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Original Research Articles

Growth and nutritional health of *Pterophyllum scalare* juveniles: Unleashing the benefits of feeding with *Artemia* sp. in aquariums

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Pterophyllum scalare is a popular ornamental fish species, but current rearing methods result in high mortality, low fertility, disease incidence, and slow growth in aquarium conditions. Research on co-feeding for ornamental fish at the juvenile or adult stage needs to be completed. This study implemented a "snacking" feeding strategy using *Artemia* sp. to evaluate changes in fish survival rate, growth performance, and enzyme activity related to digestion, antioxidants, and immunity in a recirculating aquaculture system. Two feeding strategies were tested: one group was fed a commercial diet plus *Artemia* sp. (0.1% of diet) as "snacking," and another group was fed only the commercial diet as normal. "Snacking" with Artemia sp. enhanced fish's survival and growth performance, with higher relative weight rate, standard length, and total length than the control group. Specific growth rates for weight, standard length, and total length were also more significant in the supplemented group compared to the control group. The activity of amylase, superoxide dismutase, and glutathione peroxidase was substantially higher in the supplemented group. Adding *Artemia* sp. as a supplement under a "snacking" feeding strategy to the control diet was beneficial for *P. scalare* juvenile rearing and can guide managing recirculating farming activities.

INTRODUCTION

The ornamental fish market is an essential segment of global aquaculture. As this industry continues to expand, the aquaculture industry has become interesting to explore.[1] The angelfish (*Pterophyllum scalare*), originally from the Amazon River in South America, is one of the most important Teleostei species for the ornamental fish trade and is well-known for its morphological diversity and color patterns.[2] Its further expansion and the consequent supply of markets primarily depend on the degree of intensification of the production systems used in the cultivation and the development of appropriate and new technologies to meet the market requirements. Angelfish feed on algae, insects, micro crustaceans, and worms. *Artemia* sp. is one of the most functional feeds for fish and can be used as a feed source to meet the feeding requirements of new species aquaculture. *Artemia* sp. is also a source of essential nutrients and enzymes and can be enriched with PUFA, vitamins, elements, and probiotics.[3] Among new strategies, enriched *Artemia* sp. with probiotics or synbiotics has also shown positive results in productive performance, survival, improvement of the immunological system, high-stress resistance, and intestinal modulation in angelfish.[4] Since the 1980s, research on prey plus inert diets (co-feeding) has mainly focused on the early larval stages of fish. These co-feeding trials were carried out to determine the extent that fish larvae would accept, digest and tolerate inert diets to progress toward the complete replacement of natural diets. In *P. scalare* fry, the effect of dietary inclusion of *Artemia* sp. nauplii resulted in higher length and weight values in fish fed with a combination of *Artemia* sp. than the commercial diet alone.[5] Also, with limited success, P. scalare lar-

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vae have been tested with abrupt weaning involving a direct transition from Artemia sp. to inert food.[6]

There is sufficient evidence supporting that *Artemia* sp. co-feeding benefits the fish larvae' biological performance. However, most research focuses on the fish's early stages. Information regarding the co-feeding of ornamental fish at the juvenile or adult stage still needs to be made available. Feeding *P. scalare* broodstock with *Artemia urmiana* shortened the spawning cycle and improved fecundity, fertilization, hatching and larval survival.[7] More recently, adult *Artemia franciscana* 50% enriched with soybean lecithin in combination with an inert diet positively affected digestive enzyme activity and reproductive performance in *Aequidens rivulatus* (green terror cichlid).[8] Due to the increased efficiency, the feasibility of co-feeding diets in broodstock farms is evident, despite the associated higher feeding costs. Developing a feeding strategy that provides suitable nutrition for fish without increasing costs is essential in this context, as it applies to both hatcheries and aquaria where ornamental fish are kept.

This study aimed to assess several physical and physiological changes in angelfish juveniles, such as survival rate, growth performance, and digestive, antioxidant, and immunological enzymatic activity, following a new feeding strategy, "snacking," in an aquarium's condition. One group of fish was fed a formulated diet alone, while another group was given the same diet supplemented with a small proportion (0.1%) of *Artemia* sp. The findings of this study could be beneficial for managing ornamental fish in aquariums' condition systems.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND MANAGEMENT

The angelfish *P. scalare* juveniles from the same batch of siblings were obtained from Hainan Nankun Marine Biotechnology Co., LTD (Hainan, China). Before the experiment, the juveniles were temporarily cultured in conditioned glass aquaria for seven days, with light: darkness (16 h: 8h). The indoor aquaria were continuously supplied with recirculating water, properly filtered with a wadding cartridge and activated carbon. The oxygen was maintained through an air pump in each aquarium. The average daily room temperature of the laboratory was 27.0 ± 1.0 °C. The glass aquaria were siphoned daily for cleaning and removal of feces. The conditions and management were the same as mentioned above during the experiment, both for the control and experimental groups. The values of the water physicochemical parameters for the recirculating system were recorded every two days for homogeneity. The details were: DO 7-8.2 mg/L, pH 6.80 to 7.01, total ammonia-nitrogen 0.1 to 0.2 mg/L, nitrate 2.0 to 7.0 mg/L, hardness 4 to 5 dGH, and the nitrite-nitrogen concentration cannot be detected. During acclimatization, fish were fed twice daily (Color Red & Blue Discus series, TZU-Feng Aquaculture Supplies CO., LTD., the composition was as follows: 460 g/kg crude protein, 120 g/kg crude fat, 30 g/kg crude fiber, 160 g/kg crude ash, 40 g/kg calcium and 30 g/kg phosphorus). Daily feeding was proportioned to represent 6-8% of fish body weight. Food was weighed, and each glass aquaria was fed a standard amount.

EXPERIMENTAL DESIGN AND SAMPLING

After 7-days of adaptation to laboratory conditions, juveniles (592.12 ± 18.20 mg in mean initial weight, 16.69 ± 1.50 mm in mean initial total length, 14.00 ± 1.32 mm in mean initial standard length) were randomly distributed in tanks for the control and the experimental groups. Tanks (600 L of practical volume) containing 50 juveniles were used for each group in triplicates, according to different feeding strategies.

Juveniles were fed for a period of 30 days using two different feeding strategies, a group of fish fed with a commercially formulated diet plus *Artemia* sp. (0.1% to formulated diet), called supplemented group (SG), and another group was fed with only formulated diet, the control group (CG). The *Artemia* sp. nauplii supplement (frozen) vitamin C- enhanced was supplied by Hainan Nankun Marine Biotechnology Co., LTD (Hainan, China), with the nutritional proportions: 7.6% crude protein, 0.8% crude fat, 0.35% crude fiber and 88.8% moisture. Fish were weighed bi-weekly to adjust the amount of formulated diet and *Artemia* sp. nauplii to be supplied. The frozen artemia was first thawed, weighed, and fed accordingly.

At the end of the experiment, fish were deprived of food for 12 h and euthanized with tricaine methanesulfonate (MS-222, Sigma-Aldrich, St. Louis, MO, USA, 0.5 ml/L), followed by dissecting fish on ice using sterile scissors to collect the tissue samples. Each fish's digestive organ tissues (intestines and stomach) and liver were carefully excised and preserved in a sterile centrifuge tube at -80 °C until further analysis. The experiments were conducted in the Laboratory of Tropical Fishery Animal Breeding and Culture at the Ethical Committee of the Biology Institute of Hainan University.

ANALYSIS OF PRODUCTIVE PERFORMANCE

The surviving fish in each group were counted to calculate the survival rate (SR). The SR was calculated following the equation:

$$SR = \left(\frac{\text{No. of live fish at the end of experiment}}{\text{No. of fish at the beginning of the experiment}} \right) x100$$

Total length (TL), standard length (SL), and weight were collected for each group at the beginning and the end of the feeding trial. The biometric data were measured using a digital vernier caliper (DL90150, Deli). The weight was measured using an electronic balance (ME104E, Mettler Toledo). The relative growth rate (RGR) and the specific growth rate (SGR) were calculated using the following equation:

$$RGR = \left(\frac{X_2\text{-}X_1}{X_1} \right) x\ 100$$

$$SGR = 100\ x\ \left(\frac{Ln\ X_2\text{-}Ln\ X_1}{t} \right)$$

where "X_1" and "X_2" are the average of weight (or TL, or SL) at the beginning and the end of the experiment, respectively, and "t" is the experimental days.

Furthermore, food conversion (FC) was evaluated using the following equation: weight gain = final weight - initial weight.

$$FC\left[\frac{g}{g}\right] = \frac{\text{Individual food consumption}\left[\frac{g}{\text{fish}}\right]}{\text{weight gain }[g]}$$

ENZYME ACTIVITY MEASUREMENT

A total of 30 samples of previously frozen tissue from each replicate were randomly selected to analyze. The tissues from the digestive organs were used to measure the activity of digestive enzymes and the liver for antioxidant and immune enzymes.

The frozen tissues were thawed, weighed, and homogenized for enzymatic assays using a mechanical homogenizer (PT1200C, Polytron) on ice in 0.2 M (w/v) of ice-cold physiological saline, and homogenates were centrifuged. Subsequently, each fish's aqueous supernatant was collected, incubated in the enzyme substrate, and read on a spectrophotometer (UV-1800BPC, LiuYi Biotechnology Co., Ltd, China) at a specific wavelength. The pepsin (PES, E.C.3.4.23.1), amylase (AMS, E.C.3.2.1.1), lipase (LPS, E.C.3.1.1.3), superoxide dismutase (SOD, E.C.1.15.1.1), catalase (CAT, E.C. 1.11.1.6), glutathione peroxidase (GSH-Px, E.C. 1.11.1.9) and lysozyme (LZM, E.C. 3.2.1.17), were used as biochemical indicators. The supernatant's protein content and enzymatic activity were measured and detected with commercial reagent kits according to the manufacturer's instructions. The AMS, PES, SOD, CAT, GSH-Px, and LZM activity was expressed as "U/mg protein", and the LPS activity was expressed as "U/g protein."

The BCA Protein Assay kit determined the soluble protein content in tissue homogenates. The digestion indicators for digestive and organ tissues included PES (No. A080-1-1), AMS (No. C016-1-1), LPS (No. A054-2-1), and the colorimetric method, starch-iodine colorimetric method, methyl halide substrate method (microplate method) were used for determination respectively. The commercial reagent kits used were produced by Nanjing Jiancheng Biological Co., Ltd. (Nanjing, China).

The antioxidant and immune indicators for the liver included SOD, CAT, GSH-Px, and LZM. The superoxide dismutase activity assay kit (Catalog, No. A001-1) was used for SOD activity measurement, determined by the xanthine oxidase method (hydroxylamine). The catalase activity assay kit (Catalog, No. A007-1) was used for CAT activity determined, and the glutathione peroxidase activity assay kit (Catalog, No. A005) for GSH-Px activity.

STATISTICAL ANALYSIS

The results were submitted to a *t-test* to detect significant differences at P values < 0.05. The DPS14.5 software (Hangzhou Rui Feng Information Technology Co. Ltd., Hangzhou, China) was used for all statistical analyses.

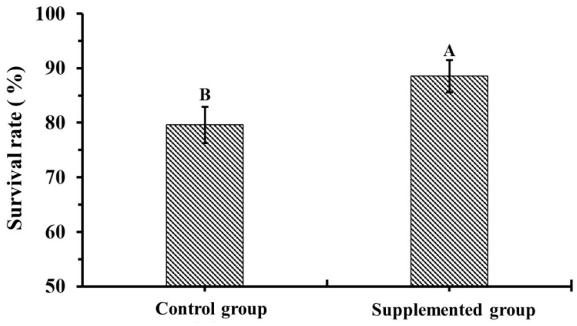

Figure 1. The survival rate of *Pterophyllum scalare* juveniles.

Different capital letters indicate a significant difference (t-test $P < 0.05$).

RESULTS

SURVIVAL RATE

The survival rates in the two fed strategies were more than 78%. The 88.53 ± 2.98% survival rate was observed in the *Artemia* sp. supplemented group, significantly higher than that in the control group (79.58 ± 3.32%, $P < 0.05$, **Figure 1**).

GROWTH AND PRODUCTIVE PERFORMANCES

The final weight, TL, and SL of *P. scalare* juveniles are shown in **Figure 2**, with the corresponding RGR and SRG. The final weight of *P. scalare* juveniles from the supplemented group was 6.14 ± 0.25 g, approximately gaining 937.34% of their initial weight in 30 days, significantly higher than that in the control group ($P < 0.05$, 5.64 ± 0.23 g, and 854.79%). Furthermore, the SGR of weight in the supplemented group was 7.80% / d, significantly higher than in the control group ($P < 0.05$, 7.52% / d). The TL and SL in the supplemented group were 57.06 ± 1.96 mm and 54.04 ± 1.21 mm, respectively, with correspondent values of 50.73 ± 1.87 mm and 46.21 ± 1.65 mm in the control group. They exhibited significance among the two feeding strategies ($P < 0.05$). The RGR of the TL and SL in the supplemented group were 243.36 ± 17.45% and 286.51 ± 24.57%, respectively, while the SGR was the 4.11 ± 0.16% / d and 4.50 ± 0.21% / d, respectively, significantly higher than that in the control group ($P < 0.05$).

Furthermore, the FC presented significantly lower values in the supplemented group (1.58 ± 0.12 g/g) compared to the control group (1.86 ± 0.10 g/g, **Figure 3**, $P < 0.05$).

DIGESTIVE ENZYME ACTIVITY

The AMS activity of *P. scalare* juveniles in the supplemented group was 2.79 ± 0.18 U/mg protein at the end of the experiment, significantly higher than that in the control group (1.96 ± 0.11 U/mg protein, $P < 0.05$). While the PES and LPS activities were without significant difference compared to the control group ($P > 0.05$), 29.27 ± 1.40 U/mg protein and 4.01 ± 0.19 U/mg protein, respectively ($P > 0.05$, **Figure 4**).

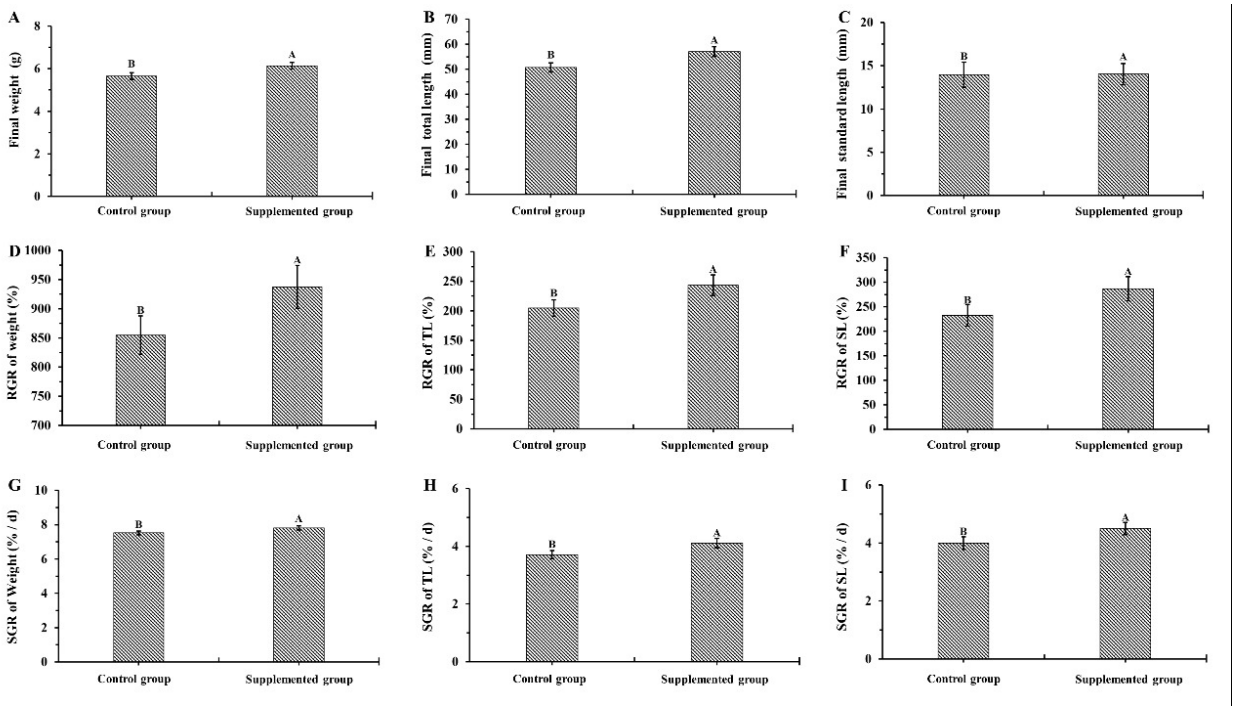

Figure 2. The growth performance of *Pterophyllum scalare* juveniles.

Different capital letters indicate a significant difference (t-test $P < 0.05$). TL = total length, SL = standard length, RGR = relative growth rate, SGR = specific growth rate.

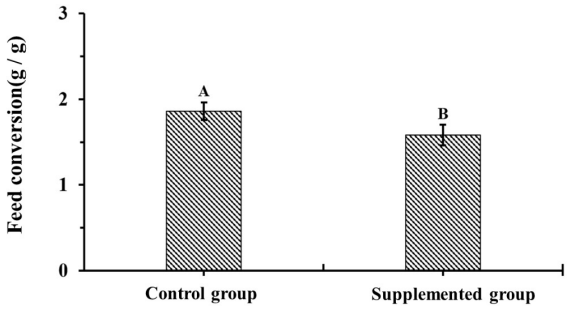

Figure 3. The feed conversion of *Pterophyllum scalare* juveniles.

Different capital letters indicate a significant difference (t-test $P < 0.05$).

ANTIOXIDANT AND IMMUNE ENZYME ACTIVITY

The SOD and GSH-Px activities in the supplemented group (35.20 ± 1.60 and 26.02 ± 1.02 U/mg protein, respectively) were significantly higher than in the control group. ($P < 0.05$, 31.19 ± 1.56, and 23.02 ± 0.82 U/mg protein, respectively). In comparison, the CAT (2.51 ± 0.13 U/mg protein) and LZM (2.29 ± 0.11 U/mg protein) activities were not significantly different compared to the control group ($P > 0.05$, Figure 5).

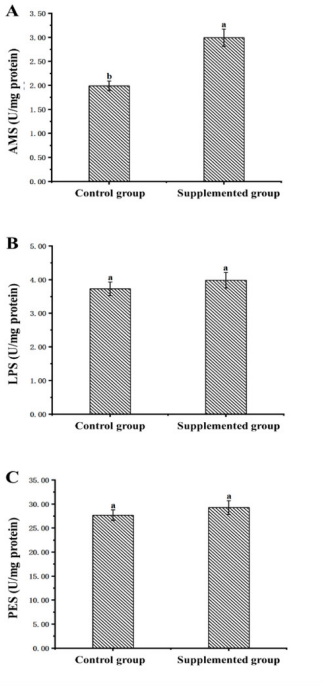

Figure 4. The digestive enzyme activities of *Pterophyllum scalare* juveniles.

Different lower-case letters indicate significant differences (t-test $P < 0.05$). (**A**) AMS activity, AMS = amylase; (**B**) LPS activity, LPS = lipase; (**C**) PES activity, PES = pepsin.

DISCUSSION

Angelfish are found in the catchment area of the Amazon River and live in waters that have a homogeneous, albeit specific, set of chemical and physical conditions (e.g., temperature 27-30°C; pH 6.5).[1] Therefore, the water physicochemical parameters in the recirculating system used in the present study were homogeneous among aquaria, where angelfish juveniles were kept and remained within the

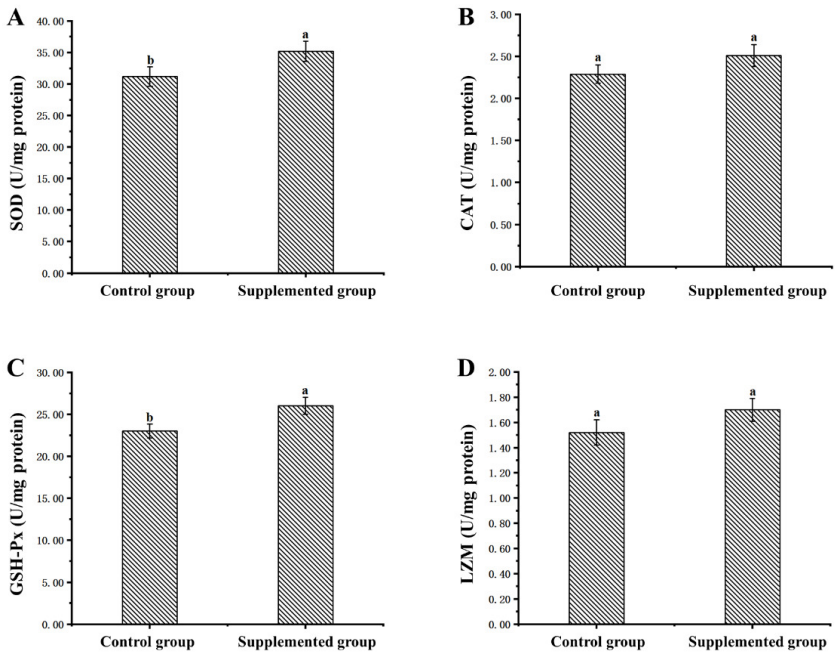

Figure 5. The antioxidant enzyme activities of *Pterophyllum scalare* juveniles.

Different lower-case letters indicate significant differences (t-test $P < 0.05$). (**A**) SOD activity, SOD = superoxide dismutase; (**B**) CAT activity, CAT = catalase; (**C**) GSH-Px activity, GSH-Px = glutathione peroxidase; (**D**) LZM activity, LZM = lysozyme.

ranges recommended for the culture of *P. scalare*. Such settings guaranteed the angelfish were living in a suitable rearing condition, with survival rates above 78% in all aquaria. Survival rate values in angelfish juvenile rearing reported by other studies vary. For instance, more than 96% survival rates were observed by Arévalo-Ibarra under different lipid and protein levels in the diet,[1] and more than 94% survival rates were observed by Azimirad, on fish feed only with *Artemia* sp.[4] The differences in survival rates between this study and those mentioned above may be attributed to the differences in the cultivation system and animal management. The recirculating water velocity in the system used in the present study was 1.2 t/h, while static aerated water conditions and water exchange were applied in the abovementioned studies. Thus, the possibility exists that a water flow may affect juvenile fish feeding behavior. However, despite the differences, the results obtained in this study indicated that *Artemia* sp. "snacking" improved the survival rate in *P. scalare* juveniles in comparison to fish that were only fed with formulated diet.

Additionally, the use of *Artemia* sp. in rearing angelfish juveniles enhances stress resistance and may be beneficial because it contains microelements, enzymes, or special nutrition components absent in formulated food.[4]

Growth performance is the main component influencing economic benefits in commercial aquaculture facilities.[9] Growth is also an ongoing process, subject to many positive and negative influences. Nutrition is one of the key factors for successful ornamental fish culturing.[10] When developing formulated diets for ornamental fish breeding and farming, it is important to consider several factors (e.g., nutrient composition, size, ease of storage and use, cost) without overlooking fish's foraging behavior when using this artificial supplement.[11] The live food from the natural conditions fetches more benefits to the fish's health since the artificial supplements may lack some nutrients present in the live food, especially during the fish's early developmental stages.[12] In case that formulated diet is fed to fish in captivity, it is preferably in combination with live food (e.g., *Moina*, *Daphnia*, *Artemia* sp. nauplii). Wild food is combined with hatchery-reared food or even a combination of different microorganisms to provide a rich source of individual fatty acids, supporting the specific nutritional requirements of marine fish larvae.[12] The rearing of marine fish larvae of the altricial type is particularly reliant on live feeds, as these larvae remain undeveloped until the yolk sac is spent. At the initial stage of feeding, the digestive system is not yet fully developed, as it does not have a stomach, and a large portion of the protein digestion takes place in the epithelial cells of the hindgut.[13] Larvae often cannot digest formulated diets and live feeds, hindering their survival and growth. Although progress has been made in developing inert diets, many important aquaculture fish species still rely on live feeds during early life stages.[12] Thus, it is not surprising that in this study, the group of *P. scalare* juveniles fed with formulated food and "snacked" Artemia sp. nauplii exhibited enhanced growth performance compared to the group that did not receive the *Artemia* sp.

Aquaculturists usually measure the growth of their specimens by using absolute growth rate (AGR), relative growth rate (RGR), and specific growth rate (SGR).[14] The RGR is a useful metric for comparing the growth of individuals of the same initial size when subjected to different treatments, as

it considers the initial size of the individuals.[15] At the commencement of the experiment in this study, *P. scalare* juveniles exhibited uniform and small initial size. The RGR values observed significantly differed among the two feeding strategies, indicating that "snacking" on frozen *Artemia* sp. nauplii promoted body size growth. The significant differences in SGR also confirmed this positive effect. When conducting nutrition-related experiments with groups of fish over a short period, it is useful to measure the percentage growth per day. This is because the weight of fish is often assumed to increase exponentially over short periods, especially small fish. In terms of weight, there is evidence that the SGR produces good fitting results for young fish.[14,15] Thus, the authors highlight that supplemented with *Artemia* sp. nauplii can enhance the *P. scalare* juvenile growth performance, including biomass and size.

Moreover, the fish group that "snacked" on *Artemia* sp. exhibited a net gain in SL up to 3.4 folds during 1 month. To the best understanding of the authors, there is no previous study on angelfish juveniles to compare this performance. However, the non-enriched *Artemia* sp. effect on the growth performance in another ornamental fish, *Poecilia latipinna*, promoted a total length gain of 6.33 ± 0.57 mm and 3.50 ± 0.32 mm in a period of two months in males and females respectively. In male and female adults, fish gained a total length of 6.33 ± 0.57 mm and 3.50 ± 0.32 mm in two months in males and females, respectively.[16] Such growth performance boost is probably caused by an elevated digestive enzyme activity that promotes the fish's acceptance of artificial feed and increases feed utilization. The *Artemia* sp. used in the present study was enriched with vitamin C, which has been proven beneficial in tissue repairing and generating enzymes of specific neurotransmitters.[17] Thus, probably promoting the breakdown of indigestible components during the experiment and ultimately boosting the *P. scalare* juvenile growth performance.

Digestive enzymes play a vital role in the digestion and posterior absorption of the ingested food, especially when artificially formulated diets are frequently used to feed the fish. The enzymes AMS, PES, and LPS are often used as indicators of living organisms' physiological status, digestive processes, and nutritional condition.[18] The feed and feeding modes, especially the food type and its component, are the key factors affecting the activities of digestive enzymes.[19] In the present study, *P. scalare* juveniles were fed a diet supplemented with *Artemia* sp. nauplii displayed higher digestive enzymatic activity than juveniles fed with the formulated diet, especially the AMS activity levels. The increased AMS enzymatic activity in the supplemented group may have improved the breakdown of indigestible components and nutrient uptake, especially the carbohydrates (starch and glycogen), contributing to a better growth rate in the fish under the tested feeding strategy.[20]

Furthermore, enzymes like amylase and trypsin, present in *Artemia* sp.,[21] may be instrumental in the enzymatic autolysis process as the nauplii pass through the digestive tract of living organisms, thus aiding in digestion. Previous studies reported such improved level of digestive enzyme activity on the black tiger prawn post larvae,[22] the green terror cichlids *Aequidens rivulatus*,[8] and so on. The difference in the carbohydrate content and source between the living prey and the inert formulated diet may explain these results.[23]

Fish rely on enzymatic and non-enzymatic components to maintain a cellular antioxidant defense system that boosts immunity and maintains a dynamic balance. Antioxidant enzymes such as SOD, CAT, POD, AKP, and GSH-Px play a crucial role in preventing cell damage caused by ROS.[24] SOD neutralizes the superoxide anion, while CAT transforms hydrogen peroxide into water and oxygen after the SOD reaction. GSH-Px reduces H_2O_2 to H_2O and lipid peroxides to their respective alcohols.[25] The innate immune system in fish can be assessed using LZM, released by leucocytes, and has antibiotic properties.[26]

In the present study, *P. scalare* juveniles that "snacked" with *Artemia* sp. nauplii displayed a higher antioxidant enzyme activity than the fish fed only with a formulated diet. The SOD and GSH-Px activities were significantly higher, while the other antioxidant enzyme activities increased slightly. This finding confirms that some enzymes may compete with or complement each other while eliminating oxidative agents. As this has already been reported in juvenile lined seahorse, *Hippocampus erectus*, fed with highly unsaturated fatty acids enriched *Artemia* sp. nauplii,[27] and in the sturgeon *Acipenser naccarii* and trout *Oncorhynchus mykiss*.[28] Moreover, GSH-Px and SOD activity was stimulated in the diet that included *Artemia* sp., implying that these two enzymes probably play a more important role than CAT and LZM in protecting juvenile angelfish against oxidative agents. Although, it is not possible to precisely pinpoint the source of the oxidative stress that triggered the enzymatic antioxidant response in the angelfish juvenile because the total oxidative stress in the *P. scalare* juveniles was not assessed. Different biotic and abiotic factors, such as age, phylogenetic position, feeding behavior, environmental factors, oxygen, temperature, and xenobiotics, can trigger oxidative stress and antioxidant defense in teleost.[29] Hence, the supplemented diet may generate a certain stress level in the fish. The activity of CAT and SOD in the liver of Senegalese sole were significantly higher when fed high-lipid diets, and low-lipid diets seemed to reduce their susceptibility to oxidative stress, indicating that energy sources can influence the oxidative status of these fish.[30]

In conclusion, feeding juvenile *P. scalare* with *Artemia* sp. can enhance their survival, growth, digestion, and antioxidation capacity, indicating that *Artemia* sp. nauplii supplementation is beneficial at the juvenile stage. Although, further detailed studies will be necessary for a better understanding of the mechanisms in which *Artemia* sp. cofeeding strategies aid in the excellent performance in the grow-out of ornamental fish. The management of angelfish reared in aquarium system conditions may benefit from the results described in this study.

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DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION PER CREDIT

Conceptualization: Xing Zheng; Data curation: Jing Mao, Yu Chen; Formal Analysis: Jing Mao, Yu Chen; Funding acquisition: Xing Zheng, Zhifeng Gu; Investigation: Jing Mao, Yu Chen; Methodology: Jing Mao, Yu Chen, Shuaiqin Lan, Ze Yin, Meng Zhang; Project administration: Xing Zheng; Resources: Xing Zheng, Zhifeng Gu, Hebert Ely Vasquez; Software: Zhifeng Gu, Xing Zheng; Supervision: Feng Yu, Hebert Ely Vasquez; Validation: Feng Yu, Xing Zheng, Hebert Ely Vasquez; Visualization: Shuaiqin Lan, Ze Yin, Meng Zhang; Writing – original draft: Jing Mao, Yu Chen; Writing – review & editing: Xing Zheng, Hebert Ely Vasquez.

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