Feed preferences of *Salminus brasiliensis* (Pisces, Characidae) larvae in fish ponds

Daniela Flávia Orísia Ribeiro, Alex Pires de Oliveira Nuñer *

Laboratório de Biologia e Cultivo de Peixes de Água Doce, Universidade Federal de Santa Catarina, Departamento de Aquicultura, Rodovia SC 406, 3532, Florianópolis, Santa Catarina, CEP 88066-000, Brazil

Received 8 March 2007; received in revised form 8 November 2007; accepted 8 November 2007

Abstract

Feed preferences of dorado, *Salminus brasiliensis*, in the early stages of life was studied in larvae, stocked 5 days after hatching in three earthen commercial ponds (810 m², 900 m² and 1480 m²) at a density of 30 m⁻². Larvae were fed on natural food from ponds supplemented with a commercial ration (40% crude protein), and for 12 days fish samples were taken daily in the morning and in the afternoon from each pond to analyze the food items present in the diet. Zooplankton available in the environment was also analyzed. Feeding was intensive since 94% of the larvae guts examined contained food. Since the commercial diet was not found in the dorado guts, the natural diet dominated the artificial food, but the cannibalistic behavior typically found in laboratory conditions was not observed. Insect larvae and cladocerans were the main prey items found in the gut of the larvae. In fish ponds dorado larvae can be considered a generalist feeder, since small amounts of different food items were consumed, but they tend to specialize in some items, according to their developmental stage.

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Keywords: *Salminus brasiliensis*; Larvae; Natural food; Fish ponds; Dorado

1. Introduction

In the early stages of development, natural food provides essential nutrients ensuring fish survival and growth (Furuuya et al., 1999), as well as playing a very important role in larvae culture and rearing of fingerlings (Kubitza, 1998).

Fish feeding can be analyzed by direct observation of the feeding behavior and/or by identifying the gut contents, which represents food availability in a certain environment (Andrian and Barbieri, 1996). Studying fish anatomy also provides basic information about the feeding habits of fish and a careful examination of the digestive apparatus gives a good idea of their favorite diet or, at least, can guide new studies regarding their feeding (Wooton, 1990).

The main factors that determine the type of prey ingested are feeding preference (Shaw et al., 2003; Hagiwara et al., 2007; Nunn et al., 2007), prey availability, its mobility and distribution in the water column, predator’s catching efficiency, water temperature and turbidity (Moore and Moore, 1976). According to these authors, changes in the feeding habits of a fish species are a function of the interactions among several environmental factors that will influence food item selection. Although fish feeding is selective, it can vary according to food availability meaning that most fish species show extremely adaptable feeding habits, using items readily available in the environment (Azevedo, 1972).

*Salminus brasiliensis* Cuvier, 1816, is a carnivorous species from its early life stages. Newly-hatched larvae are on average ± standard deviation) 4.96±0.18 mm long (Zaniboni-Filho et al., 2004), and when larvae reach a length of 6.75 mm, there is a total absorption of the vitelline sac (Nakatani et al., 2001). Santos and Godinho (2002) reported that larval cultured at temperatures of 23–25 °C, absorbed the vitelline sac by the third day after hatching. In the wild, 3-day-old larvae start feeding on plankton, microcrustaceans, cladocerans, copepods and larvae of Odonata and Chironomidae (Morais Filho and Schubart, 1955). In captivity this species develops cannibalism, which
usually decimates large scale production (Zaniboni-Filho, 2004). As feeding the larvae during the first days of life is one of the main constraints in the large-scale production of juvenile fish, the present study aimed to describe the diet of dorado larvae stocked in commercial fish ponds to promote better management of the species.

2. Materials and methods

The experiment was carried out in December/2003 with dorado larvae obtained by induced spawning of broodstock using carp pituitary extract (CPE) as the hormone in a previous dosage (0.25 mg CPE kg⁻¹ of broodstock) followed by two dosages (0.5 and 5.0 mg CPE kg⁻¹) applied in an interval of 12 h (Zaniboni-Filho and Barbosa, 1996).

After hatching, larvae were kept for 5 days in laboratory conditions and fed with Prochilodus lineatus larvae, the usual method of commercial management that assures larvae survival.

On the fifth day after hatching (DAH), dorado larvae averaging (±standard deviation) 10.73±0.26 mm in length and 10.1±1.4 mg in weight, were transferred to commercial earthen ponds at a density of 30 larvae m⁻². Three ponds were used: P1 (810 m²; 1.60 m mean depth), P2 (900 m²; 1.80 m mean depth) and P3 (1480 m²; 1.40 m mean depth), filled by gravity with unfiltered water from a reservoir located in the hatchery. Prior to stocking, ponds were treated with lime (60 g m⁻²) and fertilized with chicken manure (300 g m⁻²) to promote disinfection, pH equilibrium and zooplankton growth, as zooplankton could be used as a food source by the larvae. A daily supplement of 4.0 kg of a commercial ration (40% crude protein) was offered in equal portions at 06:00, 12:00, 18:00 and 24:00 h in each pond.

In order to analyze the gut contents, larvae samples (n=5 to 8) were collected from each pond in the morning (08:00 h) and in the afternoon (18:00 h). Sampling was carried out for 12 days, the period required for larvae to reach commercial size. A zooplankton net was used for capturing larvae during the first 6 days followed by a 1.5 mm mesh net for the next 6 days. The sampled larvae were kept in a 4% buffered formaldehyde solution for later analysis.

The total length (mm) of each of the larvae was measured with a caliper, and the wet weight (g) was obtained with a 0.1 mg precision analytical scale. The gut contents were analyzed on Petri dishes under a stereomicroscope, and the food items were identified using the keys described by Macan (1975), Merret and Cumniss (1984), Borror et al. (1992) and Roldán (1988).

Electivity of fish larvae were analyzed by the Ivlev’s index, Eᵢ = (rᵢ - pᵢ) / (rᵢ + pᵢ), where Eᵢ is the Ivlev’s index of the food type i, rᵢ is the percentage

<table>
<thead>
<tr>
<th>Group/species</th>
<th>Pond 1</th>
<th>Pond 2</th>
<th>Pond 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copepods</td>
<td></td>
<td></td>
<td></td>
<td>2775.4</td>
</tr>
<tr>
<td>Nauplii</td>
<td>384.8</td>
<td>572.0</td>
<td>539.0</td>
<td>1495.8</td>
</tr>
<tr>
<td>Cyclopoida</td>
<td>340.8</td>
<td>122.5</td>
<td>212.0</td>
<td>675.3</td>
</tr>
<tr>
<td>Calanoida</td>
<td>6.8</td>
<td>476.5</td>
<td>121.0</td>
<td>604.3</td>
</tr>
<tr>
<td>Cladocerans</td>
<td></td>
<td></td>
<td></td>
<td>948.2</td>
</tr>
<tr>
<td>Bosmina sp.</td>
<td>92.3</td>
<td>125.0</td>
<td>238.0</td>
<td>455.3</td>
</tr>
<tr>
<td>Diaphanosoma sp.</td>
<td>122.0</td>
<td>88.0</td>
<td>230.3</td>
<td>440.3</td>
</tr>
<tr>
<td>Bosminopsis sp.</td>
<td>6.0</td>
<td>30.3</td>
<td>0.0</td>
<td>36.3</td>
</tr>
<tr>
<td>Ceriodaphnia sp.</td>
<td>4.0</td>
<td>0.5</td>
<td>2.5</td>
<td>7.0</td>
</tr>
<tr>
<td>Disparalona dadayi</td>
<td>2.3</td>
<td>1.3</td>
<td>2.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Moina sp.</td>
<td>0.8</td>
<td>1.5</td>
<td>1.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Total</td>
<td>959.5</td>
<td>1417.5</td>
<td>1346.3</td>
<td>3723.6</td>
</tr>
</tbody>
</table>
of food type $i$ in larval gut and $p_i$ is the percentage of the food type $i$ in the environment (Ivlev, 1961). This index varies from $-1.0$ to $+1.0$, with positive values indicating preferred items, negative electivities indicating avoided items, and zero values indicating items that are not particularly selected. Ivlev’s index was applied only for cladocerans and copepods, for which all data were available, and for larvae that did not consume fish larvae prey (6 to 16-DAH).

Gut fullness was used to assess the larval feeding activity, adopting the following scale: empty (gut less than 25% filled), partially empty (25–50%), partially full (50–75%) and full (over 75%).

At the same time and place of larvae sampling, 20 l of pond water was filtered in a 50 µm mesh net to determine the zooplankton available in the environment. This volume was concentrated to 180 ml and kept in a 4% buffered formaldehyde solution. Quantitative and qualitative analyses of the zooplankton community were performed under a light microscope from the organisms present in 2 ml sub-samples of the concentrated samples, and the results were expressed as organisms l$^{-1}$. Identification keys by Koste (1978), Reid (1985), Paggi (1995) and Elmoor-Loureiro (1997) were used to identify the zooplankton.

The concentrations of dissolved oxygen and total ammonia, temperature and pH were analyzed with an YSI-6600 multiparameter probe. Analyses were done in all ponds in the morning and in the afternoon, and a 24-hour cycle analysis was carried out in each pond with 1-hour intervals between readings, to evaluate daily variations.

The chi-square test (Zar, 1996) was used to analyze gut fullness, zooplankton density in ponds and occurrence of food items in larval guts. The lengths and weights, for each sampled day, were analyzed using ANOVA ($\alpha=0.05$) and their relation with time was expressed by regression analyses (Zar, 1996).

3. Results

The water temperature and the dissolved oxygen concentration were higher in the afternoons and similar among ponds. In contrast, the total ammonia concentration was higher in the mornings (Fig. 1). The dissolved oxygen concentration remained high from the sixth day of sampling, coinciding with a reduction in the total ammonia concentration, which remained well below lethal levels for dorado juveniles (Gazzola, 2003). There was a small change in pH due to the initial liming that buffered the pond water. Therefore, the observed variations in water quality parameters in the ponds remained within acceptable limits for freshwater fish culture (Boyd, 1990).

Since rotifers were not consumed by dorado larvae during the experiment they were not included in the analysis. Without the rotifers, copepods were the zooplanktonic group with the largest density in all ponds, a condition derived by the high number of nauplii present (Table 1). If nauplii were ignored the densities of copepods and cladocerans were similar. Among cladocerans Bosmina sp. and Diaphanosoma sp. were the species with higher densities.

A total of 591 larval guts were examined, of which 556 (94%) contained food. Five kinds of food items were found in fish guts: $P$. lineatus larvae, from now on named fish larvae (fL), insect larvae (IL), cladocerans (C), copepods (Co) and adult insects (Ai). Cladocerans and insects partially digested were named cladoceran paste (Cp) and insect paste (ip) respectively, and were analyzed separately from the IL and C to allow visual observation of the beginning of digestion by dorado larvae.

The daily analyses showed that larvae from different ponds ingested food items in different quantities ($P<0.05$), possibly due to the different ($P<0.05$) daily density of the zooplankton groups in ponds (Fig. 2).

On the first day of sampling fish larvae, insect larvae, cladocerans and copepods were found in the guts of 5-day-old dorado larvae (Fig. 3a). Insect larvae became predominant in most 6-DAH dorado guts, and a greater reduction in the occurrence of fish larvae was also noticed (Fig. 3b). From age 7-DAH, fish larvae were not found in the analyzed guts.

Cladoceran paste was recorded for the first time, although low in frequency and abundance, in 8-DAH larvae in P2. The frequency and abundance of insect larvae stayed high whereas cladocerans were present and abundant. Copepods and adult insects were found in the larvae from P1 and P3, but in low frequency (Fig. 3c).

Cladocerans were preyed more intensely by 10-DAH dorado larvae. The insect larvae remained frequent, although in low abundance in most larvae, while few copepods and adult insects were selected (Fig. 3d). However, in 11-DAH dorado larvae cladocerans tended to be less frequently eaten and an increase in the frequency of insect larvae was observed, although in low abundance. Copepods and adult insects were rare, while cladocerans paste was present again in P2, but low in frequency and abundance (Fig. 3e). In 13-DAH larvae guts the number of cladocerans decreased while numbers of insect larvae increased, following an increase in cladocerans and insects pastes. Overall, the
occurrence of copepods and most cladocerans in the guts was low (Fig. 3f).

Insect paste prevailed with high frequency and abundance in the guts from 14-DAH dorado larvae (Fig. 3g) and 16-DAH larvae (Fig. 3h). Cladocerans and copepods were almost absent, however an important increase was observed in the cladoceran paste.

The pattern for cladocerans and copepods consumption was registered by Ivlev’s index (Fig. 4), that showed the preference for cladocerans, compared to copepods, by dorado larvae until 12-DAH. In older larvae cladocerans were alternately substituted by copepods in ponds 1 and 2, whereas in pond 3 the substitution pattern was more stable, with copepods always being selected.
The daily analyses of gut fullness showed a higher frequency of dorado larvae with the gut partially empty and partially full, with no difference (P>0.05) observed between morning and afternoon samples (Fig. 5) or among larvae from the different ponds. Furthermore, natural food predominated over the artificial diet, which was not observed in the gut contents. Similar condition was found to larvae of *Piaractus mesopotamicus* and *Brycon orbignyanus* to which artificial food was found in guts only at the age of 17 and 13-DAH, respectively (Senhorini and Fransozo, 1994; Senhorini et al., 2002).

The total length and wet weight of larvae varied between 7.6 and 49.6 mm, and 0.0031 and 1.65 g, respectively, but daily growth and weight gain did not differ (P>0.05) among the ponds during this study (Fig. 6).

### 4. Discussion

Fish larvae prevailed in the guts of dorado larvae with 5-DAH during the morning samples, since it was the sole food available in laboratory conditions. In the afternoon, after the larvae were transferred to outdoor ponds, most larvae fed on large amounts of insect larvae, maintaining the consumption of larger food, but cladocerans were also important in the diet.

Most South-American fish larvae consume very low amounts of rotifers and protozoans, preferring larger zooplankton such as cladocerans and copepods (Atencio-Garcia et al., 2003), a condition directly related to the larger size of the mouth since opening (Dostati et al., 1999; Rowlands et al., 2006; Nunn et al., 2007), especially for those larvae with carnivorous feeding habit that also present larger length at hatching (Sato et al., 2003). These organisms are excellent natural food items for fish, especially during the first 30 days of life, since cladocerans and copepods contain 56.5% and 52.3% crude protein, respectively (Furuya et al., 1999). In the present study, preference for cladocerans was recorded until the 11th DAH, since most of the guts analyzed contained these organisms, what could be related to the adequacy of their nutritional value to dorado larvae until this stage.

This preference started to shift on 11th DAH, when amounts of insects begun to be recorded in the guts, mainly between the 14th and 16th DAH, showing the importance of these organisms as food sources for freshwater fish. The selection of larger preys was intensified in the diet of 12-DAH and older dorado larvae, also accompanied by the increase of copepods consumption. Galina and Hahn (2004) emphasize the importance of insects, as well as other invertebrates, in fish feeding. Regarding the change in diet, Arcifa and Meschiatti (1993) observed that tucunáre (*Cichla* sp.) feed on insects when juvenile (<87 mm) and on fish when adult. Several studies showed that *Leporinus friderici* and *Leporinus affinis* feed highly on items of animal origin, mainly represented by insects and their larval phases (Braga, 1990; Ferreira et al., 1998; Balassa et al., 2004), which was also recorded in this trial.

Insects provide significant amounts of protein and lipids, 59.1% and 12.2%, respectively (Mera et al., 1999), and are rich in minerals such as phosphorus and potassium (Conconi and Rodríguez, 1977), representing a valuable source of food.

Vega-Orellana et al. (2006) studied the activity of some digestive enzymes in larvae of *S. brasiliensis* fed on larvae of...
P. lineatus and observed that the initial activity of pepsin was recorded at 3-DAH, with increased activity at 7-DAH. In the present study, the first observations of completely digested food in guts, represented by cladoceran paste, occurred only in one pond, with very low frequency and abundance, in 8-DAH dorado larvae. Although the observation period was similar in those studies, in the present study weight gain increased only after 11-DAH and mainly later on, when the partially digested food became more frequent and abundant. Therefore more efficient digestion was observed after that time, suggesting that full digestion capability developed after production of the first digestive enzymes, which can be related to the increase in protein requirements as fish grew. Dorado larvae showed similar behavior, primarily ingesting cladocerans and later feeding on insect larvae as they grew.

Despite that increase in digestive capability, artificial food was not used by dorado larvae during the study suggesting that until 16-DAH natural food supported larvae development in the environmental conditions registered in the ponds.

Active predation of dorado larvae on fish larvae, including intense cannibalism, is a common condition in many dorado hatcheries (Zaniboni-Filho, 2004). However, in the present study, no parts of dorado larvae were found in the guts of the fish larvae, which means that cannibalism, a usual condition in the laboratory, did not occur, probably as a result of the adequate quantity and nutritional quality of the natural food present in the ponds.

5. Conclusions

In fish ponds S. brasiliensis larvae fed exclusively on the natural food available, mainly on cladocerans and insect larvae. Dorado larvae showed preference for cladocerans until 12-DAH, and after that insect larvae and paste were mainly found in the guts, a condition related to a more efficient digestion registered after that age. The natural food produced in all ponds prevented the emergence of cannibalism, a behavior usually found in laboratory conditions.

Dorado larvae seemed to be generalist feeders, since they ingest variable amounts of different foods with certain items predominant according to the phase of development.

Acknowledgments

The authors thank Consórcio Machadinho and Tractebel Energia for the financial support and Drs. Norma Segatti Hahn and Evoy Zaniboni-Filho for the important suggestions added to the text.

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