LIVE AND DRY DIETS FOR CURIMATÃ-PIOA (*Prochilodus costatus*) LARVAE

DIETAS VIVAS E SECAS PARA LARVAS DE CURIMATÃ-PIOA (Prochilodus costatus)

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ABSTRACT

Freshwater Neotropical fish larvae hatch with an immature digestive tract. Once they shift from endogenous to exogenous feeding, they undergo a critical period in which they still lack digestive enzymes and face difficulties capturing prey, making them susceptible to mortality. Therefore, this study evaluated the effect of different feeding regimes on *Prochilodus costatus* larvae. Five-day-old larvae were subjected to four feeding regimes: Artemia nauplii; powdered dry feed; plankton sieved through a 300 µm mesh (P300); and plankton sieved through a 300 µm mesh plus dry feed (P300IF), in a completely randomized design with four treatments and three replicates each. Survival did not differ among diets. Larvae fed Artemia nauplii showed higher total length, weight, and biomass. Artemia nauplii were larger than wild plankton and provided greater biomass, increasing larval growth. Larvae fed dry feed and plankton plus dry feed showed similar performance for all parameters, demonstrating some capacity to assimilate dry feed. Larvae fed only plankton had a smaller total length than the other treatments, due to the lower plankton biomass, despite the number of prey per larva being the same as in the Artemia treatment. Thus, characterization of planktonic organisms is necessary to optimize feeding. Larvae showed the ability to ingest dry feed from the onset of exogenous feeding. However, this ability was limited, and live food remained essential, although future dry diets may be improved to meet larval requirements. Despite the limited assimilation of dry feed, when combined with live food, larvae grew more than those fed exclusively on plankton. In conclusion, larvae should be fed Artemia nauplii or other planktonic organisms providing adequate biomass and suitable prey characteristics to support growth. Further studies with dry feed are recommended.

KEYWORDS: Artemia nauplii, zooplankton, dry feed, larviculture, ontogeny.

RESUMO

Larvas de peixes de água doce, neotropicais, eclodem com o trato digestório imaturo, e assim que transitam da alimentação endógena para a exógena passam por um período crítico, quando ainda carecem de enzimas e têm dificuldades para capturar suas presas, sendo suscetíveis a mortalidade. Portanto neste experimento avaliou-se o efeito de diferentes regimes alimentares para larvas de *Prochilodus costatus*. Larvas com cinco dias pós-eclosão foram submetidas a quatro regimes alimentares: náuplios de *Artemia*; alimento seco em pó; plâncton peneirado em tela de 300 µm (P_{300}) e plâncton peneirado em tela de 300 µm acrescido de alimento seco (P_{300}AI), em um delineamento experimental inteiramente casualizado com quatro tratamentos e três repetições cada. As sobrevivências das larvas alimentadas com as diversas dietas não diferiram. As larvas apresentaram maior comprimento, peso e biomassa, quando alimentadas com náuplios de *Artemia*. Os náuplios de *Artemia* apresentaram maior porte do que o do plâncton selvagem, sendo um alimento com maior biomassa, o que resultou em um maior crescimento das larvas. As larvas alimentadas com alimento seco e plâncton acrescido de alimento seco apresentaram rendimentos semelhantes entre si para todos os parâmetros, demonstrando a capacidade da larva em assimilar o alimento seco. Lavas alimentadas somente com zooplâncton, apresentaram um comprimento total menor que nos demais, devido a baixa biomassa planctônica, apesar da quantidade de presas por larva ter sido a mesma dos náuplios de *Artemia*. Portanto, é necessário a caracterização dos organismos planctônicos para se adequar a alimentação. As larvas mostraram capacidade de se alimentar do alimento seco, desde o início da alimentação exógena. Entretanto, essa capacidade foi limitada, sendo ainda necessário o alimento vivo, apesar de que alimentos secos futuros tendam a melhorar, vindo a atender as necessidades dessas larvas. Apesar das larvas não terem apresentado uma boa capacidade de assimilar o alimento seco, quando ele foi ofertado juntamente com o alimento vivo, as larvas cresceram mais, do que as larvas alimentadas exclusivamente com plâncton. Por fim, as larvas devem ser alimentadas com náuplios de *Artemia* sp. ou outro organismo do plâncton que oferte biomassa adequada, e tenha características de preza, para suportar o crescimento das larvas. Outros estudos com alimento seco devem ser conduzidos.
PALAVRAS CHAVE: náuplio de *Artemia*, zooplâncton, alimento seco, larvicultura, ontogenia.

INTRODUCTION

Studies on ichthyoplankton characterizing continental species are essential for understanding fish ecology and fisheries biology[1].

The early development of fish larvae is a highly dynamic process. Its study can provide valuable information on ontogeny, behavior, and taxonomic traits for the identification of natural spawning areas, which are essential for population monitoring[2], as well as for establishing culture protocols[3].

Fish larvae exhibit limited swimming and vision capacity, with underdeveloped structures and reduced prey-capturing ability, leading to high mortality rates. During the initial stages of development, fish larvae undergo a "critical period," which corresponds to the transition from endogenous to exogenous feeding and is associated with high mortalities[4]. This "critical period" was initially proposed to explain interannual fluctuations in fish populations, as larval mortality could result from feeding failure due to hydrological and biological factors[5]. Later, it was also attributed to functional changes and organ immaturity, rendering larvae more sensitive to environmental stressors, impairing food acquisition, and increasing mortality[6].

Controlled-environment studies have been used to understand better how these phenomena occur in the wild[7]. In this context, laboratory larviculture of Neotropical fish enables control over feeding, water quality, stocking densities[8], among other factors. Rearing fish from identified adults has allowed the description of developmental stages of many species, supporting ecological studies and facilitating larval identification in their natural habitats[1,9]. Moreover, rearing eggs and larvae up to advanced developmental stages enables the characterization of ontogeny and species identification[1]. However, larval rearing remains challenging for many species due to limited information on the onset of exogenous feeding and optimal rearing conditions[10].

Identifying appropriate larval diets remains a significant challenge, and characterizing the ontogeny of the digestive system is fundamental to overcoming this bottleneck[3]. First feeding in many fish larvae relies on live food[3,11,12]. Live foods such as phytoplankton and zooplankton are indispensable in the larviculture of many fish species due to their high nutritional value[13,14], and their ingestion promotes the production of digestive enzymes[15]. Nevertheless, for practical and economic reasons, aquaculture seeks to replace live food with dry feed as early as possible[3].

Fish larvae are visual predators, and their prey capture, growth, and survival are influenced by prey movement[16,17], environmental–prey color contrast, sibling cannibalism, light intensity[18-20], photoperiod[20,21], and turbidity[20,22], which reinforces the importance of live food.

Among the morphological changes that most influence survival and growth during early development is the formation of the digestive tract, whose complexity varies according to species and feeding habits, and undergoes changes during ontogeny[23]. Thus, studies on early larval feeding have been conducted to achieve faster growth and higher survival rates by comparing different diets[24-33].

The curimatã-pioa (*Prochilodus costatus*) is an endemic migratory species of the São Francisco River Basin in Brazil[34], initiating its reproductive migration annually in response to rainfall regimes[35]. It has potential for commercial freshwater aquaculture[20] and is one of the most heavily exploited species in the São Francisco River[36], particularly in the Três Marias region, Minas Gerais, alongside the pintado (*Pseudoplatystoma corruscans*, Siluriformes: Pimelodidae) and the pacamã (*Lophiosilurus alexandri*, Siluriformes: Pseudopimelodidae), both stenohaline species of the São Francisco River, southeastern Brazil[10].

Although some studies have investigated larval feeding in other *Prochilodus* species, such as the level and type of food intake in *Prochilodus scrofa*[37], dietary protein requirements in *P. scrofa*[38], the need for live food in *P. scrofa* larvae[39], food selectivity in *P. lineatus*[40], and the effects of starvation in *P. lineatus*[41], no studies to date have addressed larval feeding in *P. costatus*. Therefore, this study evaluated different feeding regimes for curimatã-pioa larvae (*P. costatus*).

MATERIAL AND METHODS

The trial was carried out in January 2007 at the Hydrobiology and Fish Farming Station of Três Marias (Codevasf, MG, Brazil) during the first 10 days of exogenous feeding, after the research was approved by the UFVJM Animal Use Ethics Committee (CEUA), under protocol number 015/2013.

Five-day-old *P. costatus* larvae (6.7 ± 0.4 mm; 1.7 ± 0.3 mg) were stocked in 12 aquaria (5 L; 15 larvae L^{-1}) under continuous aeration (dissolved oxygen > 5 mg L^{-1}) and a mean temperature of 26.7 ± 0.4 °C at 9:00 h. The aquariums were cleaned daily by siphoning, at which time 10% of the water volume was renewed.

Four feeding regimes were tested in a completely randomized design (three replicates): Artemia: *Artemia* nauplii, IF: powdered dry feed, P300: plankton filtered through a 300 µm mesh, and P300IF: plankton + dry feed.

Artemia nauplii were hatched daily in 30‰ saline water, separated from cysts, and maintained at 10‰ until use. Feeding levels were 900 nauplii $larvae^{-1}$ day^{-1} (days 5–10) and 1,350 nauplii $larvae^{-1}$ day^{-1} (days 11–15).

Zooplankton was collected daily from fertilized ponds with a 65 µm net, filtered (300 µm), quantified, and supplied in equivalent numbers to the nauplii offered. Samples were preserved in 5% formalin for taxonomic identification[42-46] (Table 1). The dry feed was a commercial powdered diet (Table 2).

At the end of the experiment, survival, total length (digital caliper, 0.02 mm precision), and biomass (analytical balance, 0.001 g precision) were measured. Mean weight was calculated as biomass per individual. Survival data were arcsine-transformed for statistical analysis, but results are presented as percentages. All parameters were subjected to ANOVA and means compared by Tukey's test ($p < 0.05$) using SigmaStat 3.5.

Table 1. Zooplankton composition supplied to *Prochilodus costatus* larvae.

Taxon		(%)
Rotifera		
	Keratella tropica	13.6
	Filinia sp.	11.3
	Conochilus sp.	7.4
	Polyarthra sp.	6.1
	Brachionus calyciflorus	2.1
	Other rotifers	2.4
Copepoda		
	Copepodites and adults	3.2
	Copepod nauplii	32.6
Unidentified		22.3

Table 2. Composition of commercial dry feed supplied to *Prochilodus costatus* larvae.

Component	(g kg^{-1})
Crude protein	≥ 550
Moisture	≤ 100
Ether extract	≥ 40
Fiber	≤ 60
Minerals	≤ 180
Calcium	≤ 50
Phosphorus	≥ 15

Feeding occurred three times daily (09:00, 13:00, 17:00 h). Forty minutes after the last feeding, uneaten food was siphoned, and 50% of the water volume was renewed.

RESULTS

Larvae fed *Artemia* nauplii showed significantly higher length, weight, and biomass than other treatments. Larvae fed dry feed and plankton supplemented with dry feed exhibited similar performance across all parameters, while those fed only dry feed showed lower total length than the remaining treatments (Table 3). Survival rates did not differ significantly among treatments.

Table 3. Mean values (± standard deviation) of survival, total length, and biomass of *Prochilodus costatus* larvae under different feeding regimes.

Feeding regime	Survival (%)	Total length (mm)	Weight (mg)	Biomass (mg)
Artemia	95.5 ± 4.1a	13.3 ± 1.1a	22.9 ± 7.0a	2010.2 ± 30.3a
Dry feed	82.6 ± 5.6a	7.3 ± 0.4b	2.3 ± 0.6b	180.4 ± 5.7b
Plankton	70.2 ± 12.4a	5.9 ± 0.5c	0.7 ± 0.1b	50.2 ± 10.5b
Plankton + Dry feed	91.5 ± 6.7a	7.1 ± 1.1b	2.2 ± 0.9b	140.7 ± 100.6b

Different superscript letters within the same column indicate significant differences according to Tukey's test at $p < 0.05$. *Artemia* = nauplii of *Artemia* sp.; Plankton = zooplankton filtered through 300 µm mesh; Plankton + Dry feed = zooplankton filtered through 300 µm mesh plus dry feed.

DISCUSSION

Larvae showed greater length, weight, and biomass when fed with *Artemia*. Larvae fed exclusively with dry feed and those fed with plankton supplemented with dry feed exhibited similar development, while those fed only with plankton showed the lowest total length. Greater growth associated with higher food availability, increased biomass, and the low capacity of fish larvae to assimilate dry feed in the first days of life has been reported.

Only on the fourth day after hatching, with the absorption of the yolk sac, the appearance of the swim bladder, terminal mouth, and formation of pectoral fins, do *curimatã-pioa* (*P. costatus*) larvae begin to move across different levels of the water column[47], a behavior associated with prey capture. Larvae of native fish species have an immature digestive system[48,49]. Although *Prochilodus argenteus* larvae display digestive activity on the third day after hatching, when the digestive tract is still a simple tube, stomach and intestine differentiation only occur on the 18th and 28th days, respectively[2]. This explains why larvae have difficulty assimilating commercial dry feed during the first days of life[33,49,50]. For this reason, live feeds, which undergo autolysis during digestion and facilitate assimilation by fish larvae, are widely used[48,49,51]. These are sometimes combined with dry feed (co-feeding), which appears to accelerate digestive system development and anticipate the larvae's ability to assimilate dry feed[52].

ARTEMIA NAUPLII

The provision of *Artemia* nauplii resulted in greater weight and length in *P. costatus* (curimatã-pioa) larvae than those fed solely with plankton or plankton supplemented with dry feed. *Artemia* nauplii have also been shown to promote higher total length and weight in *Pimelodus maculatus* (mandi amarelo)[26], *Piaractus mesopotamicus* (pacu)[28], and *Steindachneridion melanodermatum* (surubim-do-Iguaçu)[29] than when fed with collected zooplankton. The supply of larger organisms, larger feed particles, and higher biomass led to better larval development in *L. alexandri* (pacamã)[33]. Similarly, the higher biomass of *Artemia* nauplii resulted in higher weight and growth rates, a pattern observed in *P. costatus* (curimatã-pioa) larvae[10], *Colossoma macropomum* (pacu)[53], *Piaractus brachypomus* (pirapitinga)[54], and also in increased weight for species such as *Pangasianodon gigas* (Mekong catfish)[55], *P. maculatus* (mandi amarelo)[26], and *P. mesopotamicus* (pacu)[28,30], which can consequently lead to greater biomass production.

The use of *Artemia* nauplii also led to higher biomass when compared to the other treatments, which showed lower and similar results among themselves. This result was expected, since survival was similar among the feeding treatments, but the larvae fed with *Artemia* nauplii exhibited higher weight. A similar finding was also reported in the larviculture of *P. brachypomus* (pirapitinga)[54] and *Carassius auratus*, where *Artemia* nauplii produced greater biomass than plankton and plankton supplemented with dry feed[24].

The plankton was composed mainly of small-sized organisms compared to *Artemia* nauplii. Newly hatched *Artemia* nauplii range from 439.3 to 517.0 µm[56]. In contrast, *Keratella tropica*, which was predominant in the samples, has a length of 190 µm[57]. Species of the genus *Filinia*, such as *Filinia longiseta*, are 177 µm long excluding elongated setae[58]; *Conochilus dossuarius* may reach 93 to 180 µm in body length with the extended foot; *Polyarthra* species range from 100 to 155 µm, and *Brachionus calyciflorus*, which was the least frequent, varies from 263 to 374 µm in length[59]. Considering that *Lecane*, a common genus, ranges from 92 to 264.5 µm[57], and that copepod nauplii are even smaller, natural plankton provided low feed biomass. It is important to note that if the organisms were half the size, they would have four times less mass by the rule of three. The difference is even more significant since the average length of plankton is less than half that of *Artemia* nauplii.

The difference in growth between larvae fed with plankton and *Artemia* could be even greater if the water had been slightly saline (2‰). Although salinity is a stressor, it keeps the nauplii alive longer, making them more attractive to the larvae and promoting greater larval growth than in freshwater. On the other hand, the difference could be minor if frozen nauplii had been used[17].

However, wild zooplankton has more desirable nutritional characteristics for larval diets than *Artemia* nauplii[18,60]. Furthermore, the nutritional value of *Artemia* is not constant; it varies geographically and seasonally[19,61]. Therefore, enrichment techniques for *Artemia* nauplii and rotifers have been developed to compensate for possible nutritional deficiencies[13]. Nonetheless, despite the superior nutritional quality of natural zooplankton, *Artemia* is a live feed that can meet the nutritional needs of fish larvae during the early stages of growth[62], as also verified in this experiment.

ARTEMIA NAUPLII AND PLANKTON

Although wild plankton proved insufficient for the proper growth of *P. costatus* (curimatã-pioa) due to the low biomass offered, it is generally considered nutritionally rich, composed of a variety of essential fatty acids, amino acids, digestive enzymes, micronutrients, etc., in addition to having size and movement that make it attractive to fish larvae[63]. Therefore, the provision of zooplankton right after the yolk sac absorption was suitable for the growth of African catfish larvae, *Clarias gariepinus* and *Heterobranchus longifilis*[64], as well as for the marine mandarin fish *Synchiropus splendidus*[65]. This outcome is generally expected, as in nature, fish larvae feed on a diversity of zooplankton species, being able to select their prey, and their abundance is often correlated with the availability and population dynamics of these organisms. However, even in nature, larval success depends on the abundance and biomass of prey[66], as observed in this study.

DRY FEED

Despite limited growth, the high survival rates of larvae fed exclusively with dry feed demonstrate the ability of *P. costatus* to ingest and assimilate dry feed from the very first day of exogenous feeding, which encourages continued research into the use of artificial diets for this species. The ability of curimatã-pioa larvae to be fed exclusively with commercial dry feed from the start of exogenous feeding had already been observed. However, survival rates varied widely (57.90% ± 52.90) compared to those fed with *Artemia* nauplii (82.00% ± 4.00)[49]. Similarly, *P. scrofa* (curimbatá) larvae showed high survival rates, equal to or greater than 80%, when fed exclusively with dry feeds containing 35–40% protein[38]. These results suggest that this capability may be a characteristic of the genus, which should be confirmed through further studies.

In some cases, dry feed was introduced after the initial provision of live feed to gradually reduce the use of live feed while maintaining favorable conditions for the larvae. *P. argenteus* larvae fed with *Artemia* nauplii for the first three days, and subsequently with various forms of the same dry feed, showed survival rates above 89.66%, demonstrating the species' ability to assimilate dry feed after a short period of live feed provision. Greater biomass and biomass gain were observed in larvae fed crumbled pelleted dry feed and crumbled extruded dry feed, compared to those fed flake dry feed and moistened extruded dry feed, which yielded the worst results[67]. This demonstrates that the physical form of the feed can lead to different outcomes. Similarly, *P. argenteus* larvae were fed *Artemia* nauplii during the first three days of exogenous feeding. Then they switched to an dry feed, which had a survival rate of 55%, suggesting that three days of nauplii provision were sufficient to mature the larvae's digestive system minimally, facilitating the assimilation of dry feed and subsequent growth[2].

Currently, there is a wide array of technologies for manufacturing microdiets for fish larvae, and many successful cases of replacing live feed with formulated microdiets in later larval stages have been reported[68]. However, artificial diets have not fully met the dietary needs of several fish larval species, and successful rearing still heavily depends on an adequate supply of nutritious live feed[69]. The shortcomings of dry feed have been associated with low survival rates and irregular larval production, as observed in *Trachelyopterus galeatus* (catfish), *Piaractus brachypomus* (pirapitinga)[54], and *Rhamdia quelen* (jundiá)[52], all native Neotropical species.

CO-FEEDING

The combined use of live and dry feed, known as co-feeding, is a technique used to facilitate the transition from live to dry feed and has been shown to promote the development of the digestive tract and enzymatic activity[70]. Consequently, larvae fed both live and dry feed typically exhibit greater development than those fed exclusively with dry feed[52,71,72], a result not observed in *P. costatus* larvae in this experiment due to the biomass offered. However, co-fed larvae performed better than those fed only with live feed[14,52,72], which was confirmed in this study.

Nonetheless, in some cases where live feed consisted of *Artemia* nauplii, the addition of dry feed did not improve growth, weight, length, or survival and, in some instances, even worsened these parameters[49]. This may be due to water quality deterioration caused by the breakdown of dry feed[33,49].

CONCLUSIONS

Larvae of *P. costatus* fed with the live feed *Artemia* nauplii grew more and achieved greater biomass, due to the larger size and higher prey biomass offered by the nauplii.

The plankton collected from fertilized tanks was composed of small-sized organisms, resulting in a lower prey biomass offered to the larvae (since the number of prey per larva was kept constant), which led to reduced growth. Therefore, it is necessary to characterize the planktonic organisms to ensure appropriate feeding.

The larvae demonstrated the ability to assimilate dry feed from the start of exogenous feeding. However, this ability was limited, and live feed is still necessary, although future developments in dry feeds may better meet larval nutritional needs. Although larvae did not show a strong capacity to assimilate dry feed when provided alone, when offered in combination with live feed, larval growth exceeded that of larvae fed exclusively with plankton.

Finally, larvae should be fed with *Artemia* nauplii or other planktonic organisms with sufficient biomass and prey characteristics supporting larval growth. Further studies using dry feed are warranted.

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CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

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