subjected to ADF resulted in similar patterns of growth performance as compared to DF, suggesting the ability of this species to compensate for growth after periods of food deprivation and refeeding.

Mean survival rates in the ADF group were slightly higher than in the DF group during the last 2 mo of the trial (Fig. 1b), but differences between the 2 feeding groups were not significant (Table 2). This study confirms findings of earlier studies in Nile tilapia *Oreochromis niloticus* (Bolivar et al. 2006; Cuvin-Aralar et al. 2012; El-Araby et al. 2020) and Atlantic cod *Gadus morhua* (Bjørnevik et al. 2021) subjected to ADF or ADF with low and high feed ration (Hezron et al. 2019). Similarly, a shorter period of starvation or intermittent feeding did not have a significant effect on production performance of milkfish *Chanos chanos* (de Jesus-Ayson and Borski 2010), hybrid tambacu (*Piaractus mesopotamicus* male × *Colossoma macropomum* female) (Paz et al. 2018), and yellow tail

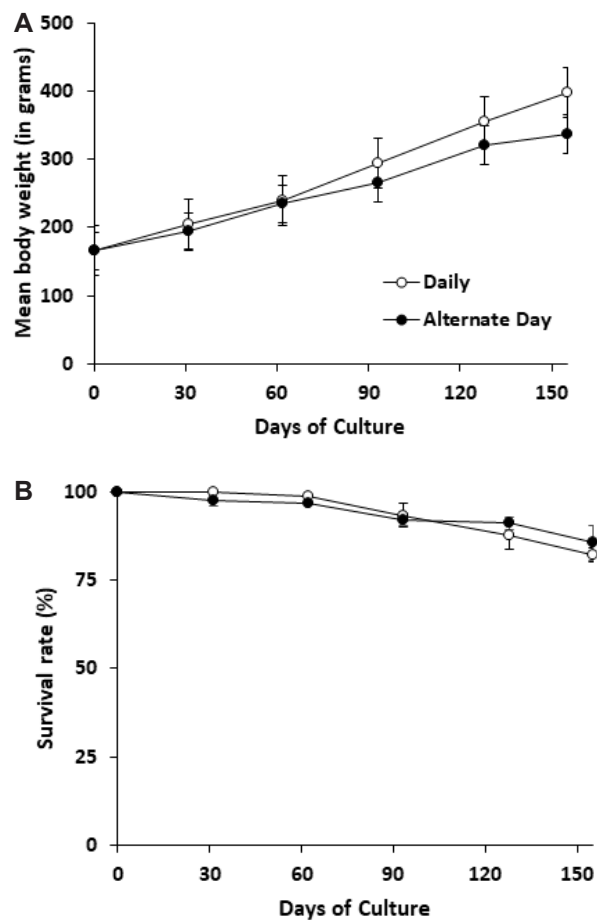


Fig. 1. Mean body weight and survival rate (b) of the Pacific shortfin eel *Anguilla bicolor pacifica* fed daily and on alternate days for 155 d in outdoor tanks. Bars represent standard deviation of the mean (n = 3).

Table 2. Growth performance, feed utilization and biometric indices (mean \pm SD; n = 3) of the Pacific shortfin eel *Anguilla bicolor pacifica* reared in outdoor tanks under two feeding strategies for 155 d. Values sharing different letter superscripts within the same row indicate a significant difference ($p < 0.05$).

Parameters	Feeding Strategy		p value
	Daily	Alternate Day	
<i>Growth performance</i>			
Final body weight (g)	399.19 \pm 45.72	336.89 \pm 25.76	0.244
Weight gain (%)	141 \pm 38	106 \pm 28	0.132
Specific growth rate (%/day)	0.56 \pm 0.10	0.46 \pm 0.09	0.108
Survival (%)	82.22 \pm 1.92	85.56 \pm 5.09	0.477
Yield (g/m ³)	6158 \pm 774	5389 \pm 160	0.289
<i>Feed utilization</i>			
Daily feed intake (% BW/day)	16.78 \pm 0.85 ^a	8.93 \pm 0.25 ^b	0.002
Feed conversion ratio	6.44 \pm 1.95	3.85 \pm 0.68	0.072
Protein efficiency ratio	0.39 \pm 0.09 ^a	0.63 \pm 0.10 ^b	0.016
<i>Biometric indices</i>			
Relative gut length	0.19 \pm 0.01	0.26 \pm 0.11	0.371
Hepatosomatic index (%)	1.35 \pm 0.10	1.24 \pm 0.14	0.357
Viscerosomatic index (%)	3.30 \pm 0.47	2.69 \pm 0.10	0.182
Digestive tract index (%)	1.42 \pm 0.47	1.31 \pm 0.57	0.097
Condition factor	0.22 \pm 0.01	0.20 \pm 0.01	0.184

Seriola ravaliana (Argüello-Guevara et al. 2018). Compensatory growth response also varies depending on fish size (Bilton and Robins 1973; Cho 2005; Cho et al. 2006), with larger fish less susceptible to starvation than smaller fish. Indeed, Bjørnevik et al. (2021) reported that feeding on alternate days resulted in an increased weight gain of Atlantic cod *Gadus morhua* weighing 704 g compared to those fed daily. Similar results were obtained by Cho (2005) and Cho et al. (2006) who subjected juvenile olive flounder *Paralichthys olivaceus*, with body weights of 16 and 53.8 g, to 2 wk of feed starvation and refeeding. In contrast, a 2 wk starvation followed by 6 wk normal feeding resulted in lower weight gain for the same species (6.4 g in weight) (Cho 2014), suggesting differences in the ability of large and small fish to achieve compensatory growth. Low basal metabolism and improved feed use efficiency could induce compensatory growth in fish (Fu et al. 2005; Foss et al. 2009; Adakli and Taşbozan 2015). However, in Nile tilapia juveniles, short cycles of fasting and refeeding do not promote compensatory growth when fed varying dietary starch:protein ratios (de Macêdo et al. 2021). Similarly, in hybrid tilapia *O. niloticus* \times *O. aureus* (Abdel-Hakim et al. 2009), fat snook *Centropomus parallelus* (Ribeiro and Tsuzuki 2010), and gilthead sea bream *Sparus aurata* (Peres et al. 2011) juveniles, longer feed deprivation periods resulted in poor compensatory growth. During fasting, fish growth is reduced mainly due to a decrease in the expression of certain genes involved in fish growth (Insulin Growth Factor or IGFs) (Zarantoniello et al. 2021). These studies indicate that

differences in compensatory growth response vary according to fish species, size, or age and duration of feed deprivation (McCue 2010).

Feeding strategies influence the feed utilization efficiency of the Pacific shortfin eel. Daily feed intake (DFI) in the ADF group was significantly lower when compared to that of the DF group (Table 2). During the rearing trial, eels fed on alternate days for 78 d consumed 0.54 kg of feed compared to almost 2 kg for those fed daily for 155 d. The reduction in total feed consumption in the ADF group while attaining a similar weight gain further supports the observed compensatory growth in this species. Improved feed use efficiency may be due to reduced feed waste through consumption of lower amounts of feed (Hezron et al. 2019; Bjørnevik et al. 2021). On the other hand, the poor growth, increased competition for feed, and mortalities could result from intermittent feeding (Abel-Hakim et al. 2006) which were not observed in this study, particularly in the ADF group. Protein efficiency ratio (PER) significantly varied between the 2 feeding strategies, with higher PER (0.63) in eels fed on alternate days than fed daily (0.39). Higher PER may also partly explain efficient feed utilization in ADF alternate day fed eels compared to those fed daily. Although not significant, feed conversion ratio (FCR) in the ADF group (3.85) was lower than in the DF group (6.44), resulting in a 40% reduction in total feed consumption (Table 2). In addition, FCR was 40% lower in eels subjected to ADF without significant loss in weight gain, suggesting improved nutrient assimilation from formulated feeds (Bjørnevik et al. 2021). Similarly,

Eslamoo et al. (2012) reported an 11.3% reduction in feed consumption for tinfoil barb (*Barbonymus schwanenfeldii*) when fed for 5 – 6 d, followed by 1 – 2 d feed deprivation. In addition, Nile tilapia subjected to 2 d of feed deprivation and 4 d refeeding resulted in 21% reduction in feed use (Gabriel et al. 2018). Improved feed utilization in the present and in other studies may suggest enhanced digestive enzymes capacity of eels during the refeeding period (Krogdahl and Bakke-Mckellep 2005; Bolasina et al. 2006; Yengkokpam et al. 2013). Accordingly, the efficient application of this feeding schedule can help eel growers reduce their production costs without compromising production output, as reported in earlier studies (Bolivar et al. 2006; Cuvin-Aralar et al. 2012; Bjørnevik et al. 2021).

Biometric Indices and Proximate Composition

Reducing metabolic costs and maintaining tissues and body chemical composition are some of the strategies used by fish to meet energy demands during and after the refeeding period (Jobling 2010). Interestingly, the relative gut length (RGL) — although not significant — was longer in ADF (0.26 ± 0.11) than in the DF (0.19 ± 0.01) group (Table 2), suggesting an increase in the digestive tract capacity of the fasting fish as a result of hyperphagic condition. Hyperphagia increased the flow of energy and metabolic substrates for somatic tissue growth during refeeding (Paz et al. 2018; de Macêdo et al. 2021). This is in line with earlier studies that suggest that fish under feed restriction have increased stomach capacity as reported in the white fish *Coregonus lavaretus* (Känkänen and Pirhonen 2009) and European seabass *Dicentrarchus labrax* (Baki et al. 2016). The 2 feeding strategies did not influence the biometric indices such as hepatosomatic index (HSI), viscerosomatic index (VSI), and digestive tract index (DTI) (Table 2). In addition, feed deprivation does not affect the biological indices — particularly the condition factor (Table 2) — suggesting similar physiological states of eels between the 2 feeding schedules. Feed deprivation also alters the mobilization of energy reserves such as triglycerides and glycogen in both liver and muscle in many fish species (Navarro and

Gutiérrez 1995; Figueiredo-Garrutti et al. 2002). In the case of *A. anguilla*, Larsson and Lewander (1973) observed a rapid utilization of liver triglycerides as energy source accompanied by a decrease in liver size during the first 95 d of food deprivation, supporting the packaging or storage of energy in the liver (Damsteegt et al. 2015). In this study, significant difference in HSI was not observed, suggesting a similar level of energy status between DF and ADF groups. However, Larsson and Lewander (1973) reported that stored liver and muscle glycogen levels during the first 95 d in an experiment with *A. anguilla* were maintained, suggesting activation of gluconeogenesis, defined as the synthesis of glucose from non-carbohydrate precursors (Nordlie et al. 1999). Similar results were reported for the American eel *A. rostrata* (Renaud and Moon 1980a; Renaud and Moon 1980b).

Whole body proximate composition between DF and ADF schedules did not exhibit any significant difference (Table 3). Although not significant, whole body protein contents were slightly higher in DF ($62.13 \pm 0.78\%$) compared to the ADF group ($60.59 \pm 3.00\%$). The comparable body proximate composition at the end of the feeding period demonstrated that energy reserves of ADF eels were restored. However, the slightly lower whole body protein content of ADF eels in this study may explain how body protein is mobilized to glucose for energy (Gabriel et al. 2018) to cope with an increased energy demand. Following Jobling's (2010) and de Macêdo et al.'s (2021) estimation of the lipid to lean body mass (L/LBM) ratio as an important predictor of compensatory growth, a similar ratio (L/LBM = 0.36; Table 3) between ADF and DF groups supports the observed compensatory growth in Pacific shortfin eel subjected to feed deprivation.

Water Quality

The water quality parameters monitored throughout the 155 d trial were shown in Table 4. Morning and afternoon water temperature and DO levels did not vary significantly between the 2 feeding groups ($p > 0.05$), and pH remained at 7.28 during the trial period. Unionized

Table 3. Proximate body composition (% dry matter) and lipid to lean body mass ratio (L/LBM) (mean \pm SD; n = 3) of the Pacific shortfin eel *Anguilla bicolor pacifica* reared in outdoor tanks under two feeding strategies for 155 d.

	Feeding Strategy		p value
	Daily	Alternate Day	
Moisture	3.83 \pm 0.07	3.72 \pm 0.46	0.703
Crude protein	62.13 \pm 0.78	60.59 \pm 3.00	0.368
Crude fat	24.99 \pm 6.34	25.12 \pm 7.68	0.918
Crude ash	7.88 \pm 0.10	8.48 \pm 0.41	0.159
L/LBM ratio	0.36 \pm 0.09	0.36 \pm 0.12	0.789

Table 4. Water quality parameters (mean \pm SD; n = 3) in outdoor tanks stocked with the Pacific shortfin eel *Anguilla bicolor pacifica* reared under two feeding strategies for 155 d.

Parameters	Feeding Strategy		p value
	Daily	Alternate Day	
Temperature ($^{\circ}$ C)			
AM	29.58 \pm 0.16	29.40 \pm 0.18	0.247
PM	29.73 \pm 0.14	29.60 \pm 0.34	0.393
Dissolved oxygen (mg/L)			
AM	3.13 \pm 0.41	3.45 \pm 0.52	0.600
PM	3.25 \pm 0.16	3.52 \pm 0.54	0.533
pH	7.28 \pm 0.01	7.28 \pm 0.02	0.748
Unionized Ammonia Nitrogen (mg/L)	0.027 \pm 0.006	0.015 \pm 0.005	0.015

AM, morning; PM, afternoon

ammonia concentrations in the ADF group were significantly lower than in the DF group ($p = 0.015$). In this study, temperature, DO, ammonia, and pH – which are considered important factors affecting eel performance – were within the acceptable limits for eel grow-out farming (Suzuki et al. 2003).

Reduction in the total feed consumption by as much as 40% in the ADF group resulted in a more favorable water quality condition such as significantly lower levels of unionized ammonia in ADF than in the DF group. Similarly, in the European eel *A. anguilla*, water quality conditions such as higher DO and lower ammonia concentrations were considerably improved as a result of feeding during nighttime, which in turn enhanced the feeding behavior (Abdel-Hay et al. 2019). Good water quality and feed management are therefore necessary to provide optimal conditions for growth and survival of eels (Cuvin-Aralar et al. 2019).

Blood Chemistry

Haematological indices reflect the fish health status and physiological condition (Adams et al. 1993). Serum biochemical indices did not significantly differ between the two feeding groups (Table 5). Serum glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), and glucose levels were numerically higher – but also not significant – in the ADF than the DF groups. Generally, neither DF or ADF Pacific shortfin eels showed

an apparent variation in their blood parameters, suggesting that the physiological and health conditions of ADF eels were not compromised. It is known that serum GOT and GPT are indicators of liver function and increased levels are responses to liver damage or environmental stress (Luo et al. 2013). However, when compared to the DF group, eels in the ADF group had numerically higher levels of GOT and GPT but did not manifest in the HSI values, suggesting that the feeding groups did not negatively alter the liver condition of eels. In contrast, the values for GOT and GPT were much higher than those reported in other eel species such as the Japanese eel *A. japonica* with 55.0 – 90.6 U/L and 15 – 28 U/L (Damusaru et al. 2018), *A. bicolor bicolor* with 100.11 – 128.44 U/L and 1 U/L (Harianto et al. 2021), and *A. marmorata* with 4.53 – 8.27 U/L and 114.88 – 183.85 U/L (Tan et al. 2018). Such differences in response in the present and other studies are most likely due to various factors such as eel species, feeding, and rearing conditions.

Liver and Intestinal Histology

Histological analyses of intestines may provide indication of short-term food deprivation status in fish (Zarantoniello et al. 2021). Food deprivation is usually linked to changes in various intestinal membrane and cells (Zeng et al. 2012; Zaldúa and Naya 2014). The intestines of elvers in the DF and ADF groups showed no

Table 5. Blood parameters (mean \pm SD; n = 3) of the Pacific shortfin eel *Anguilla bicolor pacifica* reared in outdoor tanks under two feeding strategies for 155 d.

	Feeding Strategy		p value
	Daily	Alternate Day	
GOT (U/L)	249.34 \pm 130.22	274.73 \pm 60.45	0.718
GPT (U/L)	223.76 \pm 159.66	259.81 \pm 192.51	0.783
Total protein (g/dL)	19.70 \pm 7.00	14.91 \pm 8.20	0.513
Glucose (mg/dL)	249.33 \pm 24.01	346.97 \pm 68.56	0.209
Cholesterol (mg/dL)	586.57 \pm 68.54	543.33 \pm 76.17	0.631
Triglycerides (mg/dL)	415.60 \pm 13.88	400.70 \pm 93.23	0.803

GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase

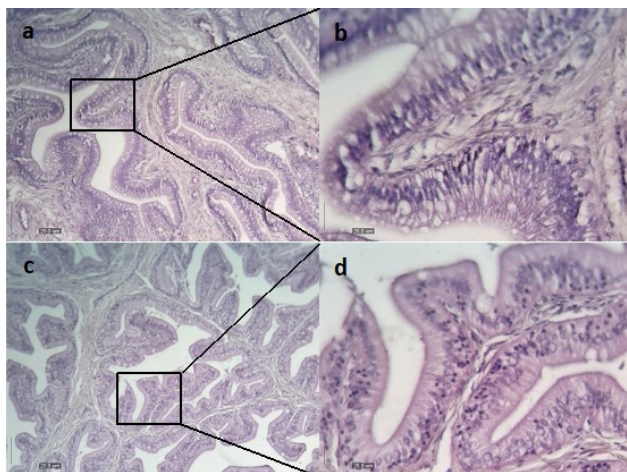


Fig. 2. Intestinal histology of the Pacific shortfin eel *Anguilla bicolor pacifica* elvers fed daily (a-b) and on alternate days (c-d) for 155 d in outdoor tanks. Hematoxylin and eosin. Magnification 40X (a, c), 400X (b, d).

alterations or inflammation of the villi (Fig. 2). In addition, intestinal pathological changes between elvers fed daily and on alternate days were absent. The normal intestinal morphology observed in this study suggests an efficient nutrient assimilation that led to no significant loss in weight gain particularly in the ADF group (Bjørnevik et al. 2021). Hepatic tissues displayed normal or healthy hepatocytes with no vacuolization between the DF and ADF groups (Fig. 3). No liver pathological alterations were observed, which implies that using the ADF strategy can still maintain the health status of the Pacific shortfin eel during culture.

Economic Analysis

The comparative simple economic analysis demonstrates the feasibility of using ADF as an economical feed management strategy in Pacific shortfin eel culture. Both feeding strategies resulted in positive total revenues of PhP 8276 and PhP 7243, respectively for the DF and ADF (Table 6). Although ADF yielded a lower volume of total harvest at 8.62 kg and a lower total revenue at PhP 7243.00, ADF efficiently reduced the costs of feeds used

Table 6. Partial cost-and-returns analysis of the Pacific shortfin eel *Anguilla bicolor pacifica* reared in outdoor tanks under two feeding strategies for 155 d.

	Feeding Strategy	
	Daily	Alternate Day
Initial weight, 90 elvers (kg)	5.01	4.96
Total feed consumption (kg)	29.40	13.90
Cost of feeds (PhP/kg)	124.95	124.95
Cost of feeds used (PhP)	3,673.21	1,736.62
Total harvest (kg)	9.85	8.62
Total revenue (PhP)	8,276	7,243
Total profit over feeds used (PhP)	4,603.22	5,506.38
Profit index	1.25	3.18

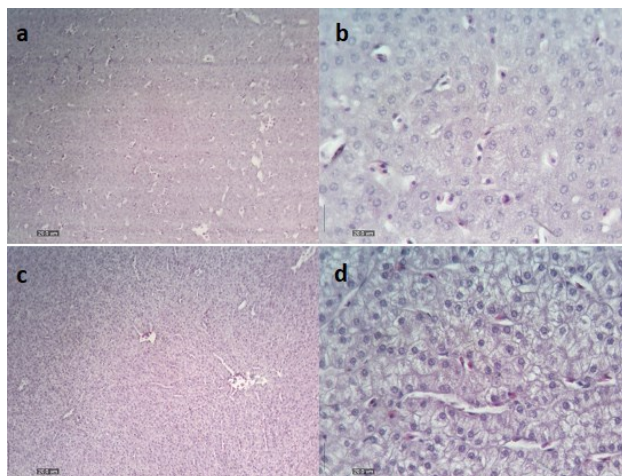


Fig. 3. Liver histology of the Pacific shortfin eel *Anguilla bicolor pacifica* fed daily (a-b) and on alternate days (c-d) for 155 d in outdoor tanks. Hematoxylin and eosin. Magnification 40X (a, c), 400X (b, d).

in rearing Pacific shortfin eel, thereby increasing the profit for eel farmers. Feeding on alternate days resulted in lower FCR as demonstrated in earlier studies (Bolivar et al. 2006; Solberg et al. 2006; Cuvin-Aralar et al. 2012; Bjørnevik et al. 2021). Therefore, eel farmers can benefit economically from this feeding practice wherein they could profit 3.18 times more per unit expenditure on feeds with ADF in contrast to DF where the profit index was only 1.25.

CONCLUSION

This study demonstrated that feeding on alternate days was found effective in promoting compensatory growth and better feed utilization, and that it had no adverse effects to blood parameters, liver, and intestinal morphology in the Pacific shortfin eel. Reduced feed conversion ratio with alternate day feeding suggests that this feeding strategy could help lower production costs of tropical anguillid eel farming.

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