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The effect of *Artemia* nauplii (*Artemia franciscana*) enriched with different commercial products on the growth performance of the larvae of freshwater angelfish, *Pterophyllum scalare* (Lichtenstein, 1823)

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Abstract

The present study was planned to determine an optimum live-feeding protocol for angelfish larvae (*Pterophyllum scalare*). Larvae with an initial weight of 0.10 mg, a length of 4.5 mm, and a depth of 1 mm were reared on three different feeding regimens for 30 days. All experimental larval groups were fed *Artemia* nauplii from the end of the yolk-sac resorption. The control treatment (Group I) was maintained on *Artemia* nauplii on days 14-28, and on *Artemia* nauplii + dry feed on days 29 and 30. Group II was reared with *Artemia* nauplii enriched with Algamac 3050 on days 14-28 and with dry feed + *Artemia* nauplii enriched with Algamac 3050 on days 29 and 30, whereas Group III was fed with *Artemia* nauplii enriched with Red Pepper on days 14-28, and with dry food + Artemia nauplii enriched with Red Pepper on days 29 and 30. The highest weight (37.8±0.51 mg) and length (15.8±0.35 mm) were determined in Group II with significant differences from the control group ($p<0.05$). However, enrichment treatments were comparable in terms of growth performance ($p>0.05$). The survival rate of the larvae in the treatments varied between 70-75% without significant differences ($p>0.05$). Overall, the study results suggest that a feeding protocol for angelfish larvae with the administration of *Artemia* nauplii during the first two weeks after hatching and then enriched *Artemia* with Algamac 3050 over the following 14-28 days followed by a gradual weaning onto dry feed.

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Introduction

There is an increasing trend in aquarium fish demand, particularly for freshwater species by the hobbyists in the world (Jones et al. 2021). Angelfish (*Pterophyllum scalare*) is one of the most popular freshwater species worldwide among ornamental fishes. Its commercial value is due to body form, shape, colour, and ability to tolerate various environmental conditions (Herath and Atapaththu 2013, Azimirad et al. 2016). However, low survival rates and poor growth are experienced in their aquaculture probably because of malnutrition of the fish, particularly during the larval stage (Valente et al. 2013, Cooke 2016). Larval rearing methods have yet to be established for angelfish. One problem with their larval culture is related to the administration of suitable food to their mouth opening and nutrient requirements (Çelik et al. 2014, Patra and Ghosh 2015). In former studies, the angelfish larvae have been fed with live feed organisms such as rotifer, *Artemia*, and cladoceran (Farhadian et al. 2014). The use of live foods has also been attracted attention in broodstock feeding to obtain high-quality sperm and eggs and larvae in many aquarium fish including angelfish, discus (*Symphysodon aequifasciatus*) and beta (*Betta splendens*) (Khanjani 2021).

The use of *Artemia* eggs during the larval rearing stage of ornamental fish has been the case since the late 1940s. Artemia has many advantages for utilization in aquarium fish aquaculture, such as high nutritional value, accessibility to the form of eggs or hatched forms with enormously changing sizes that can be fed to every development stage from larvae to broodstock (Das et al. 2012). In contrast to favorable features and its use in larval fish rearing, some shortcomings of *Artemia* must be improved, especially in terms of meeting the essential nutrient requirements of fish larvae. Therefore, it has been reported that live feeds should be enriched with some synthetic substances, such as essential fatty acids and amino acids, according to the requirements of the species (Fernández-Reiriz et al. 1993). *Artemia* is almost completely devoid of essential fatty acids (Navarro et al. 1992). Therefore, it should be enriched to provide higher levels of essential fatty acids, Eicosapentaenoic acid (EPA, 20:5n−3) and Docosahexaenoic acid (DHA, 22:6 n−3) for better growth and survival of fish larvae (Smith et al. 2002, Zakeri et al. 2011, Navarro et al. 2014, Kandathil Radhakrishnan et al. 2020, Gümüş et al. 2022). Commercial products such as Culture Selco, Protein Selco, Red Pepper, Algamac 3050, or custom-made emulsions are widely used to enrich *Artemia* in fish hatcheries (Kotani et al. 2016, Campoverde and Estevez 2017, Eryalçın 2018, Ahmadi et al. 2019).

There are comprehensive studies on zooplankton related to the feeding behavior of fish larvae (Nandini and Sarma 2000, Sarma et al. 2003, Graeb et al. 2004). Yet, the studies on the feeding behaviour and growth performance of angelfish (*P. scalare*) larvae are limited. Considering that *Artemia* should be enriched with essential fatty acids before being used as food for larvae (Samat et al. 2020, Madkour et al. 2022), in the present study, two commercial products (Algamac 3050 and Red Pepper) consisting of high EPA and DHA contents were selected as enrichment materials of *Artemia* nauplii to determine the growth parameters and survival rates of freshwater angelfish larvae to fill the information gap.

Materials and Methods

The study was carried out in the Live Feed Laboratory of Egirdir Faculty of Fisheries (EFF), Isparta University of Applied Sciences, Isparta, Turkey.

A pair of male and female brood fish in the Aquarium Unit of EFF were stocked in a separate glass aquarium (70x40x25 cm) for breeding. Following the mating, the brood fish were taken from the aquarium, and the hatched larvae were used as experimental fish after three days. The hatched larvae average live weight, length, and depth were 0.10 mg, 4.5 mm, and 1.0 mm, respectively. 180 angelfish larvae were randomly distributed to nine aquariums (19x23x34 cm) (20 individuals in each aquarium). In the study, the treatments were tested in triplicated tanks. Each experimental aquarium was filled with 14 L of dechlorinated water. Over the experimental duration, fish excrements and uneaten feeds were siphoned out every morning before feeding, and the water discharged was renewed with dechlorinated water. The angelfish larvae were abundantly fed twice daily in the mornings and evenings. Over the experiment period, the average water temperature, oxygen, and pH were 27±1 ºC, 7.05±0.07 mg L-1, and 7.4±0.3, respectively.

Artemia (*Artemia franciscana*) used in the experiment was obtained from Inve Aquaculture (Belgium). Algamac 3050 (Aquafauna Bio-Marine Inc., Hawthorne, CA, USA) in the form of microparticle and Red Pepper (Bernaqua NV, Belgium) in the form of emulsion were used as enrichment supplements. The dry powdered feed used in the experiment contained 47.5% crude protein, 6.5% crude fat, 10.5% crude ash, 2% crude fiber, and 6.0% moisture (Tetra Discus, GmbH Company, Germany). The nutritional contents of the commercial products used in the study are given in **Table 1**.

Table 1 Nutritional content of the products used in the study (Anonymous 2022a, b, c)

Nutrient	Algamac 3050	Red Pepper	Dry Feed
Moisture (%)	2.1	68	6.0
Protein (%)	17.6 (18.0)*	6.5 (20.3)*	47.5
Lipids (%)	56.2 (57.4)*	14.0 (43.8)*	6.5
Ash (%)	8.2 (8.38)*	3.0 (9.39)*	10.5
Fibre (%)	-	1.7 (5.32)*	2.0
DHA (%)	43.27	5.5	-
EPA (%)	2.88	0.5	-
ARA (%)	-	0.1	-
DHA/EPA	15.0	11.0	-

*Values in parenthesis are based on dry-matter basis.

The *Artemia* culture water was prepared using the Instant Ocean brand sea salt. The production of *Artemia* that underwent decapsulation was carried out at 30% salinity, 25±1 ºC water temperature, under 2000 lux light, and aeration. After their production, the *Artemia* was enriched with either Algamac 3050 (0.2 g/l for 100,000 nauplii) or Red Pepper (0.75 g/l for 500,000 nauplii) for 12 hours, following the manufacturers' instructions. In the enrichment process, 5 L glass containers were used. After the enrichment process, *Artemia* was harvested using a plankton net with a 150-micron mesh size. The experimental groups, according to the feeding regimens applied to the angelfish larvae, are shown in **Table 2**.

Table 2 Experimental feeding regimens for the angelfish larvae

Days	Group I	Group II	Group III
1 -3 days	No feeding	No feeding	No feeding
4 -14 days	Artemia nauplii	Artemia nauplii	Artemia nauplii
15 -28 days	Artemia nauplii	Artemia nauplii enriched with Algamac 3050	Artemia nauplii enriched with Red Pepper
29 -30 days	Dry feed + Artemia nauplii	Dry feed + Artemia nauplii supplemented with Algamac 3050	Dry feed + Artemia nauplii supplemented with red pepper

A precision balance was used to measure the treatments' larval weights at the study's beginning and end. A ruler was used for the total length measurement, whereas a caliper was used for the measurement of body depth.

Growth parameters in the experiment were determined using the following formulas.

Specific growth by weight or length = (Ln (Wt or Lt - Ln Wi or Li /t) x 100

Wt and Lt: Average absolute weight (mg) and length (mm) at the end of the trial

Wi and Li: Average absolute initial weight (mg) or length (mm)

t: Measurement period

Ln: Calculated according to the logarithm to based (Ricker 1979).

The thermal growth coefficient (TGC) is calculated according to Jobling 2003.
TGC (mg1/3/°C Day) = [(3√Wt) –(3√Wi) / (t x T)] x 1000
where the abbreviations are given above.
T: Water temperature

Survival ratio (%): (Nt/Nt-1) x 100
Nt = The number of fish at the end of the trial
Nt-1= The number of fish at the beginning of the trial

Statistical analysis

All of the statistical analyses of the findings from the treatments were carried out using the SPSS 21.00 software program 3 (SPSS Inc., Chicago, IL, USA). The data are expressed as mean ± standard deviation (SD). Before the one-way variance analysis, the data's normality and homogeneity of variances were confirmed with the Shapiro-Wilk and Levene tests, respectively. Tukey's multiple comparison test was used to discriminate the significantly different treatments. A significant level of p=0.05 was considered.

Results

The present study investigated the effects of the feeding regimens starting from the first feeding period to 30 days after hatching in angelfish. The average final weight, length, depth, and survival rates of the treatments are displayed in **Table 3**.

Table 3 Growth performance and survival rate of angelfish larvae

	GROUP I	GROUP II	GROUP III
Weight (mg)	36.1±0.88^{b}	37.8±0.51^{a}	37.1±0.39^{ab}
Length (mm)	14.9±0.39^{b}	15.8±0.35^{a}	15.6±0.23^{ab}
Depth (mm)	4.88±0.07	4.91±0.07	4.9±0.06
SGR_W (%)	4.26±0.60^{b}	4.43±0.62^{a}	4.36±0.62^{ab}
SGR_L (%)	3.99±0.39^{b}	4.18±0.07^{a}	4.14±0.23^{ab}
Survival ratio (%)	70.0±7.00	75.0±2.50	75.0±5.00
TGC (mg1/3/°C day)	3.51±0.09^{b}	4.14±0.06^{a}	4.12±0.07^{ab}

* The values with different superscripts in the same row are statistically significant (p<0.05).

The specific growth rates of weight and length are given in **Figures 1 and 2**. At the end of the experiment, the control group reached a weight of 36.1±0.88 mg, a length of 14.9±0.39 mm, and a depth of 4.88±0.07 mm. The best growth performance in the study was determined in Group II with values of 37.8±0.51 mg weight, 15.8±0.35 mm length, and 4.91±0.07 mm depth, significantly different from Group I in terms of weight and length. The larvae in Group III showed numerically better growth variables than the control but without significant differences from Group I and Group II (p>0.05).

The survival rates at the end of the study ranged between 70.0±7.00% in the control group and 75.0±5.00% in Groups II and III, but the treatment differences were not scientifically significant.

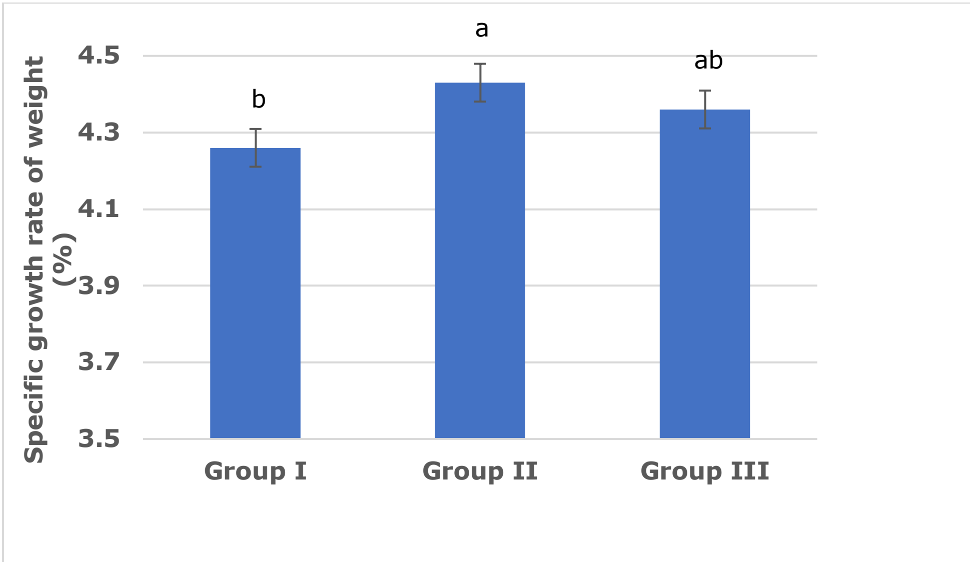

Figure 1 The specific growth rate of weight at the end of the trial (%)

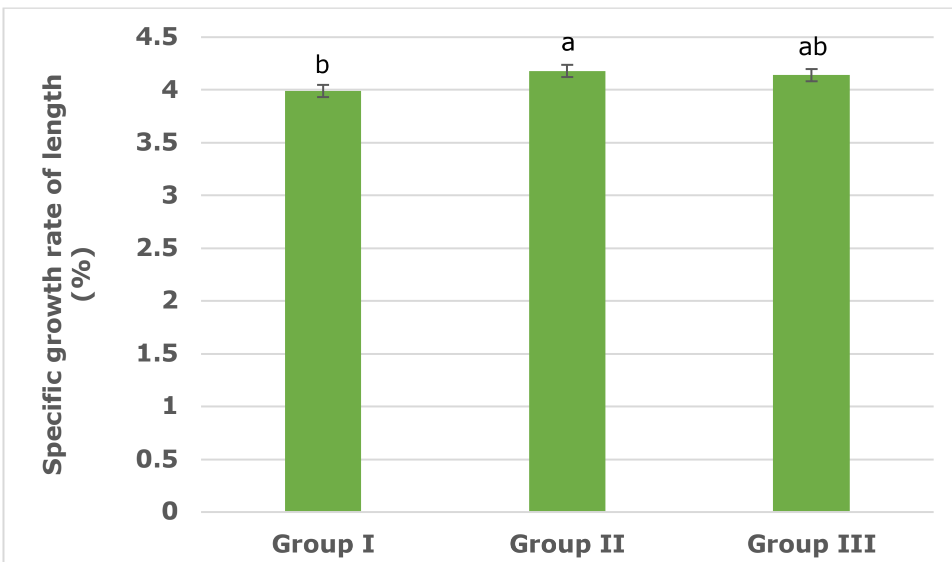

Figure 2 The specific growth rate of length at the end of the trial (%)

Discussion

Zooplanktonic organisms such as rotifers and *Artemia* are commonly used to feed fish larvae. Although extensive studies have been carried out on this subject in marine larval fish, limited information is available on angelfish larvae. (Ortega-Salas et al. 2009, Farahi et al. 2011, Herath and Atapaththu 2013, Patra and Ghosh 2015, Pereira et al. 2016). In the present study, angelfish larvae were fed live feed with or without enrichment to investigate whether an enrichment process is required.

Previous studies on *Heterobranchus longifillis* (Kerdchuen & Legendre 1994), haddock (*Melanogrammus aeglefinus*) (Blair et al. 2003), southern flounder, zebrafish *Danio rerio*

(Carvalho et al. 2006), *Paralichthys lethostigma* (Faulk and Holt 2009), Whishker catfish (*Macronema bleekeri*) (Dan 2008) have reported that the larvae fed with solely live feed exhibited relatively higher growth performances than those fed with solely dry feed. This was attributed to the fact that larvae do not have well-developed digestive systems with functional digestive enzymes at the beginning of the feeding. Exogenous enzymes existing in live feeds could play a supportive role in digestion (Person 1989). It was observed that fish larvae consumed *Artemia* nauplii faster than dry feed, and a few minutes after consumption of *Artemia* by larvae, they can be easily distinguished in the larval digestive system thanks to their orange color (Herath and Atapaththu 2013). A lower consumption rate of dry diets was shown as the influencing factor behind the lower growth performance of fish larvae (Muguet et al. 2011). Also, the physical properties of dry diets, which are not appropriate for the early larval stage, have been shown as factors for lower growth performance (Sarkar et al. 2006).

Former studies showed that the growth performance of angelfish larvae was not affected by varying feeding frequencies (Ribeiro et al. 2012). Further proof was provided by Kasiri et al. (2012), who found a comparable growth performance from angelfish larvae fed either twice or four times a day. Therefore, in the present study, feeding twice daily was performed as feeding frequency.

The survival rates of angelfish larvae in the present study were between 70 and 75.0%, within the ranges of reported values in the literature. For instance, Ortega-Salas et al. (2009) have reported the survival rate of angelfish larvae fed with *Artemia* nauplii as 66.25%. Farahi et al. (2011) fed angelfish larvae with non-supplemented *Artemia* nauplii or enriched with probiotic bacteria and found the highest survival rate of 60% in the probiotic-supplemented group. Herath and Atapaththu (2013) fed angelfish larvae with only *Artemia* nauplii or dry feed and found survival rates between 51 and 73%. Patra and Ghosh (2015) fed angelfish larvae with *Artemia*, rotifer, moina, and ceriodaphnia and reported the highest survival rate, 74.67%, in larvae fed with *Artemia* nauplii. Lipscomb et al. (2020) fed angelfish larvae with *Artemia* and reported a survival rate of 62.7%. Briefly, the survival rates of the present study are quite acceptable for larval rearing in angelfish when the best survival rates of the literature are considered.

The present study showed that feeding angelfish larvae fed Artemia enriched with Algamac-3050 significantly increased the growth performance compared with those fed unenriched *Artemia*. Enrichment with Red Pepper, however, resulted in numerically better values in terms of growth without significant differences from the control. The better performance by Algamac-3050 may be due to its higher amounts of EPA, DHA, and lipid than Red Pepper (**Table 1**), suggesting that the use of *Artemia* enriched with a suitable nutrient composition for freshwater angelfish larvae is highly important. The fatty acid contents (%) of *Artemia* enriched with Algamac 3050 for 12 hours increased ARA from 0.6 to 2.8, EPA from 1.7 to 5.6, DHA from 0.1 to 16.5, SFA from 16.0 to 19.8 and PUFA from 35.0 to 51.8, respectively (Ritar et al. 2004). Likewise, enrichment with Red Pepper for 12 hours elevated ARA to 2.4, EPA to 5.4, DHA to 7.0, SFA to 23.2, and PUFA to 47.5 (Campoverde and Estevez 2017). Previous studies on Murray cod (*Maccullochella peelii peelii*) and seahorse (*Hippocampus abdominalis*) larvae found that Algamac 3050 performed best in terms of growth performance among several *Artemia* enrichment products (Woods 2003; Francis et al. 2019), which is consistent with our study. However, meagre (*Argyrosomus regius*) and sterlet (*Acipenser ruthenus*) larvae showed higher growth performance when fed Red Pepper enriched *Artemia* (Campoverde and Estevez 2017; Lundova et al. 2018).

The growth data regarding the specific growth rate of total length at the end of the trial are compared with those reported by the previous studies (**Table 4**). Although several experimental factors should be considered, including the diets used, water temperature, and experiment duration, an attempt to compare the SGLW, SGRL, and TGC results of the present study with those published in the literature was made to understand better the efficiency of feeding protocol and enrichment products in the present study. All growth variables of the present study and survival rate are consistent with the higher values reported by the studies (for instance, Ortega-Salas et al. 2009, Herath and Atapaththu 2013) (**Table 4**), suggesting that both experimental condition and feeding protocols in this experiment are appropriate for angelfish larvae.

Table 4 Comparison of the best growth performance of the present study with previous studies on angelfish larvae in terms of weight and length

Reference	**Duration of experiment (day)**	**Water temperature (°C)**	**Initial weight (mg)**	**Initial length (mm)**	**Calculated SGR$_W$ (%)**	**Calculated SGR$_L$ (%)**	**Calculated TGC ($mg^{1/3}$/°C day)**	**Survival rate in best performing treatment (%)**
Ortega-Salas **et al.** (2009)	135	24-30*	Nd	Nd	4,36	1,96	4,09	66,25
Farahi **et al.** (2011)	20	26	Nd	Nd	Nd	3,47	Nd	60,00
Herath and Atapaththu, (2013)	28	Nd	0,1	4,5	5,03	4,15	4,24	70,00
Patra and Ghosh, (2015)	20	26-29	Nd	Nd	Nd	4,47	Nd	74,67
Lipscomb **et al.** (2020)	14	Nd	Nd	4,65	Nd	1,82	Nd	62,7
Present study	30	30	0,1	4,5	4,43	4,19	3,86	75,00

Nd: Not detected. When the authors did not report both initial weight and length, the initial mean weight or length values of the present were considered.
SGR$_W$: Specific growth rate by weight.
SGR$_L$: Specific growth rate by length.
TGC: Thermal growth coefficient.
* Average temperature was assumed as 27°C.

The type, nutrient content, and size of the live fish feed used in the larval stage of angelfish can affect the growth and survival rate of the larvae. Considering the limited number of studies conducted in the larval period of angelfish, the results of the present study are very important for larval angelfish larval rearing. Angelfish larvae fed for 14 to 30 days with *Artemia* nauplii enriched with Algamac 3050 or Red Pepper yielded a similar growth performance. Still, only larvae on Algamac 3050 were significantly higher than those on the control. Therefore, the results suggest a feeding protocol for angelfish larvae with the administration of *Artemia* nauplii during the first two weeks after hatching and then enriched *Artemia* with Algamac 3050 over the following 14-28 days followed by gradual weaning onto dry feed. Future studies on rearing angelfish larvae should be focused on the influence of optimum duration of feeding period with enriched *Artemia* and weaning protocols that can yield better growth and survival.

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