Latin American Journal of Aquatic Research, 51(2): 338-342, 2023
DOI: 10.3856/vol51-issue2-fulltext-2993

Short Communication

Effect of periods of delayed first exogenous feeding in goldfish *Carassius auratus* (Linnaeus, 1758) larvae

Jonas H. de S. Motta[1], Eduardo Luis Cupertino Ballester[2], André B. de Souza[3] Marcelo F. Polese[3] Marcella C. Radael[4], Leonardo S. Glória[5] & Manuel V. Vidal Jr.[5]

[1]Universidade Estácio de Sá, Campos dos Goytacazes, Rio de Janeiro, Brasil
[2]Universidade Federal do Paraná, Palotina, Paraná, Brasil
[3]Instituto Federal do Espírito Santo, Piúma, Espírito Santo, Brasil
[4]Universidade Federal do Oeste do Pará, Monte Alegre, Pará, Brasil
[5]Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Brasil
Corresponding author: Eduardo Luis Cupertino Ballester (elcballester@yahoo.com.br)

ABSTRACT. Four treatments were established to evaluate the effect of delayed first exogenous feeding and subsequent feeding periods on the development of goldfish larvae. The fasting and feeding periods were arranged as follows: T1 (0DFA:30DF), T2 (4DFA:26DF), T3 (8DFA:22DF), and T4 (12DFA:18DF), where DFA are the days of fasting and DF are the days of feeding. The larvae were kept in community tanks for each repetition, with 75 larvae per tank (3.75 larvae L^{-1}). There was no significant difference in total length or final weight between larvae submitted to 0, 4, and 8 days of feed deprivation. However, larvae submitted to 12 DFA had higher values of the same variables than the other treatments. No significant difference in larval survival was observed between T1, T2, and T3, but T4 negatively influenced survival. Under the conditions evaluated, the point of no return was 4 DFA. Dead larvae were partly eaten in the fasting treatments. The delayed first feeding should be avoided in large-scale productions since it significantly reduces survival during cultivation. This article results from research funded by the Pescarte Environmental Education Project (PEA/IBAMA).

Keywords: *Carassius auratus*; larvae; point-of-no-return; fasting; cannibalism; kinguio; aquaculture

Fasting is a natural process in many species, including salmonids, eels, bird species, and snakes. The effect of fasting on the animals depends on factors such as the duration of fasting and the animal's age (Wang et al. 2006).

In aquaculture, the delay in first exogenous feeding is a serious problem since it significantly reduces the survival of fish larvae (Gisbert et al. 2004, Garcia et al. 2020). A persistent delay in first exogenous feeding can lead to severe starvation of the animal that can no longer respond, even if food is available, and dies. Blaxter & Hempel (1963) called this stage the point-of-no-return (PNR).

According to data published by FAO (2022), species of the genus *Carassius* are the world's seventh-largest production group of freshwater fish. Among these species, *C. auratus* (Linnaeus, 1758) is used in polycultures for human consumption (Wang 2000) but is more important as an ornamental fish that is widely displayed in lakes and aquaria (Frimodt 1995, Copp et al. 2005).

Identifying the PNR and zootechnical performance data of teleost larvae in response to the challenge of delayed first feeding is fundamental for developing more sustainable and competitive production protocols.

Corresponding editor: Cesar Lodeiros

Therefore, the main objective of the present study was to analyze the effect of delayed first exogenous feeding on goldfish larvae held in shoals.

This article results from research funded by the Pescarte Environmental Education Project (PEA/ IBAMA), a mitigation measure required by the Federal Environmental Licensing conducted by IBAMA.

The experiment was conducted in a water recirculation system comprising 16 tanks with a useful volume of 20 L. The water flow in the tanks was regulated so that the total volume was renewed at least 20 times per day. Filtration was compartmentalized into three steps: mechanical filtration (acrylic wool, filtration area of 1600 m^2), biological filtration (mBBr media, 0.4 m^3), and filtration by ultraviolet radiation (36-W UV lamp). A heater with a 200-W thermostat (±1°C) was used to maintain the water temperature within that established for the experiment. Aeration was controlled by a radial compressor (190 L min^{-1}), with the air being transported through polyvinyl chloride tubing. An air diffuser (porous stone) was installed in each tank (experimental unit).

Uneaten feed and feces were removed daily from the tanks and mechanical filter by siphoning. The walls and bottom of the tanks were rubbed with a sponge to prevent the formation of bacterial film and periphyton formation. Furthermore, the UV filter helped control bacteria and algae in the system.

Four treatments (T1, T2, T3, T4), with four repetitions each, were established to evaluate the effect of delayed first exogenous feeding (days of fasting, DFA) and subsequent feeding periods (days of feeding, DF) on the development of goldfish larvae. The treatments were randomly distributed across the 16 tanks of the recirculation system, and the tanks were considered experimental units. The fasting and feeding periods were arranged as follows: T1 (0DFA:30DF), T2 (4DFA:26DF), T3 (8DFA:22DF), and T4 (12DFA: 18DF).

Seventy-five goldfish *C. auratus* larvae from the same spawning still in the free embryo stage, with an initial weight of 0.9 ± 0.01 mg and an initial length of 5.5 ± 0.2 mm, were allocated to each of the 16 experimental units. Once horizontal swimming was observed, the larvae were examined under a microscope (Nikon® Eclipse e200) for possible mouth opening. After observation of these two events, the larvae were considered fit to receive the exogenous feed, and the experiment was thus started.

During the feeding period, the fish were fed *ad libitum* four times a day (08:00, 11:00, 14:00, 17:00 h) with commercial feed (45 g kg^{-1} crude protein; 9 g kg^{-1} fat; 3 g kg^{-1} crude fiber), fractionated and sieved through a 200 µm sieve, as well as newly hatched brine shrimp at a concentration of 200 nauplii $larvae^{-1}$; dead larvae were removed as soon as observed to minimize cannibalism.

Water quality parameters were randomly measured in four tanks of the recirculation system, always at 10:00 h. Dissolved oxygen and temperature were measured daily (YSI 550 A ± 0.01). Ammonia-nitrogen (NH_3-N) was measured once a week (Hanna HI83203, Nessler method), and the pH was measured three times per week (pHtek PHS-3E ± 0.02).

At the end of the experimental period (30 days), all animals were weighed to obtain the total length, final weight, and survival. For the final weight and the total length, mixed linear models were adjusted using the MIXED procedure of the Statistical Analysis System (SAS System, Inc., Cary, NC, USA). If a significant difference was detected, the Tukey test was applied. Survival of the individuals was analyzed using the Kaplan-Meier method (Kaplan & Meier 1958).

The PNR was determined as proposed by Blaxter & Hempel (1963), who define PNR when 50% of the larvae reach a point of irreversible starvation. Even if food is available, the larva cannot grasp the food and dies at this stage. Thus, in the present experiment, the PNR was defined when 50% mortality was observed in the treatment.

The mean values and standard deviation of dissolved oxygen, temperature, pH, and NH_3-N observed throughout the experiment were 5.96 ± 0.09 mg L^{-1}, 28.36 ± 1.2°C, 7.09 ± 0.4 and 0.16 ± 0.01 mg L^{-1}, respectively. The values were within the expected range for the species and did not influence the variables analyzed in the present experiment.

The larvae of goldfish lose the ability to search for food after a delayed first exogenous feeding, and even if food is available, they cannot ingest it. Thus, survival was influenced by delayed first feeding (Table 1).

Most deaths occurred during the period of feed deprivation. Mortality was higher than 50% in T2, T3, and T4. Under the present environmental conditions, T2 (4 DFA) was defined as the PNR. There was no significant difference in the survival of larvae submitted to T1, T2, and T3 ($P > 0.05$). However, T4 negatively influenced survival ($P < 0.05$). In the treatments with high mortality rates (T2, T3, and T4), cannibalism of dead but not of live larvae was observed.

Delayed first feeding affected the total length of fish ($P < 0.05$), as seen in Figure 1a. There was no signifi-

Table 1. Survival of goldfish larvae submitted to the treatments consisting of different periods of delayed first exogenous feeding and subsequent feeding. Different letters indicate a significant difference between treatments (P < 0.05). SE: standard error.

Treatment	Mean ± SE (%)	Confidence interval (%)	
		Upper	Lower
T1	82.67 ± 2.19[a]	86.46	77.81
T2	44.33 ± 2.87[b]	49.68	38.42
T3	11.33 ± 1.83[c]	14.85	7.67
T4	4.33 ± 1.18[d]	6.61	2.00

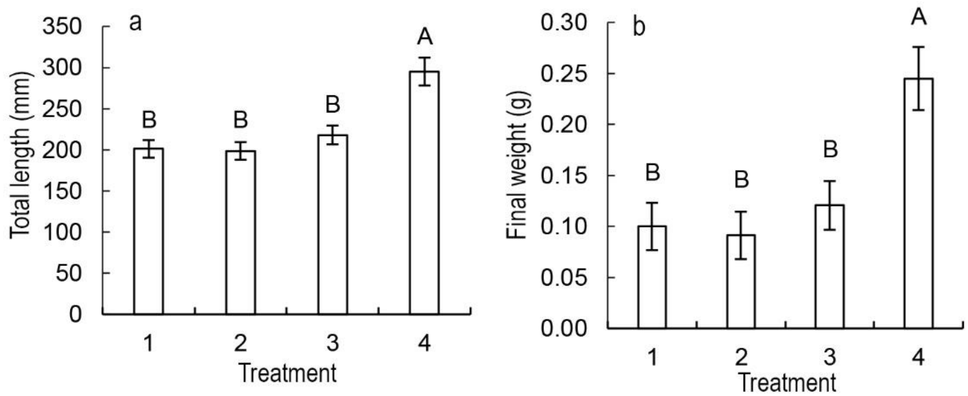

Figure 1. Effect of delayed first exogenous feeding and subsequent feeding on the zootechnical performance indices of goldfish larvae. The larvae were deprived of first feed for 0, 4, 8, and 12 days in treatments 1, 2, 3, and 4, respectively. Different letters indicate a significant difference between treatments (P < 0.05).

cant difference in length at the end of the experimental period between larvae submitted to 0, 4, and 8 days of feed deprivation (P > 0.05). However, larvae deprived of exogenous first feed for 12 days had a greater total length than the other treatments (P < 0.05).

Treatment had a significant effect on final weight (P < 0.05). Figure 1b shows that larvae submitted to 12 days of feed deprivation had a higher final weight than larvae of the other treatments (P < 0.05). No significant difference in final weight was observed for the treatments of 0, 4, and 8 days of feed deprivation (P > 0.05).

In the present experiment, fasting influenced the zootechnical performance data, in contrast to the findings of Motta et al. (2021). The high mortality observed in the experiment probably affected these data (total length and final weight). The significant effect of fasting on the survival of goldfish larvae decreased the stocking density, which may have influenced the zootechnical performance of the larvae. According to Jobling (1995) and Motta et al. (2020), competition for food interferes with the performance of fish larvae and juveniles.

According to Abe et al. (2021), juvenile fish can be fasted to minimize production costs; however, such a protocol is not recommended for the larval stage. In the latter stage, teleost fish have sufficient energy reserves to survive during the early stages of development (Blaxter 1969); however, this reserve is small, and larvae need to shift quickly to exogenous feeding. Otherwise, they may die. Thus, even short fasting during this phase significantly decreases survival during cultivation (Garcia et al. 2020). Initial fasting has been reported to result in progressive deterioration of the digestive system and atrophy of skeletal muscle fibers (Gisbert et al. 2004).

Interestingly, although survival below 5% was observed, 100% mortality was not found in any treatments. This fact is possibly related to the cannibalism observed in treatments with high mortality rates (T2, T3, and T4). According to De Andrade et al. (2004), cannibalism significantly affects survival during the larviculture phase. More resistant larvae fed on dead larvae and stayed alive until feed became available. Another possibility to consider is using bacterial film and periphyton as food for larvae.

However, since a daily cleaning was performed in tanks precisely to avoid this bacterial film formation, this is unlikely to be a valid option in the present experiment.

Cannibalism and survival may also have influenced the PNR. Analyzing the survival data for T2, which were close to 50%, and the information on cannibalism, it is difficult to establish the PNR reliably. Lima et al. (2017) found that, at a temperature of 29.3ºC, the PNR of *Rhamdia voulezi* occurs on day 6 after hatching. However, the authors observed cannibalism in the experimental units by day 4 after hatching; as in the present experiment, this fact may influence the analysis of this finding.

Possibly, a method that analyzes the individual instead of the shoal, minimizing the effect of intraspecific competition and cannibalism is more suitable to assess the PNR for goldfish. From a commercial point of view, i.e. in large-scale productions, the data obtained in the present experiment may be used since producers are not interested in understanding the event's effect on the individual.

The delayed first feeding should be avoided in large-scale productions since it significantly reduces survival during cultivation, a fact that would make production infeasible.

ACKNOWLEDGMENTS

Researcher/scholarship holder Jonas Henrique de Souza Motta thanks the UNESA for granting the research grant through the Programa Pesquisa Produtividade UNESA 22/23. This article results from research funded by the Environmental Education Project (PEA) Pescarte, a mitigation measure required by the Federal Environmental Licensing conducted by IBAMA.

REFERENCES

Abe, H.A., Reis, R.G.A., Paixão, P.E.G. & Fujimoto, R.Y. 2021. Effect of short-term fasting and re-feeding on growth performance of larvae and juveniles *Pyrrhulina brevis*, an amazon ornamental fish. Journal of Applied Aquaculture, 34: 894-906. doi: 10.1080/10454438.2021.1904084

Blaxter, J.H.S. 1969. Development: eggs and larvae. In: Hoar, W.S. & Randall, D.J. (Eds.). Fish physiology. Volume III. Academic Press, Cambridge, pp. 177-252.

Blaxter, J.H.S. & Hempel, G. The influence of egg size on herring larvae (*Clupea harengus* L.). 1963. Journal du Conseil International pour l'Exploration de la Méditerranée, 28: 211-214.

Copp, G.H., Wesley, K.J. & Vilizzi, L. 2005. Pathways of ornamental and aquarium fish introductions into urban ponds of Epping Forest (London, England): the human vector. Journal of Applied Ichthyology, 21: 263-274.

De Andrade, L.S., Hayashi, C., Souza, S.R. & Soares, C.M. 2004. Canibalismo entre larvas de pintado, *Pseudoplatystoma corruscans*, cultivadas sob diferentes densidades de estocagem. Acta Scientiarum. Biological Sciences, 26: 299-302.

Food and Agriculture Organization (FAO). 2022. The state world fisheries and aquaculture 2022. Towards blue transformation. FAO, Rome.

Frimodt, C. 1995. Multilingual illustrated guide to the world's commercial warm water fish. Fishing News Books, Oxford.

Garcia, L.M.B., Sayco, M.J.P. & Aya, F.A. 2020. Point-of-no-return and delayed feeding mortalities in firs-feeding larvae of the silver therapon *Leiopotherapon plumbeus* (Kner) (Teleostei: Teraponidae). Aquaculture Reports, 16: 100288. doi: 10.1016/j.aqrep.2020.100288

Gisbert, E., Conklin, D.B. & Pidrahita, R.H. 2004. Effects of delayed first feeding on the nutritional condition and mortality of California halibut larvae. Journal of Fish Biology, 64: 116-132.

Jobling, M. 1995. Simple indices for assessment of the influences of social environment on growth performance, exemplified by studies on Arctic charr. Aquaculture International, 3: 60-65.

Kaplan, E.L. & Meier, P. 1958. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association, 53: 457-481.

Lima, A.F., Andrade, F.F., Pini, S.F.R., Makrakis, S. & Makrakis, M.C. 2017. Effects of delayed first feeding on growth of the silver catfish larvae *Rhamdia vouleze* (Siluriformes: Heptapteridae). Neotropical Ichthyology, 15: e160027.

Motta, J.H.S., Glória, L.S., Polese, M.F., Souza, A.B., Neto, M.A.C. & Vidal Jr., M.V. 2020. Stocking density influences common carp larval development. Can restocking processes activate compensatory growth consequent to previous high stocking density? Spanish Journal of Agricultural Research, 18: e0608. doi: 10.5424/sjar/2020183-15652

Motta, J.H.S., Glória, L.S., Souza, A.B., Fosse-Filho, J.F., Polese, M.F. & Vidal Jr., M.V. 2021. Effect of fasting on freshwater angelfish *Pterophyllum scalare* (Lichtenstein; Pisces: Cichlidae) development. Latin

American Journal of Aquatic Research, 49: 125-135. doi: 10.3856/vol149-issue1-fulltext-2499

Wang, T., Hung, C.C.Y. & Randall, D.J. 2006. The comparative physiology of food deprivation: from feast to famine. Annual Review of Physiology, 68: 223-251.

Wang, W. 2000. Culture and enhancement of fishes. China Agriculture Press, Beijing, pp. 267-275.

Received: October 7, 2022; Accepted: December 9, 2023