

Original Research Articles

Early weaning protocols for the brackishwater nursery culture of the tropical anguillid eel *Anguilla marmorata* (Quoy & Gaimard, 1984)

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Feeding trials with *Anguilla marmorata* during the nursery culture were conducted to establish optimized protocols for transitioning from live to formulated diets. This study evaluated different weaning schemes over three phases of the nursery culture: from live feed (*Artemia*) to raw fish meat-based mash (FMT), from FMT to dry fish meal-based mash (FML), and from FML to dry formulated crumble diet. Specific growth rate (SGR), percent weight gain (%WG) and survival of *A. marmorata* elvers were assessed across treatments. The optimized weaning scheme for transitioning *A. marmorata* glass eel feeding from *Artemia* to FMT requires a 6.25% reduction of live feed daily for 16 days, resulting in the highest growth performance (SGR=4.49, %WG=109.25%) and survival (98.37%). Dietary transition from FMT to FML feeding, a 5% daily reduction of FMT with FML, over 20 days was found optimal. This treatment yielded superior growth performance with an SGR of 1.59, %WG of 68.80%, and survival of 62.00% ($p<0.05$). The final phase, transitioning from FML to a formulated crumble diet, was optimized with a 25% daily reduction of FML over 4 days. Elvers on this feeding regime exhibited the highest growth (SGR=2.49, %WG=41.81%) and survival (100.00%). These weaning protocols effectively facilitated the dietary transition of *A. marmorata* during the nursery stage from live feed to dry-formulated diets within a 40-day period, enhancing the growth and survival of *A. marmorata* cultured in the brackish water nursery system.

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INTRODUCTION

Anguillid eels, belonging to the Genus *Anguilla* (Family *Anguillidae*), inhabit marine, freshwater, and brackish water environments, with 19 known species/subspecies.^{1,2} These eels are a valuable fisheries resource consumed globally, making them a target species for aquaculture. Among the Anguillid species, temperate eels such as *A. anguilla* and *A. japonica* are the most popular cultured and marketed species, holding high market value. However, the global eel farming industry relies entirely on the availability of wild glass eels, as captive production of glass eels has not yet been achieved.³ This reliance has led to a drastic decline in wild stocks of these temperate eels, prompting regulations to limit glass eel collection.^{4,5} The global eel trade has shifted due to the listing of *A. anguilla* in Appendix II of the Convention on International Trade in Endangered Species

of Wild Fauna and Flora (CITES),⁶ resulting in increased sourcing and demand for tropical anguillid eels for aquaculture.

In tropical countries like the Philippines, eel aquaculture is receiving increased attention for its high export potential, particularly to neighboring countries in the East Pacific Region such as China, Taiwan, Hong Kong, Japan, South Korea, Vietnam, and Australia.⁷ In 2022, the eel was among the top exported fishery commodities in the Philippines, accounting for 7.9% (22,352 MT) of the total volume of exported fishery products and 4.5% (Php 21.9B or \$53.9M) of the total exported fishery product value.⁸

The giant mottled eel, *A. marmorata*, has the widest geographical distribution among anguillid species, thriving in tropical waters from the Eastern Pacific Ocean to the Western Indian Ocean.^{1,9-12} Despite its wide distribution and availability, *A. marmorata* glass eels are considered bycatch

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to *A. bicolor pacifica*, which has a higher market value due to its taste and meat equality that is comparable to the popular *A. japonica*.¹³ Nevertheless, *A. marmorata* maintains a thriving export market in China and Taiwan, where it is sold frozen, processed, and alive.⁷

Despite the abundance and potential of the tropical eel *A. marmorata* in aquaculture, understanding of its biology is still limited.¹⁴ Cadiz and Traifalgar¹⁵ explored the nursery culture of *A. marmorata* concerning optimal salinity, but suitable diets and feeding protocols have not yet been fully developed. Eels are known to be predatory, feeding primarily on carnivorous diets such as planktonic crustaceans during their early life stages.¹⁶ Since artificial propagation of eel larvae has not yet been developed, the eel aquaculture industry is dependent on wild-caught glass eels that are adapted to natural food present in their environment. This condition makes wild-caught glass eels weaning to an artificially formulated diet during the nursery stage challenging. Typical diets in the nursery culture of tropical anguillid eels are moist compounded dough-type feeds, which can deteriorate water quality, therefore requiring intensive water management due to nutrient leaching. This condition could also promote the outbreak of bacterial and fungal diseases that could significantly affect the nursery culture of eels. Alternatively, the use of dry-formulated feeds in eel nurseries offers a significant advantage due to the ease of handling, preparation, storage, and reliability of its nutrient composition and quality. However, as eels are not adept at consuming dry feeds, weaning using a formulated crumble diet during the nursery culture is crucial for the adaptability of this organism to pelleted feeds in the grow-out phase of culture.

Previous studies have shown the feasibility of weaning *A. japonica*, *A. anguilla*, *A. australis*, and *A. bicolor*¹⁷⁻²⁰ onto artificial diets. For instance, De Silva et al.¹⁸ investigated the weaning of *A. australis* to artificial diets using fish roe. Moreover, Hirt-Chabbert et al.¹⁹ examined various feeding stimulants to successfully wean *A. anguilla* glass eels and elvers to pelleted diets for optimum growth. However, weaning strategies for *A. marmorata* were not totally explored, and the feasibility of different weaning strategies for this species was not fully investigated. During the early stage, glass eels struggle to get accustomed to formulated feeds, resulting in weight loss or slow growth. Typically, first feeding to artificial feeds results in lower survival rates and large variation in growth, with only 25-40% reaching the market table size.²¹ This makes it harder for eel farmers to effectively facilitate the weaning of *A. marmorata* to artificial diets and efficiently culture this species. A better understanding of the possible weaning strategies of *A. marmorata*, from live feed to formulated solid feeds, will help optimize their culture, enhance growth and survival during the early weaning stages, and improve production in the nursery culture of this species. Thus, this study evaluated different weaning schemes for *A. marmorata* glass eels and elver. Specifically, this study evaluates the effect of different weaning strategies—transitioning from live feed to dry-formulated feeds—on the growth performance and survival of *A. marmorata* glass eels.

MATERIALS AND METHODS

EXPERIMENTAL ANIMAL

Wild-caught *A. marmorata* glass eels (av. wt. 0.11 g) were procured from eel collectors in General Santos City, South Cotabato. The procured glass eels were stocked in quarantine tanks, allowed to rest for 24 hours, and then treated with formalin (150 mg L⁻¹ for 30 minutes) as a prophylaxis. Glass eels were further sorted to collect *A. marmorata* based on the pattern of caudal fin pigmentation.²² After sorting, the animals were acclimated to a salinity of 18 ppt in 10-tonner tanks and fed ad libitum twice daily with *Artemia* nauplii at the Multi-species Hatchery Complex of the University of the Philippines Visayas-Miagao, Iloilo Philippines.

EXPERIMENTAL CONDITIONS AND WEANING PROTOCOLS

Three feeding trials were conducted to transition glass eels from live feed to dry-formulated feeds. The treatments include weaning from (i) live feed to raw fish meat-based mash (Trial 1); (ii) raw fish meat-based mash to dry fish meal-based mash (Trial 2); (iii) dry fish meal-based mash to formulated dry crumble (Trial 3). The best results from each trial were used in the subsequent trials to achieve the optimum weaning protocol for the nursery culture of *A. marmorata*.

Treatments in all three experiments were carried out in 90L polyethylene (PE) tanks, each filled with 40L of UV-filtered brackish water, 18 ppt, and supplied with mild aeration. Eels in all experiments were maintained at a salinity of 18 ppt,¹⁵ the temperature at 28°C, dissolved oxygen (DO) above 4 mg/L, and ammonia maintained at 0.08 mg L⁻¹. Salinity and temperature were measured daily, while ammonia and DO were measured every three days. All experiments were conducted in a flow-through system with a flow rate maintained at 5.71 mL sec⁻¹.

In Trial 1, *A. marmorata* glass eels with an initial average body weight (ABW) of 0.10 g were weaned from live feed to a raw fish meat-based mash. Newly hatched *Artemia* nauplii was used as live feed for this experiment, and the formulated feed (raw fish meat-based mash, FMT) consisted of pre-prepared minced fish meat, potato starch, yeast, and vitamin and mineral mix. There were five treatments performed in triplicates, totaling 15 tanks, with 50 glass eels stocked in each PE tanks, in a completely randomized design (CRD): (a) 100% live feed until termination; (b) combination of live feed and raw fish-meat based mash, with a gradual reduction of live feed by 6.25% day⁻¹, (c) combination of live feed and raw fish-meat based mash, with a gradual reduction of live feed by 12.50% day⁻¹; (d) combination of live feed and raw fish-meat based mash, with a gradual reduction of live feed by 25.00% day⁻¹; and (e) 100% raw fish-meat based mash until termination. Glass eels were fed twice a day (9 AM and 4 PM) at a feeding rate of 15% body weight (BW). Size sampling to adjust the feed allocation

and cleaning of culture tanks was done every four days, and the experiment lasted for 20 days.

In the 2nd trial, *A. marmorata* glass eels (ABW= 0.15 g) were reared in PE tanks for weaning from raw fish meat-based moist mash to dry fish meal-based mash. The composition of the formulated diets was the same as in Trial 1, except that fish meal was used instead of raw fish meat in the dry fish meal-based mash (FML). One thousand glass eels were randomly distributed in four different treatments in quintuplicate, with 50 eels stocked in each PE tanks, and fed 15% of BW (twice a day: 9 AM and 4 PM) with the following treatments for 28 days: (a) 100% raw fish meat-based mash until termination; (b) combination of raw fish meat-based mash and dry fish meal-based mash, with a gradual reduction of raw fish meat-based mash by 5% day⁻¹ (c) combination of raw fish meat-based mash and dry fish meal-based mash, with a gradual reduction of raw fish meat-based mash by 10% day⁻¹; and (d) 100% dry fish meal-based mash until termination. Sampling was done every seven days.

The 3rd trial was conducted using 36-day-old *A. marmorata* elvers, originating from the glass eel stocking and weighing an average of 0.56 g, were used for the weaning experiment from the dry fish meal-based mash to formulated crumble feed. The experiment was done in triplicate (30 elvers stocked in each PE tank) with the following treatments: (a) 100% dry fish meal-based mash until termination; (b) 100% formulated crumble feed until termination; (c) combination of dry fish meal-based mash and formulated crumble, with dry fish meal-based mash gradually reduced by 10% day⁻¹; and (d) combination of fish meal-based mash and formulated crumble, with dry fish meal-based mash gradually reduced by 25% day⁻¹. Elvers were fed 15% of their BW, twice a day (9 AM and 4 PM). Sampling was done every four days, and the experiment lasted for 16 days.

DATA COLLECTION AND ANALYSIS

Prior to the start of each experiment, 40 randomly selected glass eels from the conditioning tank were sacrificed and sampled for initial weight, length, and mouth gape. Mouth gape was measured using a digital caliper. During each sampling period in the three experiments, ABW and survival were recorded. ABW was obtained by weighing glass eels by group per replicate and survival was determined by individually counting eels per tank. At the end of each experiment, 10 eels per treatment were euthanized using tricaine methanesulfonate (MS-222) and sampled for final length and mouth gape. ABW, specific growth rate (SGR), and survival (%) of eels per treatment were also measured. The aforementioned growth indices were calculated as follows:

$$ABW = \frac{\text{total weight}}{\text{number of samples}}$$

$$SGR = \frac{\ln \text{Final weight} - \ln \text{Initial weight}}{\text{number of days}} \times 100$$

$$\% \text{Survival} = \frac{\text{final number}}{\text{initial number}} \times 100$$

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS version 26. The data were tested for normality using the Shapiro-Wilk test. Following this, a one-way analysis of variance (ANOVA) was conducted to compare the means of the different growth indices of *A. marmorata* reared under different treatments in each feeding trial. Tukey's multiple comparison procedure was used as the post hoc test to determine the statistical significance of the differences between means at 95% confidence level.

RESULTS

EFFECT OF WEANING FROM LIVE FEED TO RAW FISH MEAT-BASED MASH (FMT) ON THE GROWTH AND SURVIVAL OF *A. MARMORATA* AT NURSERY STAGE

Weaning from live feed to fish meat was completed after sixteen (16) days, achieving 100% replacement. A significant difference between treatments was observed in the ABW of glass eels ($p < 0.05$). At the final sampling, the highest ABW was recorded in the treatment with 100% live feed until termination (ABW=0.21 g). However, this was not significantly different from the treatment with 6.25% reduction of live feed day⁻¹ (ABW=0.19 g), which was the second highest in terms of body weight. This was followed by the treatment with 100% FMT (ABW=0.13 g), the treatment with a 25% reduction of live feed day⁻¹ (ABW=0.12 g), and finally, the treatment with a 12.5% reduction of live feed day⁻¹ (ABW=0.11 g).

The initial average body length of the glass eels was 48.60 mm. A significant difference in body length was observed between treatments at the final sampling ($p < 0.05$). In the final sampling, glass eels in the treatment fed with 100% live feed for 16 days had the longest body length (ATL=52.26 mm). However, this was not significantly different from the treatment with a 6.25% reduction of live feed day⁻¹, which had the second longest in body length (ATL=50.82 mm) (Table 1).

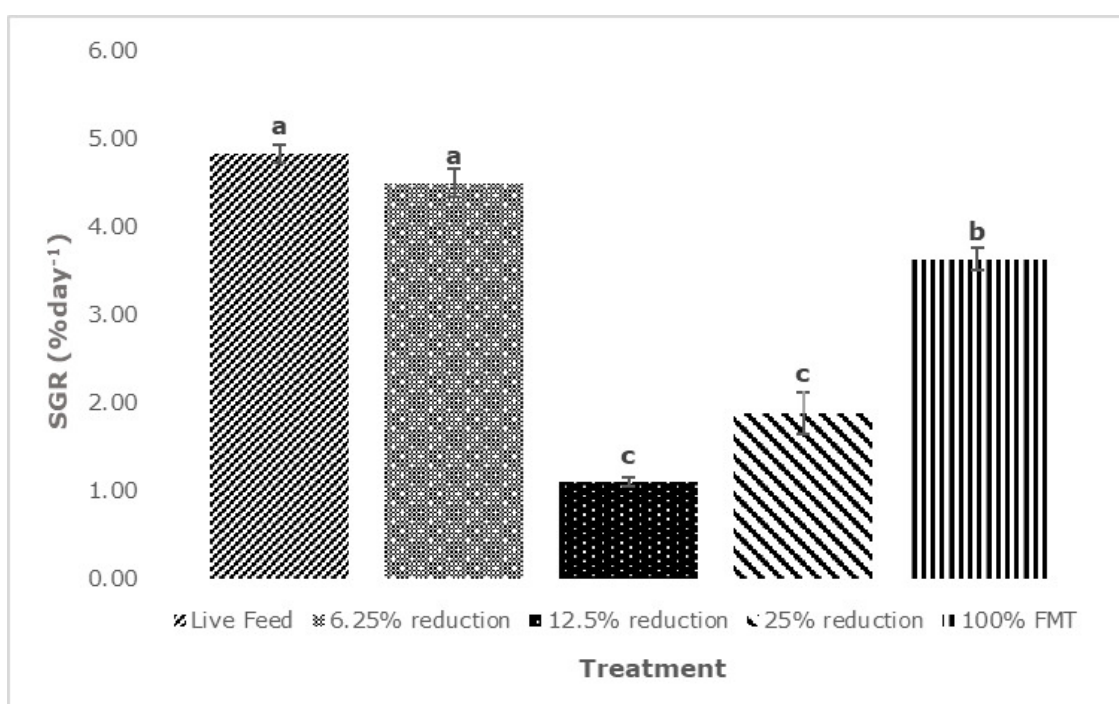
The specific growth rate (SGR) results of the trial showed that glass eels fed with 100% live feed have the highest SGR. However, this was not significantly different from the second highest SGR, observed in the treatment with a 6.25% reduction of live feed day⁻¹. Meanwhile, glass eels fed with 100% FMT and those in the treatment with a 25% reduction of live feed day⁻¹ recorded SGRs of 3.63 and 1.87, respectively. The lowest SGR was recorded in the treatment with a 12.5% reduction of live feed day⁻¹, with an SGR of only 1.09 (Figure 1).

The highest weight gain (%WG) in the first weaning trial was observed in glass eels fed with 100% live feed (115.00%), this was followed by 6.25% reduction of live feed day⁻¹ (109.25%), 100% FMT (88.00%), a 25% reduction of live feed day⁻¹ (35.00%), and finally, a 12.5% reduction of live feed day⁻¹ (19.09%) (Figure 2). There was a significant difference in the weight gain of glass eels among the treatments ($p < 0.05$), although the difference between glass eels

Table 1. Initial and final weight and length of *A. marmorata* weaned at different rates after 16 days (Live feed to FMT).

Growth Parameter	Treatments				
	100% Live Feed	6.25% reduction	12.5% reduction	25% reduction	100% FMT
Initial average weight (g)	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Final average weight (g)	0.21 ± 0.02 ^a	0.19 ± 0.05 ^a	0.11 ± 0.02 ^b	0.12 ± 0.02 ^b	0.13 ± 0.02 ^b
Initial average total length (mm)	48.60 ± 0.00	48.60 ± 0.00	48.60 ± 0.00	48.60 ± 0.00	48.60 ± 0.00
Final average total length (mm)	52.26 ± 0.46 ^a	50.82 ± 0.57 ^{ab}	49.25 ± 0.07 ^b	48.90 ± 0.50 ^b	48.65 ± 0.37 ^b

Values are the means of the replicates ± SD (n=3). Values with different superscript letters are significantly different from each other (p<0.05).

**Figure 1. Specific growth rate of *A. marmorata* weaned at different rates after 16 days (Live feed to FMT).**

Values are the means of the replicates ± SEM (n=3). Bars with different superscript letters are significantly different from each other (p<0.05).

fed with 100% live feed and those with 6.25% reduction of live feed day⁻¹ was not statistically significant (p< 0.05).

The survival rate of eels at different weaning rates was also assessed, with significant differences observed between treatments (p<0.05) (Figure 3). The highest survival rate was recorded from glass eels fed with 100% live feed (98.78%), followed by the 6.25% reduction of live feed day⁻¹ (98.37%), 25% reduction of live feed day⁻¹ (94.72%), and 12.5% reduction of live feed day⁻¹ (84.76%). The lowest survival rate was observed in glass eels fed directly with 100% FMT (82.62%). No significant difference was found between the treatment with the highest survival (100% live feed) and the treatment with a 6.25% reduction of live feed day⁻¹.

Based on the results of this experiment, the optimal approach for weaning glass eels from live feed to FMT is to replace live feed with raw fish meat-based mash at a rate of 6.25% day⁻¹.

EFFECT OF WEANING FROM RAW FISH MEAT-BASED MASH (FMT) TO DRY FISH MEAL-BASED MASH (FML) ON THE GROWTH AND SURVIVAL OF *A. MARMORATA* AT NURSERY STAGE

The final average body weight (ABW) and average total length (ATL) for all treatments are summarized in Table 2. *A. marmorata* glass eels were weaned from raw fish meat-based mash (FMT) to dry fish meal-based mash (FML) with a gradual reduction of raw fish meat by 5% and 10% per day. Treatments including 100% FMT and 100% FML throughout the culture period were also evaluated. The results revealed significant differences between treatments for both growth parameters. Specifically, eels fed with a 5% reduction of FMT day⁻¹ exhibited the highest ABW and ATL after 28 days (p<0.05).

Specific growth rate (SGR) and percent weight gain (%WG) were assessed at the end of the experiment. The

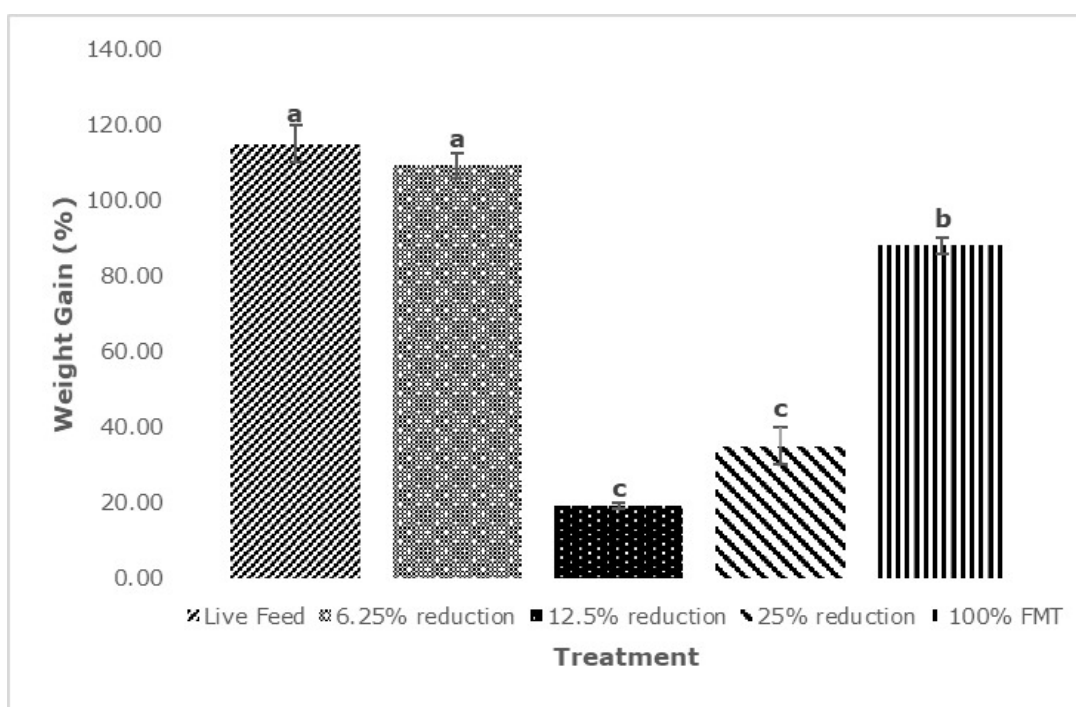


Figure 2. Percent weight gain of *A. marmorata* weaned at different rates after 16 days (Live feed to FMT).

Values are the means of the replicates $\pm$ SEM (n=3). Bars with different superscript letters are significantly different from each other ($p < 0.05$).

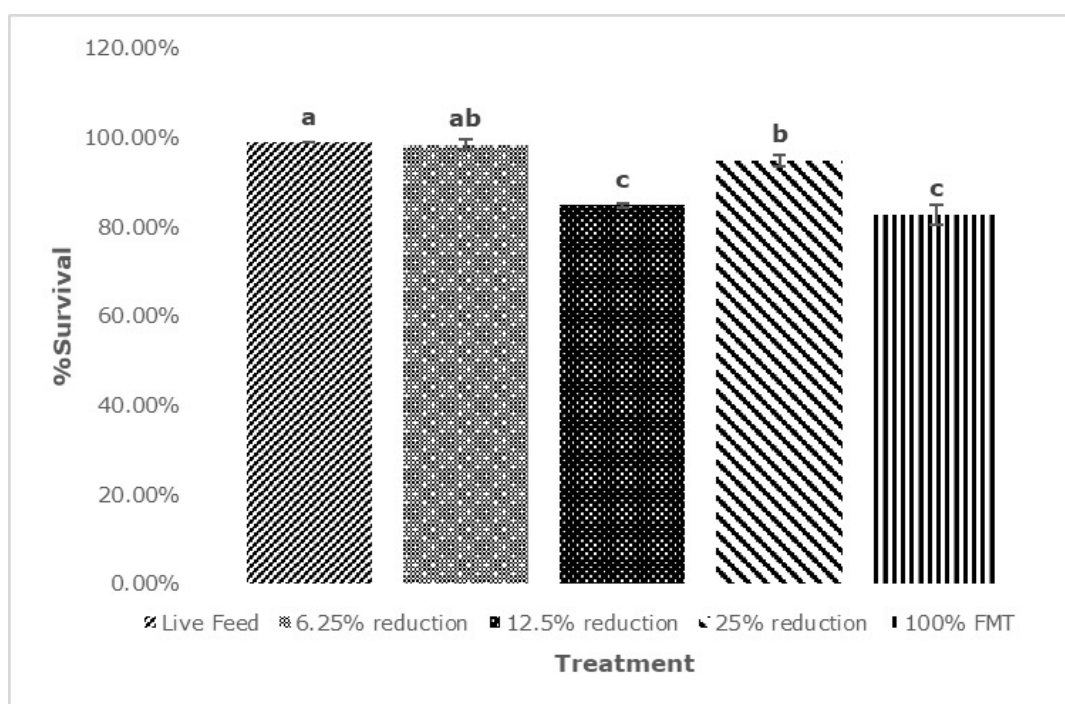


Figure 3. Percent survival of *A. marmorata* weaned at different rates after 16 days (Live feed to FMT).

Values are means of replicates $\pm$ SEM (n=3). Bars with different superscript letters are significantly different from each other ($p < 0.05$).

SGR and %WG of *A. marmorata* elvers are depicted in [Figure 4](#) and [Figure 5](#), respectively, with significant differences observed between treatments ($p < 0.05$).

Eels fed with a 5% daily reduction of FMT exhibited the highest SGR at 1.87 ± 0.04 , followed by those with a 10% daily reduction of FMT ($SGR = 1.59 \pm 0.3$). Eels fed with 100%

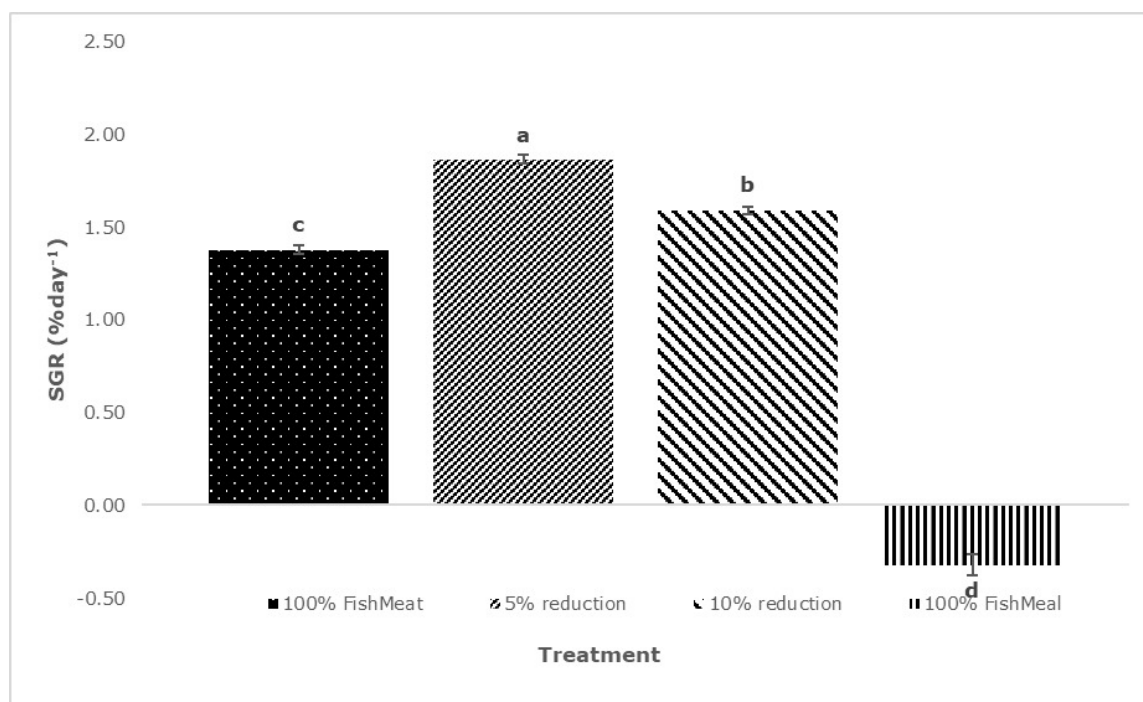
FMT had a moderate SGR of 1.38 ± 0.03 , while those fed 100% FML showed negative SGR at -0.32 ± 0.08 ($p < 0.05$).

Meanwhile, eels subjected to a 5% daily reduction of FMT achieved the highest %WG at 68.80, followed by those with a 10% daily reduction of FMT (%WG=56.29). Eels fed exclusively with 100% FMT recorded a %WG of 47.00, while

Table 2. Initial and final weight and length of *A. marmorata* weaned at different rates after 28 days (FMT to FML).

Growth Parameter	Treatment			
	100% FMT	5% reduction	10% reduction	100% FML
Initial average weight (g)	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00
Final average weight (g)	0.21 ± 0.00 ^{bc}	0.28 ± 0.02 ^a	0.23 ± 0.01 ^{ab}	0.14 ± 0.01 ^c
Initial average total length (mm)	51.76 ± 6.11	51.76 ± 6.11	51.76 ± 6.11	51.76 ± 6.11
Final average total length (mm)	55.68 ± 1.31 ^{bc}	61.35 ± 2.05 ^a	60.45 ± 0.78 ^{ab}	52.89 ± 0.40 ^c

Values are the means of the replicates ± SD (n=5). Values with different superscript letters are significantly different from each other (p<0.05).

**Figure 4. Specific growth rate of *A. marmorata* weaned at different rates after 28 days (FMT to FML).**

Values are means of replicates ± SEM (n=5). Bars with different superscript letters are significantly different from each other (p<0.05).

those fed with 100% FML exhibited a negative %WG at -8.67. The %WG findings corroborate the SGR results, with the highest %WG observed in the 5% reduction group and the lowest in the 100% FML group (p<0.05).

The percent survival of *A. marmorata* elvers was evaluated at the end of the experiment, and the results are depicted in Figure 6. Significant differences in survival rates were observed between treatments (p<0.05).

Eels subjected to a 5% daily reduction of FMT exhibited the highest percent survival at 62.00%. This was followed by eels with a 10% daily reduction in FMT, which had a survival of 51%, and eels fed 100% FML, with a survival of 41.00%. The lowest percent survival was observed in eels fed exclusively with 100% FMT, at 21.00%. These results highlight the superior survival performance of eels transitioning with a 5% daily reduction in FMT compared to other treatments (p<0.05).

A significantly low percent survival was observed in eels fed exclusively with 100% FMT. This low survival may be attributed to the use of fish meat as a feed ingredient, which

can act as a potential carrier of pathogens. This hypothesis is supported by the bacterial infections observed in deceased eels in tanks with 100% FMT. Figure 7 illustrates the results of the microbiological analysis conducted following the mass mortality event. The analysis revealed that tanks fed with 100% FMT were infected with three species of *Vibrio*: *V. harveyi*, *V. rotiferanus*, and *V. vulnificus*.

Based on the observed growth performance and survival across all treatments, the optimal approach for transitioning *A. marmorata* from raw fish meat-based mash (FMT) to dry fish meal-based mash (FML) is to gradually replace fish meat with fish meal at a rate of 5% per day.

EFFECT OF WEANING FROM DRY FISH MEAL-BASED MASH (FML) TO FORMULATED CRUMBLE FEED ON THE GROWTH AND SURVIVAL OF *A. MARMORATA* AT NURSERY STAGE

The initial and final growth parameters, including body weight and average total length, are summarized in Table

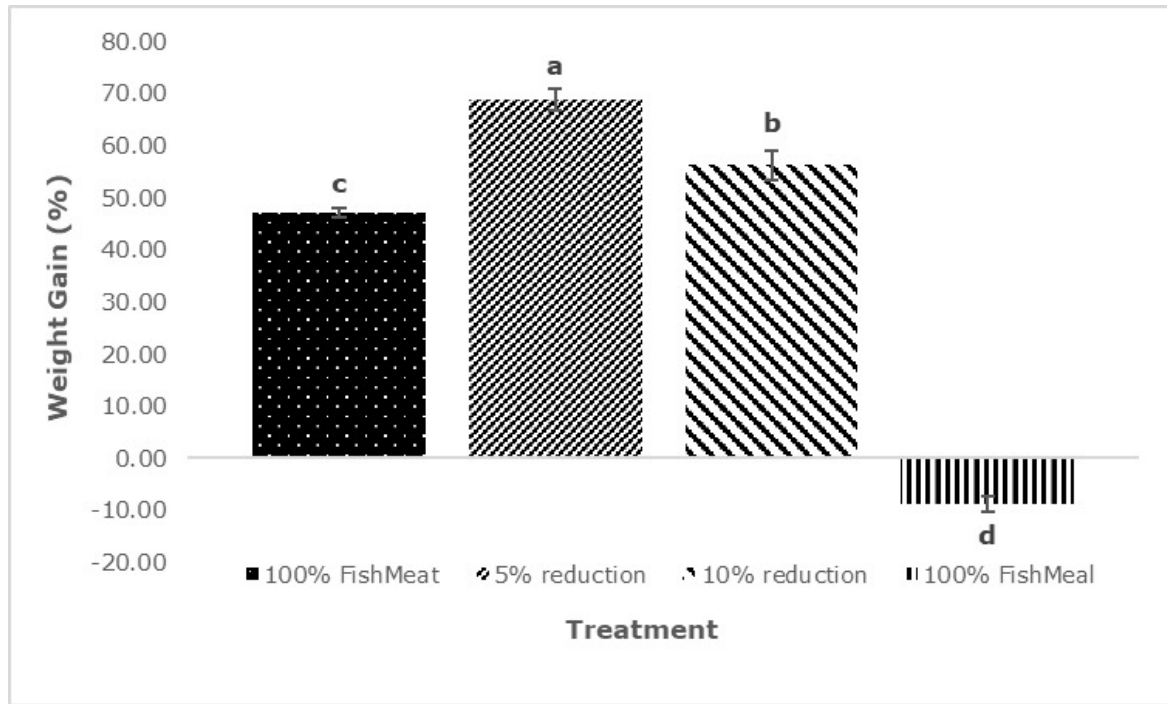


Figure 5. Percent weight gain of *A. marmorata* weaned at different rates after 28 days (FMT to FML).

Values are means of replicates $\pm$ SEM (n=5). Bars with different superscript letters are significantly different from each other ($p < 0.05$).

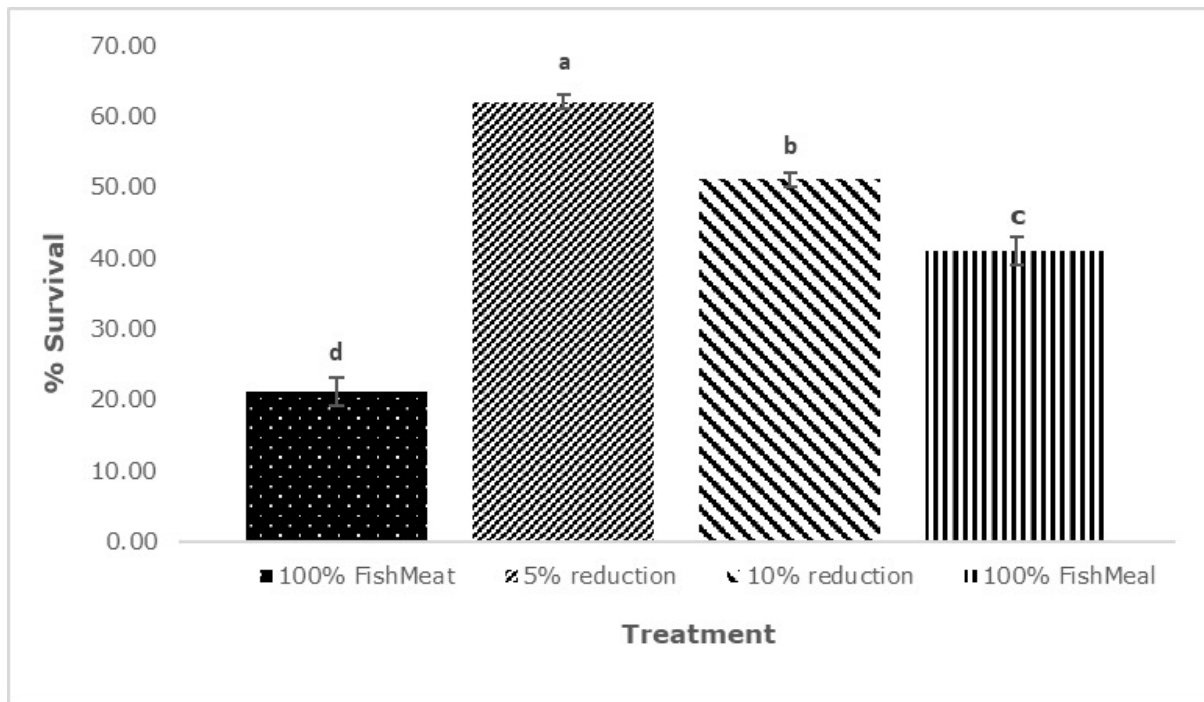


Figure 6. Percent survival of *A. marmorata* weaned at different rates after 28 days (FMT to FML).

Values are means of replicates $\pm$ SEM (n=5). Bars with different superscript letters are significantly different from each other ($p < 0.05$).

3. The weaning scheme lasted for a maximum of 10 days, with the study continuing until the 16th day of the trial. The results indicate significant differences between treatments for both final average body weight and final average total length ($p < 0.05$). Specifically, the treatment with a 25% daily reduction of FML, gradually substituted by crumble,

resulted in the highest average body weight and total length.

Specific growth rates and weight gain were also evaluated in the feeding trial. [Figures 8](#) and [9](#) illustrate significant differences in SGR and %WG among the different weaning schemes from FML to crumble diet ($p < 0.05$).

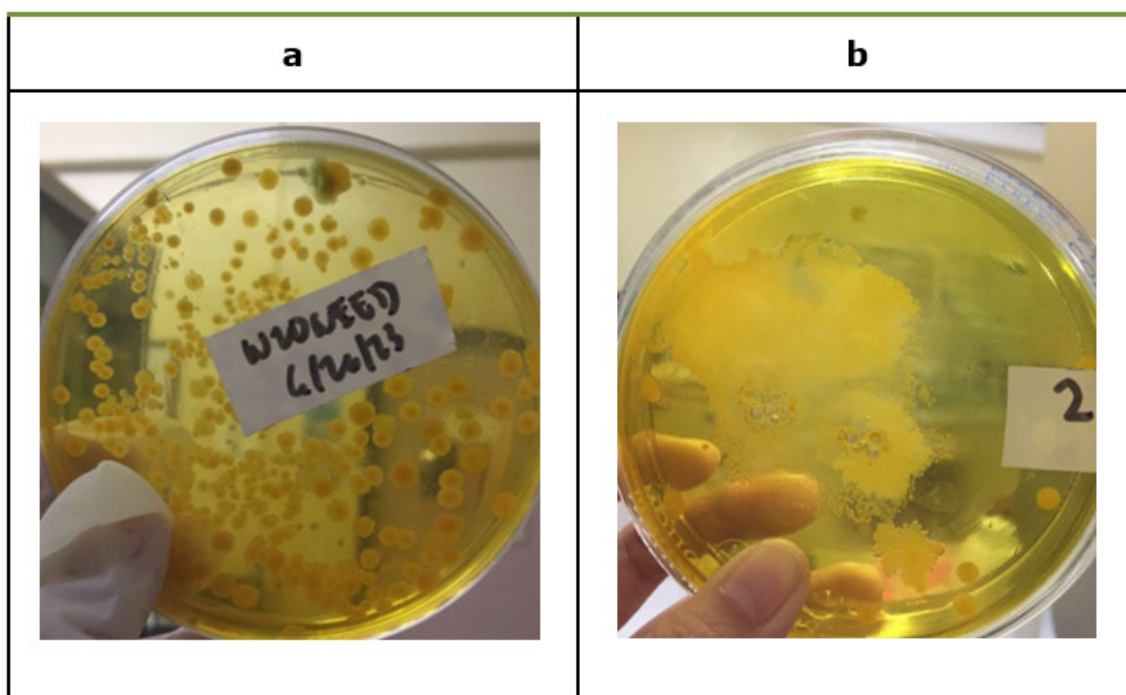


Figure 7. Microbiological analysis of water samples after *Vibrio* occurrence (a) 100% FMT (b) 5% reduction fish meat day⁻¹

Table 3. Initial and final weight and length of *A. marmorata* weaned at different rates after 16 days (FML to crumble).

Growth Parameter	Treatments			
	100% FML	10% reduction	25% reduction	100% crumble
Initial average weight (g)	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
Final average weight (g)	0.20 ± 0.00 ^d	0.23 ± 0.00 ^c	0.28 ± 0.00 ^a	0.25 ± 0.01 ^b
Initial average total length (mm)	52.10 ± 0.80	52.10 ± 0.80	52.10 ± 0.80	52.10 ± 0.80
Final average total length (mm)	53.51 ± 0.98 ^c	56.77 ± 0.53 ^b	60.19 ± 1.81 ^a	57.18 ± 0.33 ^b

Values are the means of the replicates ± SD (n=3). Values with different superscript letters are significantly different from each other (p<0.05).

The highest SGR was recorded in the treatment with a 25% daily reduction of FML, gradually substituted by the crumble diet. This was followed by the 100% crumble treatment and the 10% daily reduction of FML treatment. In contrast, the lowest SGR was observed in the 100% FML treatment (p<0.05).

The results for the %WG mirrored those observed for SGR. Elvers subjected to the treatment with 25% daily reduction of FML, gradually replaced by crumble, demonstrated the highest %WG at 41.80. This was followed by elvers fed with 100% crumble (%WG=24.61) and those with a 10% daily reduction of FML (%WG=15.51). Conversely, the lowest %WG was recorded in elvers fed 100% FML (2.70) (p<0.05).

Throughout the 16-day feeding trial, all treatments showed 100% survival, with no mortalities observed. Consistent with growth performance results, the best outcomes were observed in elvers subjected to a 25% daily reduction of FML, gradually replaced by crumble. This weaning scheme demonstrated the optimal growth performance,

making it the most effective approach for transitioning elvers from FML to crumble.

DISCUSSION

Anguilla eels remain a challenging species for aquaculture, primarily due to the complexities in their life cycle and dependency on wild stocks for seed supply.²³ The capture of glass eels during their onshore migration, and the subsequent feed weaning process are particularly critical and difficult stages of eel aquaculture.^{3,18} High mortality rates during the early weaning in the nursery culture are common, largely due to eels struggling to adjust to dietary changes.^{3,24} The results from this study demonstrate that a gradual weaning approach yields the best growth and survival outcomes for *A. marmorata* elvers in nursery culture.

In the first experiment, glass eels fed exclusively on *Artemia* nauplii exhibited the highest growth performance. This preference can be attributed to the diet's similarity to their natural food in the wild. The 6.25% daily reduction

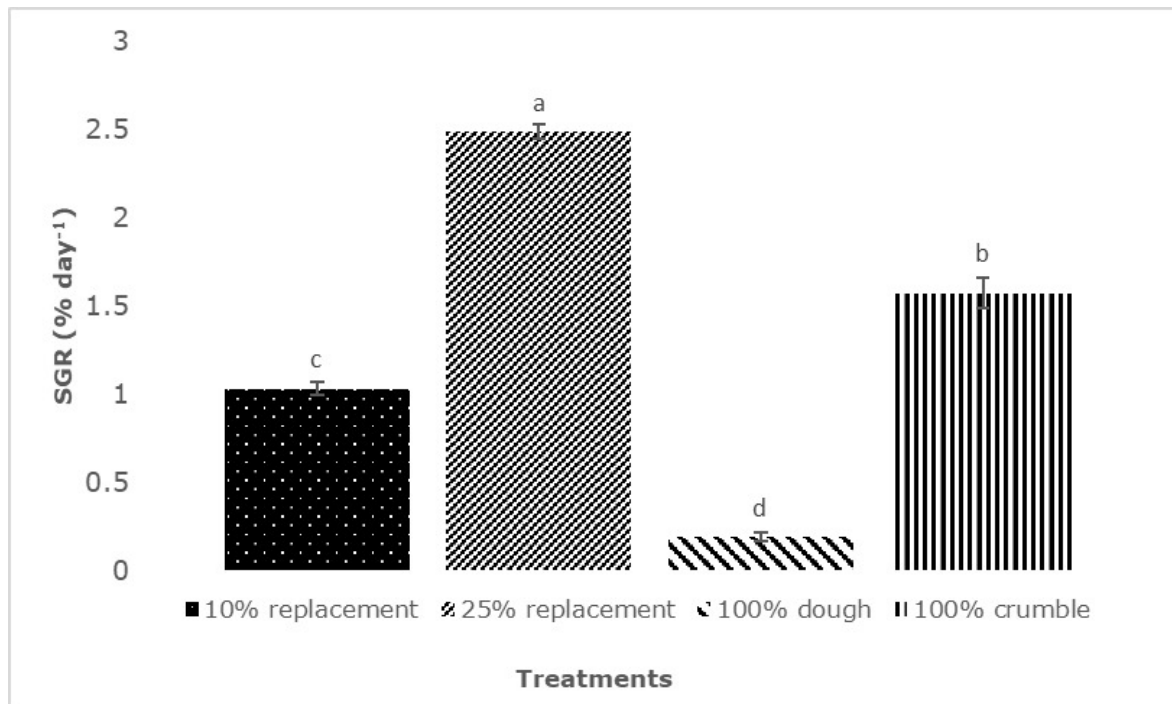


Figure 8. Specific growth rate of *A. marmorata* weaned at different rates after 16 days (FML to crumble).

Values are the means of the replicates $\pm$ SEM (n=3). Bars with different superscript letters are significantly different from each other ($p < 0.05$).

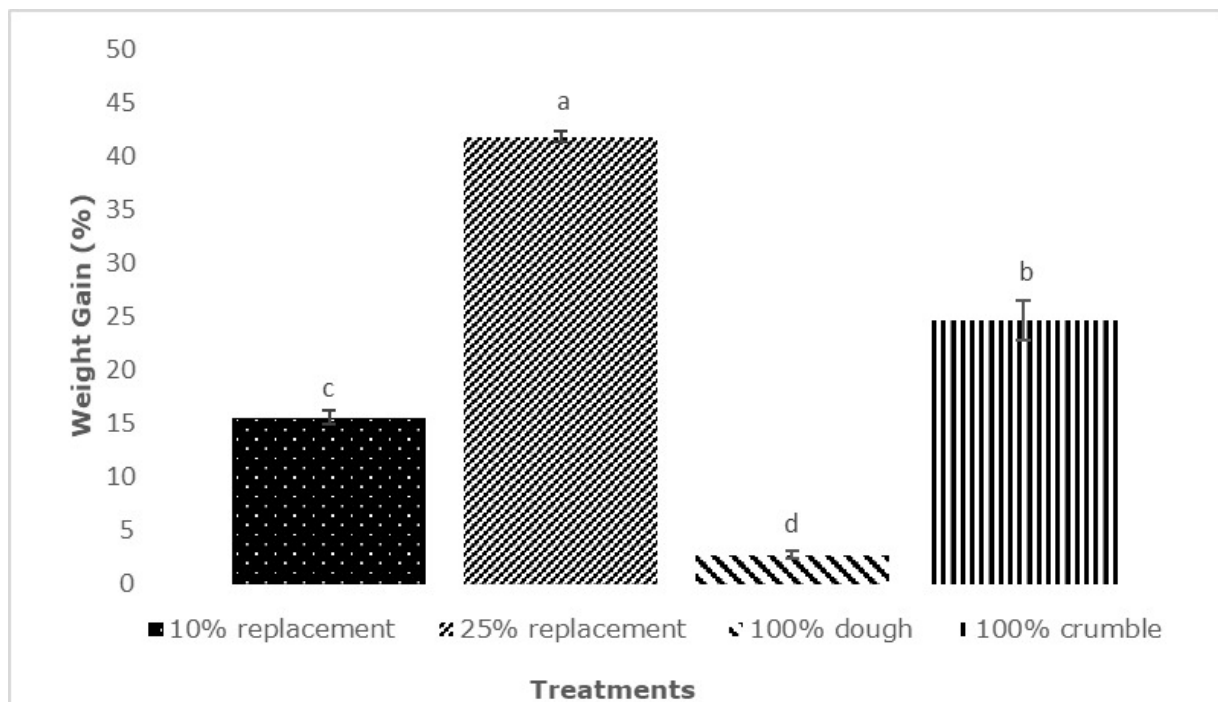


Figure 9. Percent weight gain of *A. marmorata* weaned at different rates after 16 days (FML to crumble).

Values are the means of the replicates $\pm$ SEM (n=3). Bars with different superscript letters are significantly different from each other ($p < 0.05$).

of live feed showed a similar growth response to the treatment with 100% live feed, indicating that a slower transition allows eels ample time to adjust to dietary changes. Conversely, faster weaning rates (12.5% and 25% daily reductions of live feed, and 100% FMT) were associated with poorer growth outcomes compared to the control group

that received live feeds. This may be due to the eel's difficulty to adapt quickly to the new diet, compounded by ongoing morphological changes during early development.^{3, 18,24} The low activity of digestive enzymes in fish during their early developmental stages may explain the poor growth performance of eels with rapid weaning. Aya et al.²⁵

observed that abrupt weaning of *Leiopotherapon plumbeus* larvae to an artificial diet from live feed resulted in significantly lower growth and survival rates compared to a gradual weaning strategy, suggesting that larvae were unable to effectively utilize the artificial diet during the early feeding stages. Additionally, rapid dietary changes can lead to decreased body weight, as eels may starve or down-regulate their digestive enzymes, resulting in mortality.²⁶ In fish larvae such as eels, *Pyrrhulina brevis*, and zebrafish, starvation can cause irreversible stress and increased mortality once a critical point is reached.^{27,28} Allowing sufficient time for diet transition is crucial to prevent such issues and ensure better growth and survival.^{3,18,26} Utilizing fish meat as a starter diet or as a main component in formulated diets has been documented to facilitate better and easier weaning of carnivorous fish species from live food to artificial diets.²⁹

In the second experiment, weaning glass eels from raw fish meat-based mash (FMT) to dry fish meal-based mash (FML) with gradual reduction of 5% and 10% fish meat per day resulted in superior growth performance as compared to the other treatments. These results align with the earlier findings by Degani and Levanon,³⁰ who suggested that incorporating raw meat into artificial feeds could improve initial growth performance. The highest SGR and %WG were observed in eels fed with 5% daily reduction of FMT, followed by a 10% reduction. This trend emphasized the importance of gradual weaning to artificial diets. Studies have consistently shown that longer weaning periods result in better growth and survival compared to abrupt transitions.³¹⁻³³ Gradual weaning allows eels to acclimate their metabolic and physiological capacities to adapt to the new feed, enhancing their feeding response and growth.

The lowest survival rate observed in eels fed solely with FMT may be attributed to bacterial infections present in the raw fish. Microbiological analysis revealed *Vibrio* species (*V. harveyi*, *V. rotiferanus*, and *V. vulnificus*) in tanks with 100% FMT, suggesting that raw fish can be a vector for pathogens despite pre-freezing.³⁴ *Vibrio* spp. are common in raw fish and warm saline environments,³⁵ and their presence in the tanks likely contributed to the observed mortalities. *Vibrio vulnificus*, in particular, is known for causing vibriosis and septicemia in eels, with symptoms including red spots, loss of appetite, and inflamed anus.^{36,37}

In contrast to the previous experiments, the third trial demonstrated that a faster weaning rate (25% daily reduction of FML) was optimal for transitioning to crumble diets. This is likely due to the advantages of early introduction to solid diets, which enhances growth performance. Dry feeds have been shown to improve growth compared to moist feeds,³⁸ and early adaptation to dry feeds facilitates easier weaning.^{39,40} Thus, 25% daily reduction of FML as a weaning scheme for transitioning from FML to crumble promotes good survival and higher growth performance for *A. marmorata* elver.

In summary, this study presents effective weaning protocols for *A. marmorata* at the nursery stage, transitioning from live feed to FMT, FMT to FML, and FML to crumble diet. The optimal schemes are: 6.25% reduction of live feed daily until reaching 100% FMT (16 days); 5% daily reduc-

tion of FMT, substituted with FML (20 days); and 25% daily reduction of FML, substituted with crumble diet (4 days). These protocols enable a successful transition from live feed to artificial crumble diets over a total of 40 days, promoting better growth, survival, and production in the nursery culture of *A. marmorata*.

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AUTHORS' CONTRIBUTION

Conceptualization: Fredson H. Huervana (Equal), Rex Ferdinand M. Traifalgar (Equal). Methodology: Fredson H. Huervana (Equal), Rex Ferdinand M. Traifalgar (Equal). Writing – review & editing: Fredson H. Huervana (Equal), Rex Ferdinand M. Traifalgar (Equal). Funding acquisition: Fredson H. Huervana (Lead). Supervision: Fredson H. Huervana. Formal Analysis: Kelee Ira B. Nodque (Equal), Cedric Jay A. Nantong (Equal), Richael P. Vargas (Equal), Rizza Mae T. Guyapale (Equal). Investigation: Kelee Ira B. Nodque (Equal), Cedric Jay A. Nantong (Equal), Richael P. Vargas (Equal), Rizza Mae T. Guyapale (Equal). Writing – original draft: Kelee Ira B. Nodque (Equal), Cedric Jay A. Nantong (Equal). Software: Kelee Ira B. Nodque (Equal), Cedric Jay A. Nantong (Equal). Data curation: Kelee Ira B. Nodque (Equal), Cedric Jay A. Nantong (Equal). Visualization: Kelee Ira B. Nodque (Equal), Cedric Jay A. Nantong (Equal), Richael P. Vargas (Equal), Rizza Mae T. Guyapale (Equal).

COMPETING OF INTEREST – COPE

No competing interests were disclosed.

ETHICAL CONDUCT APPROVAL – IACUC

Ethical review and approval were not required for the animal study because this study adheres to the Philippine National Standard (PNS) on the Code of Good Aquaculture Practices (GAQP) for nursery fish culture (BAFS, 2017). Protocols on rearing, handling, and animal welfare were strictly followed based on the guidelines stipulated in Philippine Republic Act Number 8485, known as the Animal Welfare Act of 1998.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

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