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Effect of food type on growth and survival of *Chirostoma riojai* Solórzano y López, 1965 (Atheriniformes: Atherinopsidae) during early development

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The aim of the present study was to analyze the effect of the diet on growth and survival of *Chirostoma riojai* larvae, using a mixed feed until 30 days after hatching. The tested diets were: 1) *Artemia* nauplii, 2) *Brachionus rubens*, 3) formulated diet (micropellets in the size of a 65 μm rotifer, AZOO™, 50% protein), 4) *Artemia* nauplii + formulated diet, 5) *Brachionus rubens* + formulated diet, 6) *Artemia* nauplii + *Brachionus rubens.* Weight, total length, notochordal length and survival rate were determined for each diet. The optimal prey size for larvae from the first feeding up to 20 days after hatching was determined. The relative growth of the mouth opening was allometrically negative with respect to the width of the jaw and allometrically positive to notochordal length. Survival, notochordal and total length, as well as weight, were higher in those larvae fed with live feed. With the inert feed diet, larvae did not survive after the yolk-feeding period, probably due to the lack of enzymes in the digestive tract.

Key words: *Chirostoma riojai*, larval rearing, optimal prey size, growth, survival.

INTRODUCTION

Chirostoma riojai is a zooplankton eating fish, endemic to the Central High Plateau of Mexico. It is of great socio-economic and cultural relevance for the "Mazahuas" Indian groups that inhabit the riparian communities on the lakes located in the high course waters of the Lerma-Santiago watershed system. This watershed system is of great importance because it is a large fish endemism center, with 58% of endemic species (Miller & Smith, 1986). Important human settlements have been established in the area, affecting its resources intensively. At present, the area is characterized by a high degree of urbanization and industrialization, affecting in different degrees the air, water and soil quality, as well as the natural communities inhabiting this area.

Chirostoma riojai, similarly to the other members of the genus, is carnivorous. Its diet, although presenting modifications along its ontogenetic development, is based on zooplanktonic organisms, mainly cladocerans and copepods (Méndez, 1996; Ordóñez, 1999). It is considered a primitive species within the Arge group, mainly because of its pigmentation and dental characteristics (Barbour, 1973). It is distributed over the high course of the Lerma-Santiago system, particularly in the Valley of Toluca, State of Mexico, from which its name "Charal of the High Lerma" derives (Mayden *et al*., 1992).

Chirostoma riojai was first described by Solórzano & López (1965). In 1997, its presence was recorded in nine localities of the High Lerma, and at present it is found in two localities, the Santiago Tilapa

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lagoon, where its holotype was recorded, and the Ignacio Ramírez dam, where pesticides, such as dieldrin and methyl-parathion have been found (Favari *et al.*, 2002). Therefore, if these adverse factors persist, this species could be endangered.

The present study was designed to investigate the effects of the diet on the growth and survival during the early development of *Chirostoma riojai*. The quality, type and size of food have been examined in order to establish a conservation methodology.

MATERIALS AND METHODS

We tested six diets to assess their effects on growth through increments in weight, notochordal (NL) and total (TL) lengths in three-days old larvae, starting with a mixed feed from this time on until 30 days after hatching. The diets used were: 1. *Artemia* nauplii (A), 2. *Brachionus rubens* (R), 3. formulated diets (B) (micropellets of the size of a 65 µm rotifer, AZOO™ with 50% protein), 4. *Artemia* nauplii + formulated diet (A+B), 5. *Brachionus rubens* + formulated diet (R+B), 6. *Artemia* nauplii + *Brachionus rubens* (A+R). One fish was placed in 0.5 l of water, with 40 replicates of each treatment and water renewal was every third day. Diets were provided *ad libitum* twice per day. The number of live larvae (survival percent) and dissolved oxygen were recorded daily. At the end of the experiments, larvae were sacrificed and their weight, NL and TL were determined. The effects of the diets were assessed using analysis of variance (ANOVA). To discriminate the efficiency of each diet, the final average values of length and weight obtained with each diet were analyzed by the Tukey-Kramer method (Sokal & Rohlf, 1981). Survival was analyzed by means of single classification analysis of variance (ANOVA), normalizing the obtained survival percent (p_i) by arcsine transformation of the square root of p_i (Sokal & Rohlf, 1981).

To determine the relation of the mouth opening to the size of the prey, we used organisms at exogenous feeding age (3 days). One cohort (F5) of 240 organisms was fed with three species of rotifers *Brachionus calyciflorus, B.plicatilis,* and *B. rubens* at a ratio of 1:1:1. Observations were made up to 20 days after hatching.

To measure TL and NL, 30 larvae were fixed at two-days intervals in 4% formaldehyde. To determine mouth opening, larvae became transparent with 1% KOH, the jaw was extracted and the bone tissue was stained with 0.5% toluidine blue and 1% alizarin red (modified from Clothier, 1950). Samples were mounted on slides and the length and width of the jaw were measured under a micrometric lens. Mouth opening was determined according to Dabrowski & Bardega (1984), where mouth size (c. o.) = tang 45° (length of jaw, c. a.). Afterwards, the relative growths between mouth opening and NL and between mouth opening and mouth width were determined.

Finally, 1 g (dry weight) of a lyophilized sample of each of the rotifer species was used to quantify contents of total nitrogen (micro-Kjendal method), protein (4.2 conversion factor) (Øie *et al*., 1997) and total carbohydrates (following the phenol method). Fatty acids were quantified by gas chromatography and with a mass spectrophotometer via compound fragmentation in a Finnigan equipment, model GCQ.

RESULTS

The tested diets had a significant effect on TL, NL and weight after 30 days (ANOVA, $p < 0.05$). Average TL of larvae varied from 14.72 ± 1.72 mm with the A + R diet to 11.54 ± 0.91 mm with R diet. The smallest change in length was observed with R+B diet.

Final NL varied with the different diets (ANOVA, $p < 0.001$). The largest increment was found with the A+R diet (13.24 ± 1.57) (Tukey, $p \leq 0.001$), whereas diet B combined with live feed did not significantly increase growth when compared with the diets having only live feed (Tukey, $p \geq 0.946$). The larvae maintained solely on B diet died after the yolk-eating period. Finally, the NL of the larvae fed with diet A was larger (11.73 ± 0.8) than that of the larvae fed with diet R (9.9 ± 0.9) (Tukey, $p \leq 0.001$) (Fig. 1).

Variation of TL was similar to that observed in NL. Larvae fed with A+R diet depicted the greatest increase in TL (14.72 ± 1.72 mm), while the smallest increase was obtained with diet R (11.54 ± 0.91 mm).

The average weight with each diet followed a similar pattern to that shown by NL and TL (ANOVA, $p < 0.001$) (Fig. 2). The A+R diet yielded the greatest increase in weight (16.05 ± 0.05 mg), whereas diet R alone produced the smallest weight gain (5 ± 0.9 mg) (Tukey, $p < 0.001$).

The diets had also a significant effect on survival. The highest survival by the end of the 30 days was ob-

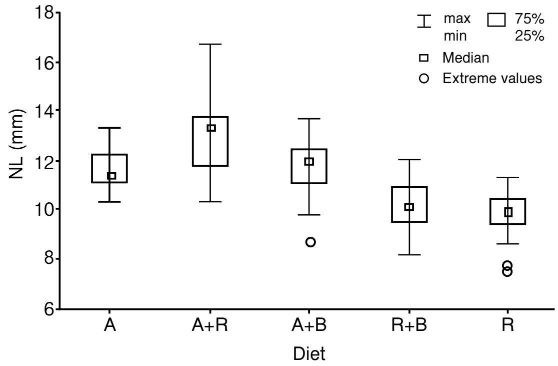

FIG. 1. Notochordal length (NL, in mm) of *Chirostoma riojai* larvae under five different diets after 30 days. A: *Artemia* nauplii*;* A+R: *Artemia* nauplii + *Brachionus rubens;* A+B: *Artemia* nauplii + formulated diet; R+B*: Brachionus rubens* + formulated diet; R: *Brachionus rubens.*

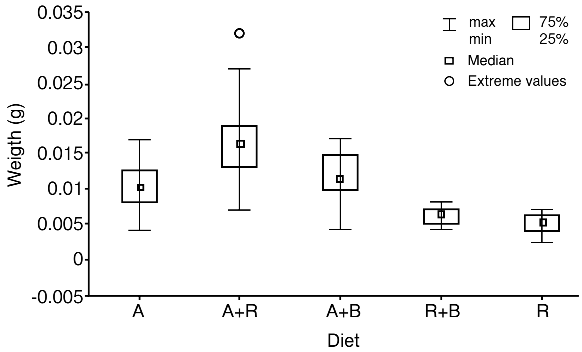

FIG. 2. Weights (W, in g) of *Chirostoma riojai* larvae under five different diets after 30 days: A: *Artemia* nauplii; A+R: *Artemia* nauplii+*B. rubens*; A+B: *Artemia* nauplii+formulated diet; R+B*: Brachionus rubens*+formulated diet, R: *Brachionus rubens*.

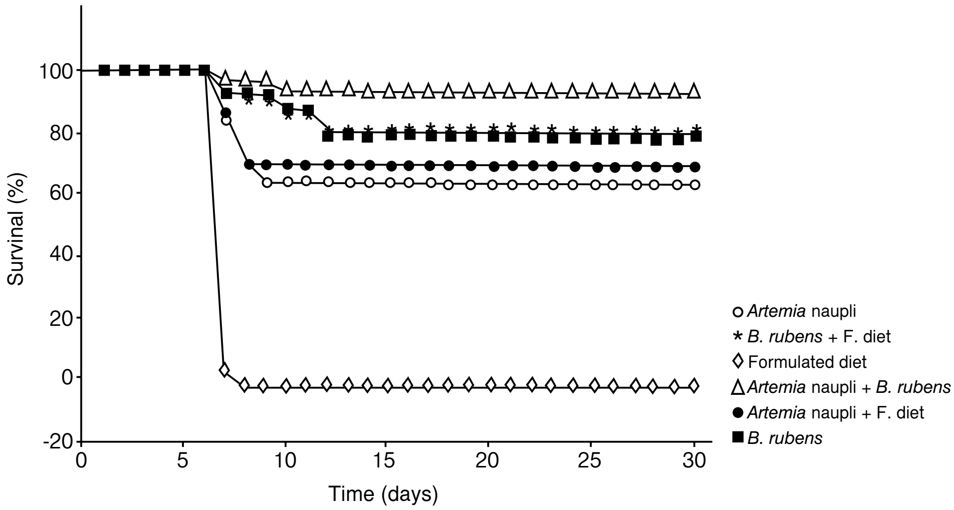

FIG. 3. Survival of *Chirostoma riojai* larvae reared with six different diets after 30 days.

served with the diets constituted of live feed, with 93.25% for the A+R diet and 80.66% for the R diet. Diets combining live with formulated feed yielded survivorship of 80.66% for larvae fed with R+B diet, 70% with diet A+B and 64.5% with diet A (ANOVA, $p < 0.001$) (Fig. 3). Larvae fed with diet B alone died at the end of the yolk-feeding period.

Diets A+B and B yielded the highest and lowest survival, respectively, whereas those diets constituted of live feed plus formulated food did not differ in survival from those in which only live feed was given (Tukey, $p < 0.05$).

The relative growth of mouth opening with respect to NL (Fig. 4) presented a positive allometric growth ($R^2 = 0.98$).

Besides, the relative growth of the mouth opening with respect to its width (Fig. 5) was positively correlated. During the study period, the speed of

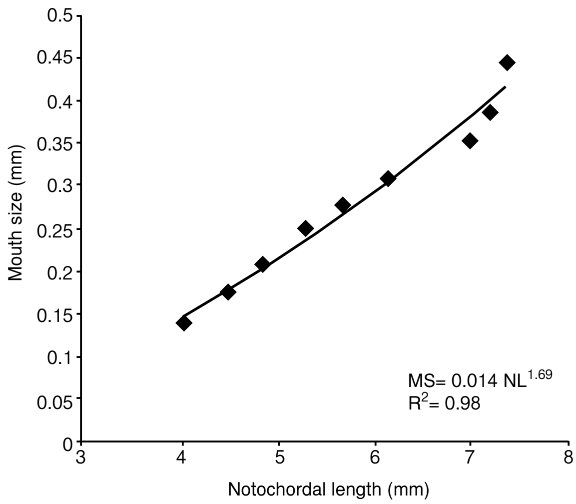

FIG. 4. Relationship between mouth size and notochordal length of *Chirostoma riojai* larvae during the first 17 days of exogenous feeding.

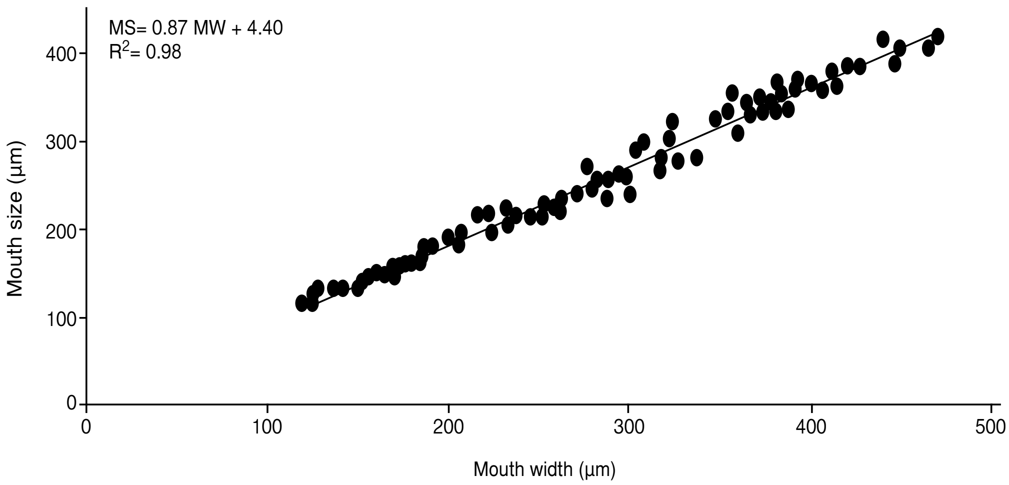

FIG. 5. Relationship between mouth size and mouth width of *Chirostoma riojai* larvae during the first 17 days of exogenous feeding.

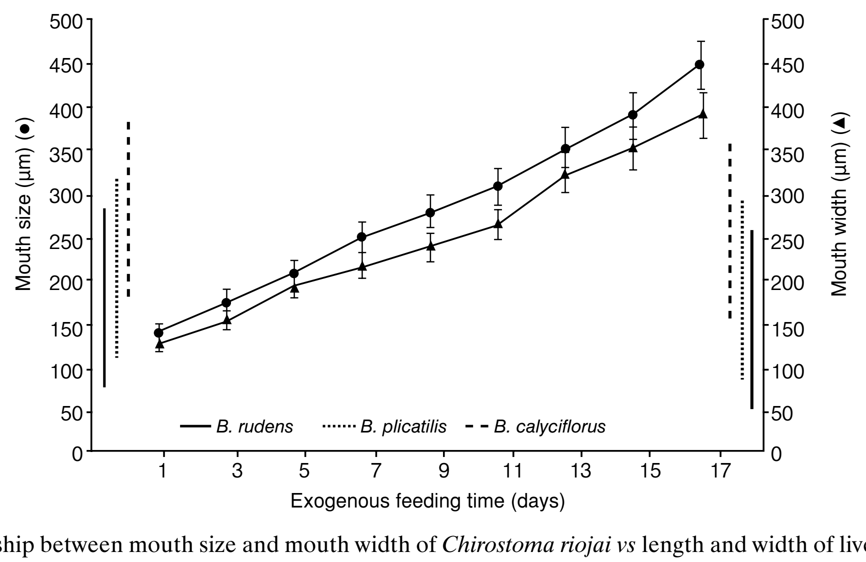

FIG. 6. Relationship between mouth size and mouth width of *Chirostoma riojai vs* length and width of live food during the first 17 days of exogenous feeding.

TABLE 1. Content of nutrients in the feed given to the larvae of *Chirostoma riojai* (given as a total percentage of the sample)

	Proteins	Carbohydrates	Lipids			
			EPA	AA	DHA	Linoleic
Brachionus calyciflorous	31.97	4.32	0.72	0.14	0.53	1.18
Brachionus plicatilis	50.93	3.21	0.26	0.03	1.33	4.00
Brachionus rubens	51.28	2.91	0.34	0.85	0.86	4.00

mouth opening growth was lower than that of width increment.

Relation of the mouth opening to the prey size

When comparing the dimensions of the three rotifer species with the width and mouth opening of the larvae (Fig. 6), it was found that the prey is accessible from the start of exogenous feeding since the width of the prey is smaller than both the opening and the width of the mouth.

Nutritional content of feed

Brachionus rubens was the species with the highest protein content (more than 50%) and *B. calyciflorus* with the lowest one (30%). The carbohydrate content was the highest in the latter species and the lowest in the former.

Regarding polyunsaturated fatty acids, *B. plicatilis* and *B. rubens* had the highest concentration of linoleic acid. In regard to essential fatty acids, *B. plicatilis* had the highest docosahexaenoic acid (DHA) followed by *B. rubens* and *B. calicyflorus*. Arachidonic acid (AA) was found to have the highest value in *B. rubens* and the lowest one in *B. plicatilis*. Eicosapentanoic acid (EPA) was highest in *B. calyciflorus*, whereas differences were minimal in B. *plicatilis* and *B. rubens* (Table 1).

DISCUSSION

Survival and growth of fish larvae are tightly related with the age of first exogenous feeding. Amount, quality and accessibility of food (Nicolsky, 1976; Wootton, 1990) are associated with this.

In this experiment, feeding started at day 3 of age, as this is the start of mixed feeding. We observed that administration of live feed at this stage is crucial. When exogenous feeding starts at later stages, close to the starvation threshold, which for this species is at day 7 at 22°C, larvae do not survive as the point of no-return has been reached. Likewise, digestion of proteins during the early stages in many fish species is characterized by a limited production of digestive enzymes by the pancreas and by an unusual pinocytic intracellular digestion. This requires a source of easily digestible nutrients, which are found in their natural zooplankton diet that contains high concentrations of digestive enzymes, free amino acids (FAA), and soluble proteins (Dabrowski & Glogowski, 1977; Geurden *et al*., 1988; Walford & Lam, 1992).

A disadvantage of inert feed is the following: if not immediately ingested, it sediments becoming inaccessible to the larvae; besides it is susceptible to dissolution due to its small particle size (0.05-0.70 µm in diameter). Loss of nutrients in such small particles starts immediately when in contact with water: particles smaller than 20 µm become inaccessible to the larvae and are lost (Kamler, 1995). This has been explained by the morphological and functional characteristics of the digestive tract, as well as by the nature of the diets. An incipient digestive tract development, a fast evacuation rate, and a low digestive enzyme production limit the digestion of the feed, especially in formulated diets. The low effects of formulated diets have been attributed to a deficiency in essential substances, such as fatty acids, amino acids, vitamins, and minerals, either separately or in combination (Bergot *et al*., 1986; Fluchter, 1982). In this sense, although microencapsulated feeds with protein cover have been designed to be easily ingested by larvae, these have not been completely functional (Dabrowski & Glogowski, 1977; Walford *et al.*, 1991) since the enzymatic activity occurring in the digestive tract is sometimes insufficient to break up this protein cover, causing damage to the intestinal wall and, consequently, death of larvae (Cunha & Planas, 1999).

Lack of exogenous enzymes is another feature that distinguishes the formulated diets from the live feed. The proteolytic activity of the zooplankton-provided enzymes is high, contributing substantially to the total enzymatic activity of the larval digestive tract (Lauff & Hofer, 1984). From these facts, it can be inferred that the enzymatic apparatus of *C. riojai* larvae in this stage is not functional or does not possess yet the necessary enzymes to digest the inert feed, since total mortality was observed once the yolk sac had been consumed. Therefore, it would be necessary to determine the type and amount of the enzymes in the digestive tract to confirm these effects.

The differences in survival observed among the diets with live feed allow us to infer that the nutritional contents of the rotifers and *Artemia* nauplii complement each other and increase survival. Individual consumption of feed is related to the size of the prey and to the mouth opening of larvae at the start of the first feeding. Some larvae (30%) die due to inaccessibility of food, as occurred with the larvae fed on *Artemia* nauplii alone in which the nauplii were only accessible to all larvae from day 9 on, when the size of the mouth allowed their ingestion.

Live feed accelerated growth, particularly the combination of *Artemia* nauplii plus rotifers, since the three analyzed variables (NL, TL, weight) exhibited lower increases with those diets in which each component was supplied alone, and larvae fed inert feed alone died after consuming the yolk sac. The greater increases in larval length and weight regarding mixed diets (live with inert feed) than those obtained with any of the two live feed diets are probably due to enzymes provided by the live feed. These enzymes break down partially the ingested inert feed (Gawlicka *et al*., 2000).

The levels of EPA, DHA, and AA acids contained in the supplied rotifers were adequate, since both growth and survival of larvae were strongly influenced by the presence of these fatty acids that are determinants for the development of the eleutheroembryo (Jaworski & Kamler, 2002; Abi-Ayad *et al*., 2000; Verreth *et al*., 1994) and are involved in the formation and maturation of the eyes and brain (Sargent *et al*., 1997; Geurden *et al*., 1988). Likewise, the present results reveal that, besides the nutritional characteristics of the feed, development of the mouth opening is fundamental for determining the time, type and size of the provided feed.

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