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The potential use of the euryhaline rotifer *Proales similis* for larval rearing of the freshwater pike silverside *Chirostoma estor estor*

Uriel Arreguin Rebolledo [a], Nandini Sarma [b], Gustavo Alejandro Rodríguez-Montes de Oca [c], María del Carmen Monroy-Dosta [d], Juan Antonio Tello-Ballinas [e], S.S.S. Sarma [b], José Cristóbal Román-Reyes [c,*]

[a] *Posgrado en Ciencias en Recursos Acuáticos, Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, Paseo Claussen S/N. Col. Los Pinos, C.P. 82000 Mazatlán, Sinaloa, Mexico*
[b] *Laboratorio de Zoología Acuática, Universidad Nacional Autónoma de México, FES Iztacala, Av. de los Barrios No.1, C.P. 54090 Tlalnepantla, Mexico*
[c] *Laboratorio de Reproducción y Cultivo de Peces, Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa, Paseo Claussen S/N. Col. Los Pinos, C.P. 82000 Mazatlán, Sinaloa, Mexico*
[d] *Universidad Autónoma Metropolitana Xochimilco, Live Food Production Laboratory, Biology degree, Secondary Production Module, Calzada del Hueso, No 1100. Col. Coyoacán, Ciudad de México. CP.04960, Mexico*
[e] *Instituto Nacional de Pesca y Acuacultura, Centro Regional de Investigación Acuícola Pesquera-Pátzcuaro, Calz. Ibarra No. 28, Col. Ibarra, C.P. 61609 Pátzcuaro, Michoacán, México*

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A B S T R A C T

Successful larval rearing of many fish species depends on the adequate selection of live food. Rotifers of the genus *Brachionus* are the most widely used in feeding protocols for freshwater and marine aquaculture. In this work, the utility of the euryhaline rotifer *Proales similis* as potential prey for the freshwater pike silverside *Chirostoma estor estor* larval rearing was investigated. The rotifers *P. similis*, *Brachionus ibericus* and *B. plicatilis* (body length: 84 ± 1.24, 193 ± 2.90 and 206 ± 4.20 μm, respectively) were used to evaluate *C. estor estor* larvae prey selectivity during the first 3 to 18 days post-hatching (DPH). Functional responses were tested at prey densities of 0.5, 1, 2, 4, 8 and 16 ind. mL^{-1}. Larval survival was evaluated using four treatments: *P. similis* (T1), *B. ibericus* (T2), *B. plicatilis* (T3) and a mixed diet of all three taxa (T4). Results showed that *P. similis* was the *C. estor estor* most selected prey during the 3 to 7 DPH when compared to *B. ibericus* and *B. plicatilis*. From 8 to 18 DPH, the selectivity for *P. similis* shifted to increase selectivity for *B. ibericus* and *B. plicatilis*. At 4 to 12 DPH, functional responses indicated a greater *P. similis* consumption by larvae at high density (16 ind. mL^{-1}). Larval survival was significantly higher in T1 than other treatments (T2 – T4). These findings provide evidence that *P. similis* can be included during the *C. estor estor* larval rearing. *Proales similis* could be therefore used at least during the 3 to 7 DPH, but the feeding protocols should be then complemented with brachionid rotifers. The present data are valuable for aquaculture practices and help approach the management and conservation issues of this endemic endangered fish species of the Central Plateau of Mexico.

1. Introduction

The Central Mexican Plateau is home to at least 18 endemic species of the genus *Chirostoma* (Pisces: Atherinidae) including the endemic freshwater pike silverside *C. estor estor* Jordan 1880, from Lake Pátzcuaro, Michoacán (Miller et al., 2009). The Pátzcuaro basin is about 116 km^2, 5 m mean depth (Gomez-Tagle et al., 2002) and surrounded by a human population of 93,265 inhabitants (Pátzcuaro municipality, INEGI, 2019). Many endemic fish populations are found in Pátzcuaro Lake, including the genera *Algansea*, *Alloophorus*, *Allotoca*, *Chirostoma*, *Goodea* and *Poeciliopsis*, which have significantly declined or even became endangered as a result of overfishing and other anthropogenic activities (Ceballos et al., 2018). Further biological and ecological knowledge of such endemic fish is critical for addressing conservation issues and promote their use in sustainable aquaculture (Domínguez-Domínguez et al., 2002; Tancioni et al., 2019).

Chirostoma estor estor, also known as whitefish, is an ideal candidate for aquaculture farming because: (a) this species is easily maintained

* Corresponding author.
E-mail address: jocrore@gmail.com (J.C. Román-Reyes).

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and reared in captivity (Martínez-Palacios et al., 2006), (b) contains a higher DHA/EPA ratio compared to other freshwater fishes, thus, benefiting human health through its consumption (Fonseca-Madrigal et al., 2012), (c) it is generally sold in regional markets at similar or even higher prices (kg^{-1}) than some other commercially important marine species (Serranidae, Lutjanidae, Sciaenidae and Carangidae) in Mexico (Martínez-Chávez et al., 2018; PROFECO, 2019), and (d) its aquaculture activity contributes to improving employment and regional economic growth in local communities (Ross et al., 2008). Since 2002, current advances in the aquaculture of *Chirostoma* have been documented in a series of studies focusing on its biology, ecology and biotechnology (Martínez-Palacios et al., 2002; Martínez-Palacios et al., 2006; Ross et al., 2008; Ríos-Durán et al., 2016; Martínez-Chávez et al., 2014, 2018).

The freshwater atherinid *C. estor estor* is a zooplanktivorous fish that during the early larval stages can be reared at salinities from 5 to 15 ppt (Martínez-Palacios et al., 2004; Ross et al., 2006), allowing a wide variety of salinity tolerant zooplankton (e.g., rotifers, copepods and cladocerans) to be included in its feeding protocols. Rotifers are important as live feed for the rearing of several marine and freshwater fish larvae (Lubzens et al., 2001). Worldwide, strains from the *B. plicatilis* species complex are most commonly used rotifers in marine aquaculture (Hagiwara et al., 2007; Snell et al., 2018), while *B. calyciflorus*, *B. rubens* and *B. angularis,* are mainly used for the rearing of freshwater fish larvae (Ogata, 2017). The rotifer selection criteria for aquaculture purposes mainly focuses on the environmental condition responses (e.g., salinity and temperature), body size, morphology, population growth rates, biogeographic distribution and ease of cultivation at low and high scale (Lubzens et al., 2001). The use of rotifers in aquaculture also depends on the gape size of the fish larvae (Conceição et al., 2010). The SS and MS morphotypes of the *B. plicatilis* species complex are used as the first prey for *Chirostoma* larvae due to their small body length, which typically ranges from 100 to 200 μm, well within the margin of the predator mouth size (Martínez-Palacios et al., 2004). Adequate prey selection is vital for improving both fish survival rate and growth (Cunha and Planas, 1999; Rao, 2003). More than one criterion has motivated researchers to explore and test several rotifer taxa in fish larviculture in this context. Rotifer taxa such as *Anuraeopsis*, *Brachionus*, *Keratella*, *Polyarthra*, and *Trichocerca* are found in the Pátzcuaro Lake (Sarma and Elías-Gutiérrez, 1999); however, only a few species (e.g., *Brachionus rotundiformis*, *B. rubens* and *B. plicatilis* (SS-SM types and L-type, respectively)) have been proposed so far in the aquaculture of atherinids (Morales-Ventura et al., 2004; Nandini et al., 2019).

In addition to *Brachionus* species, several euryhaline rotifers have been successfully grown in laboratories (Chigbu and Suchar, 2006); however, only a few such as *Proales similis* are under evaluation for larviculture (Wullur et al., 2011). The euryhaline rotifer, *P. similis*, was introduced into marine aquaculture as a promising species in fish larviculture (Wullur et al., 2009). It is ideal for use during the first feeding of fish larvae that grow at low and high salinities, such as some members of the Atherinidae and Lutjanidae family, respectively (Martínez-Palacios et al., 2004; Alvarez-Lajonchère et al., 2012; Rebolledo et al., 2018). Large scale production of this rotifer is possible at 25 to 30 °C. In mass culture, it may reach from 500 to 2500 ind. mL^{-1} at 2 – 25 ppt salinity, while at 35 ppt it takes longer to reach the peak population density of up to 1500 ind. mL^{-1} (Román-Reyes et al., 2017). *Proales similis* is a minute rotifer (body length and width ca. 85 μm and 45 μm, respectively); the body size is about 20 to 60% smaller than the SS and SM morphotypes of the *B. plicatilis* species complex (Mills et al., 2017; Snell et al., 2018). According to the literature, *P. similis* is suitable for larvae whose mouth size is equal to or greater than 90 μm (Yúfera and Darias, 2007). Hagiwara et al. (2014) suggested that the absence of a rigid lorica (iloricate) of *Proales* may facilitate ingestion by fish larvae.

Ecological studies such as feeding behavior are useful tools to select ideal live food in fish aquaculture (Wells et al., 2008). For example, prey selectivity indicates appropriate prey type (size and morphology) according to consumer age. It is known that adequate prey selection optimizes energy intake in larval fish (Rao, 2003). Functional response studies can provide useful data about the optimal feed concentrations applied in fish culture (Ma et al., 2013; Rabe and Brown, 2000). The success of the selected prey is also validated through a survival bioassay of the fish larvae (van der Meeren, 1991; Figueroa-Lucero et al., 2004).

Proales similis has only been used in marine fish larvae rearing such as *Epinephelus septemfasciatus* (Serranidae), *Centropyge ferrugata* (Pomacanthidae), *Cheilinus undulatus* (Labridae) and the eel *Anguilla japonica* (Anguillidae), whose mouth size is too small to optimally feed on other rotifer taxa (Wullur et al., 2009; Hagiwara et al., 2014). In the present work, the use of the euryhaline rotifer *P. similis* as potential prey for the freshwater pike silverside *C. estor estor* was evaluated for the first time. These results will help enhance aquaculture and conservation efforts of this endemic species by improving larval survival and, therefore, juvenile production.

2. Materials and methods

2.1. Rotifer and microalgal cultures

The rotifers *P. similis*, *B. ibericus* (isolated from an estuarine system, Sinaloa, Mexico) and *B. plicatilis* (Strain CRIP-Patzcuaro) were cultured separately in 500 mL Erlenmeyer flasks at 10 ppt salinity and 25 °C. Synthetic brackish water was prepared by dissolving 10 g L^{-1} amount of artificial sea salt (Instant Ocean®, Spectrum Brands Inc., USA) in distilled water. The marine microalgae *Nannochloropsis oculata* was used as food ($3 – 5 \times 10^6$ cells mL^{-1}). Under these laboratory conditions, it was possible to reach a population density above 200 ind. mL^{-1} of brachionid rotifers and up to 500 ind. mL^{-1} of *Proales*. Microalgae were cultured in 2 L Erlenmeyer flasks using artificial seawater (35 ppt) enriched with F/2 medium (Guillard, 1975). Stock cultures were maintained under 12/12 h light/dark photoperiod provided by commercial light bulbs (ca. 1000 lx), 22 °C. We harvested the microalgae during the exponential phase of growth, normally 6 to 8 days after inoculation and centrifuged the culture at 3000 rpm for 5 min. The final product was kept refrigerated (4 °C) and used as food.

2.2. Fish larvae

Chirostoma estor estor larvae were obtained from a broodstock (2+ years old) maintained in captivity by the Regional Fisheries Research Center (CRIP-Pátzcuaro) Michoacán, Mexico. Eggs were collected by using artificial substrate. Approximately 1000 eggs were counted per breeder female (per week). Fertilized eggs (70%) were collected during their first annual reproductive season (March–April) and incubated at 10 ppt in order to reduce the risk of *Saprolegnia* infection, and later at 5 ppt (until eyes appeared) to improve hatching success (Martínez-Palacios et al., 2004). Eggs were maintained at 25 °C in McDonald jars connected to a flow-through system. Under these conditions, most eggs hatched within seven days. Then, fish larvae were transferred to a recirculation system (80 L fiberglass tubes (60 × 60 × 12 cm)) at a density of 40–50 larvae/L. Water was maintained at 5 ppt and 25 °C as a general batch for upcoming feeding trials. Since the experiments started from 3 to 4 days post-hatching (DPH), salinity used was 5 ppt to rear *C. estor estor* larvae in order to avoid stress in the larvae by an abrupt salinity change, which could influence on the feeding response (Martínez-Palacios et al., 2004). A mixture of the test rotifers was given to the test larvae batch, ad libitum, as food.

2.3. Prey selectivity experiments

Proales similis, *B. ibericus* and *B. plicatilis* had a mean body length (in parenthesis, size range in μm) of 84 ± 1.24 μm (62 – 98), 193 ± 2.90 μm (152 – 200) and 206 ± 4.20 μm (171 – 228), respectively. Prey selectivity tests were performed daily, from 3 to 18 DPH. The bioassays were

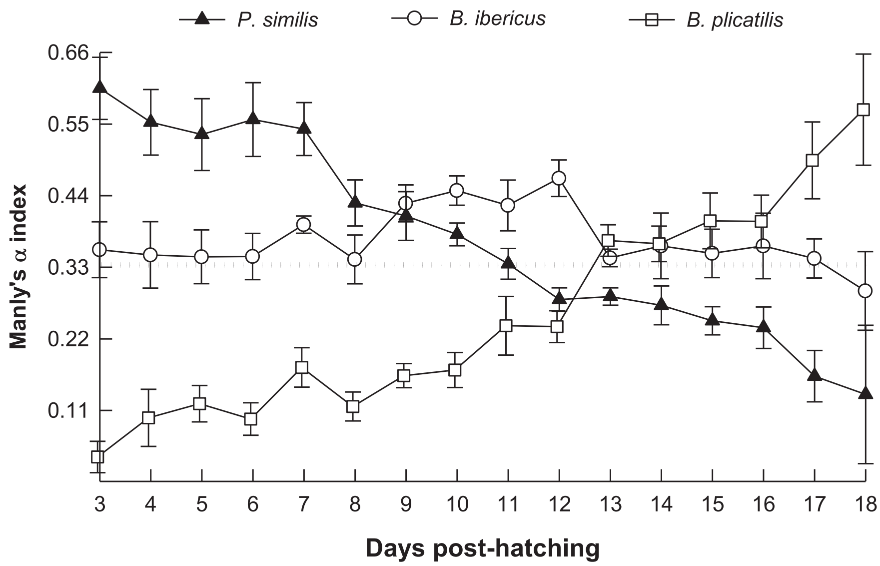

Fig. 1. Feeding preference (<0.33 Manly's α) of *C. estor estor* larvae during the first 18 days post-hatching offered three rotifers of prey (*P. similis*, *B. ibericus* and *B. plicatilis*). Values shown are a mean ± SD.

carried out in cylindrical transparent containers (4.5 cm diameter by 5 cm height).

Before beginning, a larvae stock was maintained in starvation for 2 h in a glass beaker (10 larvae/L). Rotifers were acclimated for at least 6 h to the experimental conditions (from 10 to 5 ppt) in order to ensure their availability as prey (Fielder et al., 2000). In total, 150 prey items were added (50 individuals of each rotifer taxa) into glass containers, previously filled with 50 mL of water at 5 ppt salinity and 25 °C (× 5 replicates). Two larvae were subsequently introduced into each container. After 45 min, the larvae were then removed, and a formalin solution (4%) was added for the subsequent remaining prey quantification. The number of residual rotifers in each container was counted under a stereomicroscope (10–63 times magnification, Nikon SMZ800, Japan). Morphological differences between *B. ibericus* and *B. plicatilis* were distinguished according to Ciros-Pérez et al. (2001), using a YS2-H microscope (Nikon Corporation, Japan). The number of ingested prey was determined based on the density differences between initial and final prey (Domínguez-Domínguez et al., 2002). To quantify preference, Manly's alpha index was calculated using the following equation (Krebs, 1999):

$$\alpha_i = \frac{r_i}{n_i} * \frac{1}{\sum^m (r_j/n_j)}$$

where α_i is Manly's α index for prey type *i*, r_i and r_j are the ratio prey species *i* and *j* in the diet (consumed by the predator), n_i and n_j are the ratio prey species *i* and *j* in the environment *i* and $j = 1, 2, 3......m$; where *m*, is the number of potential prey species. A value of $\alpha > 1/m$ indicates that the prey species *i* is preferred in the diet, while a value of $\alpha < 1/m$ demonstrates that the species of prey *i* is avoided in the diet. In this data set, $1/m = 0.33$ as there were three prey taxa.

2.3.1. Larval mouth size

A total of 60 larvae were anesthetized with 0.4 mL 2-phenoxyethanol L^{-1} (Sigma Aldrich Chemical Co, USA) and measured to determine the mouth opening during the first 18 DPH. Measurements were processed and analyzed using an Opticam® photomicroscope. The maximum mouth opening (MMO) was calculated estimating an angle of 90° by the following equation (Shirota, 1970): $MMO = UJL \times \sqrt{2}$. Where: UJL = Upper Jaw Length.

2.4. Functional response experiments

Our experimental design followed established protocols from previous studies (Houde and Schekter, 1980; Morales-Ventura et al., 2004). Rotifer taxa were introduced separately at densities of 0.5, 1, 2, 4, 8 and 16 ind. mL^{-1}. Fish larvae were not fed, as described in the previous section. Two larvae were introduced into each container of 50 mL (× 3 replicates). After 45 min, the remaining prey density was counted within each container. The difference between the initial and final prey count corresponded to the prey consumed by the fish larvae (Nandini and Sarma, 2000). Bioassays were conducted every two days (4 to 18 DPH). Data obtained was transformed using the Michaelis-Menten equation (Lampert and Sommer, 1997): $y = ax / (b + x)$, where *x* is the number of prey offered, and *y* is the number of prey consumed, *a* is the *y*-intercept and *b* is the slope.

2.5. Larval survival

All larvae used in the survival bioassays were obtained from a single egg batch. Survival was assessed during the first 16 DPH. Bioassays were conducted using cylindrical transparent containers (12 cm of diameter by 10 cm in height). A total of 25 larvae (3 DPH) were introduced within each container (2 L). In all, four treatments (× 3 triplicates) were established: *P. similis* (T1), *B. ibericus* (T2), *B. plicatilis* (T3) and a mixture of the three taxa (T4). The rotifer density was 20 ind. mL^{-1} of each taxa for T1, T2 and T3, while seven individuals of each rotifer species were mixed (=21 ind. mL^{-1}) for T4. Every three days *N. oculata* was added to the culture containers at 1×10^6 cells mL^{-1} in order to feed the rotifers. Trials were maintained at 5 ppt salinity, 25 °C and under a photoregimen of 12 L/12D. The pH of the rearing medium was 7.5 ± 0.3; meanwhile, the dissolved oxygen levels were 6.0 ± 0.2 mg L^{-1} and the total ammonia concentrations were below 0.5 mg L^{-1}. Every three days, the 75% of the medium was removed and replaced with fresh culture medium.

2.6. Statistical analysis

Prey selectivity data was used to derive Manly's α (Krebs, 1999). A three-way analysis of variance (ANOVA) was used to quantify the differences among the treatments based on functional response experiments. For survival experiments, one-way ANOVA was used to

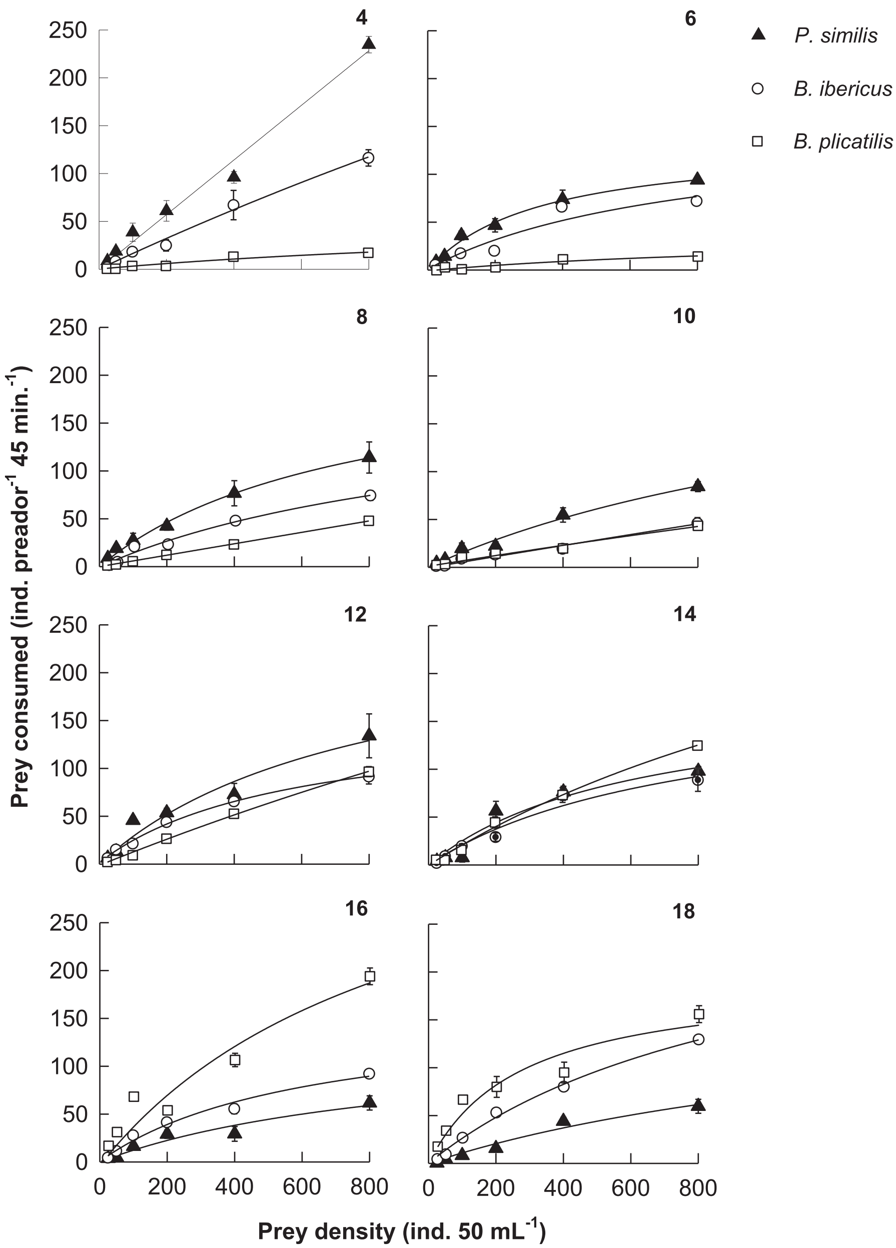

Fig. 2. Functional response curves of *C. estor estor* larvae during the first 4–18 days post-hatching offered three rotifers of prey (*P. similis*, *B. ibericus* and *B. plicatilis*). Values shown are a mean ± SD, and the transformation of the curve using the Michaelis-Menten equation are shown.

determine significant differences among treatments. A probability level of $p < 0.05$ was considered as being statistically significant. All the above analyses were performed with SigmaPlot 10.0 (Systat Software, Inc). Data were expressed as mean ± SD based on 3 – 5 replicate recordings.

3. Results

3.1. Prey selectivity

As can be seen in Fig. 1, prey selectivity by *C. estor estor* showed a shift based on the larval age and offered taxa. For example, Manly's preference index (0.54–0.60) indicated that 3 to 7 DPH larvae showed a higher selection for the iloricate rotifer *P. similis*. At the same larval age, the loricate rotifer *B. ibericus* was also the preferred prey item when compared to *B. plicatilis*, but 40% less than *P. similis*. Manly's index was low for *B. plicatilis* (0.04–0.18) at 3 to 7 DPH of *Chirostoma* larvae. From 8 DPH onwards, prey selectivity changed and increased for *B. ibericus* (0.34–0.46), while it started decreasing for *P. similis* (0.40 to 0.13). Manly's index decreased for *B. ibericus* at 13 DPH, but increased for *B. plicatilis* (0.36–0.56).

3.1.1. Larval mouth size

The maximum mouth opening increased from 97 ± 10 μm to 388 ± 15 μm during the first 3 to 18 DPH (Fig. 2). The minimum and maximum size was 85 μm and 410 μm, respectively. From day 3 to day 8 post-hatching, the gape size increased from 97 ± 10 to 177 ± 18 μm, while from 10 to 18 DPH it increased from 195 ± 23 to 388 ± 15 μm.

3.2. Functional response

The functional response curves of *C. estor estor* larvae indicated that prey consumption increased with increasing prey density in the medium, and in most cases, an asymptote curve was reached (Fig. 3). Prey consumption by *C. estor estor* larvae corresponded to a Type II response.

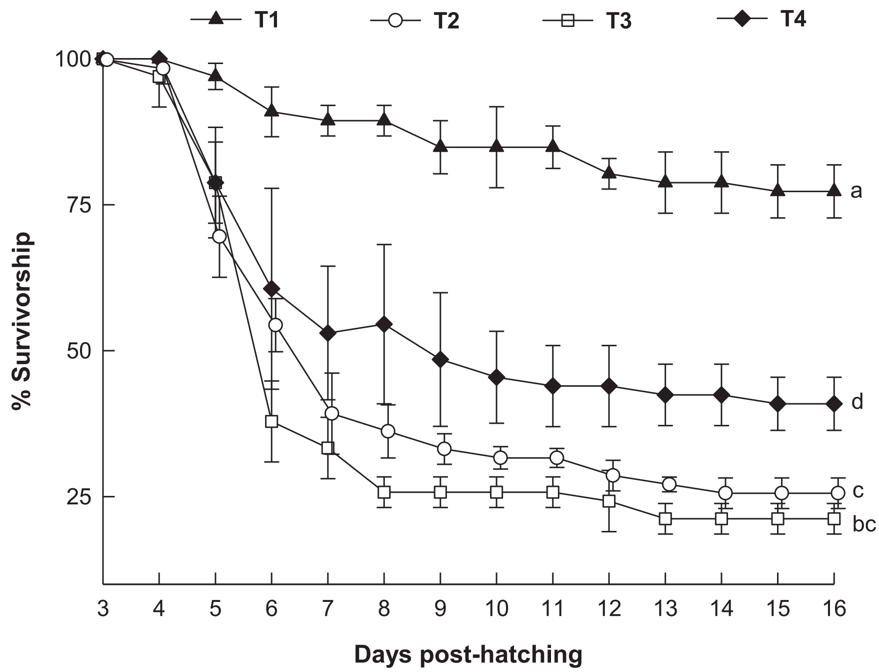

Fig. 3. Daily survival of *C. estor estor* larvae fed with different diets. *P. similis* (T1); *B. ibericus* (T2); *B. plicatilis* (T3); and mixed diet (T4). Values shown are a mean ± SD. Different letters indicate significant differences in relation to larval survival at 16 DPH (p <0.05).

Table 1
Three-way analysis of variance (ANOVA) performed on the maximal prey number consumed, prey type and the larval age of *C. estor estor*.

Source	DF	SS	MS	F
Prey (A)	2	11,497.14	5748.57	54.39***
Age (B)	3	8490.34	2830.11	26.77***
Density (C)	5	252,892.19	50,578.43	478.55***
A × B	6	78,633.88	13,105.64	124.00***
A × C	10	10,320.74	1032.07	9.76***
B × C	15	7872.56	524.83	4.96***
A × B × C	30	58,138.44	1937.94	18.33***
Residual	144	15,219.33	105.69	

DF = degrees of freedom, SS = sum of squares, MS = mean squares, F = F-ratio.
*** $p < 0.001$.

The whitefish showed significant differences in prey consumption in relation to prey type, prey density, and age of the age of the predator as well as their interaction (p <0.001, 3-way ANOVA, Table 1). Larvae at 4 DPH showed a significant increase (p <0.05, ANOVA and Tukey tests) in *P. similis* consumption (120 ind. predator^{-1}); 50 and 90% more than *B. ibericus* and *B. plicatilis*, respectively. While the consumption was more numerically for *P. similis* until 12 DPH, the larvae began to increase their consumption of *B. ibericus* and *B. plicatilis* too. From 4 to 10 DPH, there was a greater consumption of *B. ibericus* by the larvae than *B. plicatilis*. A shift was observed at 13 to 18 DPH, when consumption for *B. plicatilis* increasing significantly (p <0.05, ANOVA and Tukey tests); 72% more than *P. similis*. In general, the iloricate rotifer *P. similis* was well accepted during the first 8 DPH, and brachionids were consumed more during later stages.

3.3. Larval survival

The survival curves of *C. estor estor* larvae are noticeably different among the distinct treatments, as shown in Fig. 4 Survival was significantly higher when larvae were fed with *P. similis* (T1, p <0.05, ANOVA and Tukey tests). For example, larval survival at 7 DPH was 89% for T1, while in the other treatments (T2 – T4) it varied from 35 to 55%. At the end of the experiment (16 DPH), survival was also significantly higher (p <0.05) in T1 (80%) when compared to the other treatments (21 – 40%). Compared to T2 (*B. ibericus*) and T3 (*B. plicatilis*) treatments, survival improved significantly (p <0.05) with a mixed-based diet (T4). The survival rate was significantly reduced (p <0.05) while using a *B. plicatilis*-based diet. In all treatments, the larval survival rate stabilized after 11 DPH.

4. Discussion

Fresh and marine waters are home to several zooplankton groups that can be useful in the aquaculture of many fish species of commercial interest (McKinnon et al., 2003; Ogata, 2017). In this study, the use as potential prey of the euryhaline rotifer *P. similis* was evaluated during the first feeding of the pike silverside *C. estor estor*. The rotifer *P. similis* is found in coastal waters of Mexico and other parts of the world (Wullur et al., 2009; Román-Reyes et al., 2017), which facilitates its availability for aquaculture purposes.

The shift to exogenous feeding is considered as a critical point in larviculture of many fish species (Yúfera and Darias, 2007). For this reason, the selection of live food is a crucial step in fish larval aquaculture (Rimmer and Glamuzina, 2019). In this work, the feeding preference results indicated that *Chirostoma* larvae presented a considerable selectivity for *P. similis* at 3 to 7 DPH, as compared to *B. ibericus* and *B. plicatilis*. Wullur et al. (2011) reported that at 4 DPH, *E. septemfasciatus* larvae also showed higher selectivity for *P. similis* with respect to *B. rotundiformis*. Afterwards, a marked shift occurred and prey selectivity was inclined towards *Brachionus* taxa, as observed in the present study. We found that *Proales* may be an ideal prey for *C. estor estor* when its mouth opening ranges from 95 to 160 μm, as predicted by Yúfera and Darias (2007). On the other hand, *Brachionus* species were well accepted while the fish mouth opening increased (170 to 420 μm). Both prey size and morphology of the tested rotifers influenced the prey selectivity of *C. estor estor*, as reported in other freshwater fish species (Rao, 2003). Based on feeding preference data, it might be inferred that *P. similis* could also be an ideal candidate for other fish larvae species of aquaculture interest from Mexico with a very small gape size. Such fish species list includes the freshwater endemic Mexican fishes, *C. humboldtianum* and *C. riojai* (Atherinopsidae) (Figueroa-Lucero et al.,

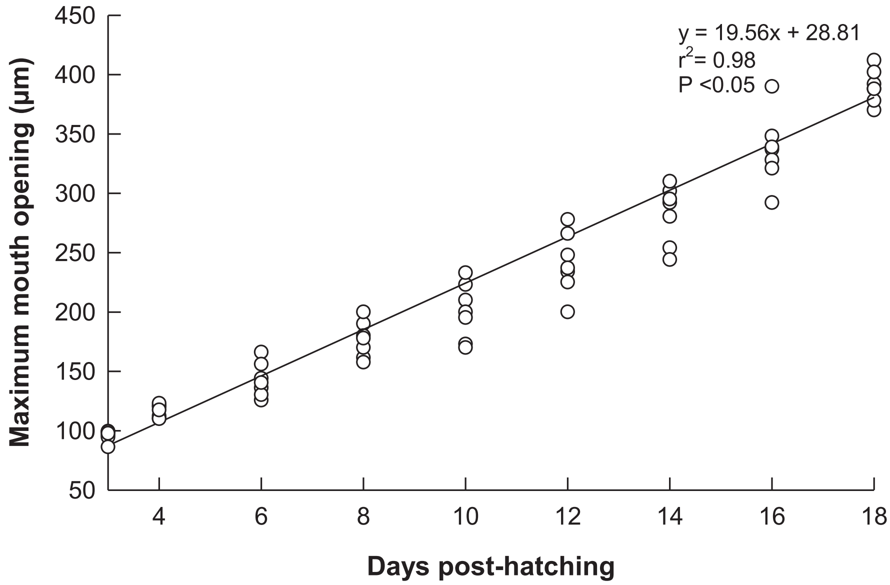

Fig. 4. Relation between the maximum mouth opening (μm) and larval age of *C. estor estor* during the first 18 days post-hatching.

2004; Morales-Ventura et al., 2004). Furthermore, we can also mention the leopard grouper *Mycteroperca rosacea,* endemic to the Mexican Pacific Ocean, the spotted sand bass *Paralabrax maculatofasciatus* (Serranidae), the spotted rose and red snappers *Lutjanus guttatus* and *L. campechanus* (Lutjanidae) respectively, the bullseye puffer fish *Sphoeroides annulatus* (Tetraodontidae), the California halibut *Paralichthys californicus* (Paralichthyidae) and the common snook, *Centropomus undecimalis* (Centropomidae) (Wells et al., 2008; García-Ortega, 2009; Martínez-Lagos and Gracia-López, 2009; Ibarra-Castro et al., 2011).

Using the rotifers *P. similis*, *B. ibericus*, and *B. plicatilis* as prey, Type II functional response, usually observed in feeding patterns of fish predators, was observed (Houde and Schekter, 1980; Rao, 2003; Peña-Aguado et al., 2009). This response indicates that the consumption rate increases with prey availability in the medium, but once a plateau is reached, it remains the same regardless of the prey density (Rao, 2003; Morales-Ventura et al., 2004). In a particular case, high rates of consumption were observed when the larvae were fed with *P. similis* at 4 DPH. This is a key point; at this larval age we suggest that high food density (16 ind. mL^{-1}) be made available; later, according to our results, this density can decrease up to 20–30% (> 6 DPH). The above-mentioned is important in terms of the cost of live food and labor in larviculture (Rabe and Brown, 2000). Functional response data also support that *P. similis* is an ideal prey during the first 4 to 8 DPH of *C. estor estor* when their mouth size ranges from 100 to 150 μm. Later, the rotifers *B. ibericus* and *B. plicatilis* can be supplied as a complementary prey. Usually, several fish species increase their ability to search, attack, capture, and ingest prey as they grow (Rao, 2003). This aspect should be considered to determine the feeding strategies necessary for fish of interest in aquaculture, including *C. estor estor* (Ross et al., 2006).

After the complete depletion of the yolk-sac, the mortality rate may increase if the larvae do not feed appropriately (Rimmer and Glamuzina, 2019). In this sense, adequate prey selectivity can improve survival larval (Cunha and Planas, 1999; Figueroa-Lucero et al., 2004). *Chirostoma estor estor* larvae fed exclusively with *P. similis* show better survival than other treatments. Mortality was critical at 4 to 7 DPH, when larvae were separately fed with either *B. ibericus* or *B. plicatilis*. Although different rotifer body sizes were included in the diet, a mixed diet was likely not favourable, as reported by Wullur et al. (2011). Nevertheless, a mixed diet improved the larval survival when compared to *B. ibericus* and *B. plicatilis*-based diet. Based on these results, the best time to provide a mixed diet to the *C. estor estor* larvae might be from 7 DPH (<7 DPH, *P. similis*; >7 DPH, brachionid rotifers and other zooplankton groups).

The rotifers *B. ibericus* and *B. plicatilis* are up to 2.35 and 2.45 times larger (in length), respectively, than *P. similis*. Regarding biomass, *B. rotundiformis* (ca. 150 μm in length) weighs around seven times more than *P. similis* (Wullur et al., 2011). The morphological aspect of *Proales*, including a soft and flexible body, may have facilitated its capture by the *C. estor estor* during the first days post-hatching which could contribute to a better survival rate as it has been reported for some marine fish larvae with a small mouth gape (Wullur et al., 2011; Hagiwara et al., 2014). In terms of fatty acid composition, brachionid rotifers may provide more energy than *Proales* (Yin et al., 2013), however, *P. similis* can also transfer an appreciable percentage of essential nutrients to larvae (Hagiwara et al., 2014). In the present study, all prey were fed with the marine microalgae *N. oculata* rich in n-3 fatty acids DHA and EPA (Renaud and Parry, 1994). These fatty acids are an essential component of the fish larvae diets, especially during its early developmental stages (Lubzens et al., 2001). Although *P. similis* can supply the above-mentioned nutrients (Wullur et al., 2011), we recommend their application during the first 7 DPH and subsequently, a transition to *Brachionus* species may well provide nutrient requirements to fulfill specific metabolic demands. Although the use of *P. similis* has often been shown to successfully improve the fish larvae rearing in aquaculture (Wullur et al., 2009; Hagiwara et al., 2014), there is still much more to explore and investigate. For example, this research did not include treatments with enriched rotifers, whereas it is well known that this technique may play a significant role in fish larvae growth and survival (Lubzens et al., 2001). Future works should consider this aspect. Further studies are also needed for a better understanding of the nutritional qualities or advantages of including *P. similis* in the first feeding stages of the pike silverside *C. estor estor*, particularly at pilot-scale and massive juvenile production.

5. Conclusion

For almost two decades, the *C. estor estor* larvae feeding protocols have been limited to the use of *B. plicatilis* rotifer species complex as prey; however, the present study demonstrated for the first time that *P. similis* could also be successfully used during the first feeding period of this fish species. *Proales similis* could be used during the first 3 to 7 DPH, while *B. ibericus* and *B. plicatilis* could be later added to complete the feeding protocol. The small body size and morphology of *P. similis* and its soft body confers a considerable advantage during the first feeding stages of *Chirostoma*. Our study highlights the potential application of this small rotifer as a first diet in the aquaculture of small gape size fish species and in conservation efforts of the endangered *C. estor estor*. Further studies need to be conducted on the utility of *P. similis* as a diet for freshwater fish species.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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