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Advances in applied research for the culture of Mexican silversides (*Chirostoma*, Atherinopsidae)

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The family Atherinopsidae[1] consists of 150 to 160 species, and most of them live in marine and brackish water. The genus *Chirostoma* is divided into two groups: *Jordani* and *Arge.* The *Jordani* group contains all of the relatively large species known as "white fish" in Mexico, while the smaller species belong to the *Arge* group and are known as "charales". All members of the genus are endemic to central México and live in fresh water, but they share many features with marine Atherinopsids because of their common ancestry (Barbour, 1973).

The Pátzcuaro Silverside (*Chirostoma estor estor*) is a fresh water fish from the Jordani group. It is an important economic resource in the region and many families live almost exclusively on its fishery. This species is currently in danger due to environmental changes and also because of its high value and demand in the regional markets that induces local fishermen to capture small juveniles and adults indiscriminately. The "charales" are also an important fisheries resource. However, during the capture it is impossible to distinguish "charales" from young Pátzcuaro silversides. All of these problems have significantly reduced the natural populations of these species. In this context, an alternative to increase *C. estor estor* populations and then to create alternative employment in the area is to develop its culture. However, the basic biology of the species is not yet fully understood, limiting the development of its culture.

C. estor estor has been characterized as a strict carnivore. Some authors have found that larvae feed on protozoans and rotifers after yolk sac consumption up to 65 mm total length and then, in the early juvenile

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[1] Nelson (1994) found that the majority of the members of the family are marine, although about 50 species are found in freshwater. The family is distributed from the tropics to temperate areas. Schultz (1948, 1950) recognized 165 species in 25 genera and 4 subfamilies: Atherinopsinae, Menidiinae, Atherioninae, and Atherininae. More recently, Dyer and Chernoff (1996) established the family Atherinopsidae which includes the genera *Chirostoma* and *Odonthestes*, two of the most studied genera in terms of culture. The subfamily Menidiinae is confined to America, principally in the tropics. It has many fresh water members in Mexico and Central America, the genera *Chirostoma, Labidesthes, Menidia* and *Poblana*, belonging to the tribe Menidiini; and *Atherinella, Nectarges* and *Xenanthjerrina*, belonging to the tribe Membradini. The eighteen species of *Chirostoma* are freshwater fish, living in the southern Mexican plateau (Barbour, 1973; Echelle and Echelle, 1984).

stages they feed on zooplanktonic microcrustaceans such as ostracods, cladocerans (*Bosmina* and *Daphnia*), copepods (*Cyclops*) as well as some insects (Rosas, 1970; Rosas, 1976). Big fish usually eat isopods (*Hyalella*) and amphipods (*Asellus*). Solórzano (1963) found that adult fish (up to 150 mm of total length), feed on small fish, crustaceans (Astacids) and insects, considering the ingestion of microcrustaceans and algae as an accidental event. According to the same author, adult fish over 200 mm of total length are basically ichthyophagous, feeding on fish and decapod crustaceans.

Chirostoma spp: Larvae descriptions and first attempts at culture

Larvae of different species of *Chirostoma spp* are quite similar with almost no differentiation in size and morphology due to their common phylogenetic origin and an early specialization (Barbour, 1973). In our laboratory, the larvae at hatching have a mean total length of 5 mm. They are transparent with a strong melanization in the eyes, they also with a line of black chromatophores along the body, very near the ventral area. The yolk sac usually has a prominent and transparent oil drop, differing from other freshwater fish larvae. One day after hatching, *C. estor* larvae reach 5.4 mm of total length, the mouth is functional and they have constant movement. The yolk sac is considerably reduced in size and the swim bladder and otoliths are quite apparent (De Buen, 1940 a, b; Morelos *et al*, 1994; Rojas and Mares, 1988).

Although *C. estor estor* is very important in the region, little has been published on its culture. The first reports on culture of the genus *Chirostoma* were made by De Buen (1940a), who described the eggs, larvae and six month old juveniles of *C. grandocule and C. bartini var Janitzio* (*C. attenuatum*) from Pátzcuaro Lake. Rosas (1970) was the first to describe the initial steps for culture of *C. estor estor* and other species of the genus.

Culture of other atherinopsids

The Atherinopsidae family has a very wide distribution in the world, and several of the larger species have been cultured. Semple (1986) described the reproductive behavior and the early development of the Australian Atherinopsid *Craterocephalus spp nov*, cultured in aquaria. Middaugh and Hemmer (1990) found the optimum salinity for growth for *Atherinopsis californiensis* and *Atherinops affinis*. Thompson and Withers (1992) cultured native Atherinopsids from Australian estuaries in aquaria, feeding them with commercial food and reporting high survival after the fifth day.

One of the most studied Atherinopsids is the Argentinian pejerrey *Odonthestes bonariensis*. There have been numerous studies of its reproductive physiology, feeding habits and ecophysiology and some important techniques for its culture have been developed (López *et al*., 1991; Stefano *et al*., 2000; Miranda *et al*., 2001; Montaner *et al*., 2001; Strüssmann *et al*., 2004).

Recent progress on *Chirostoma spp*

Eggs and first larvae

Segner *et al.* (1994) suggested that some specific fish larval features, such as the digestive tract development at the beginning of the exogenous feeding, can be a consequence of the size of the animal. Fishes with big eggs have an extended yolk-sac period and their larvae

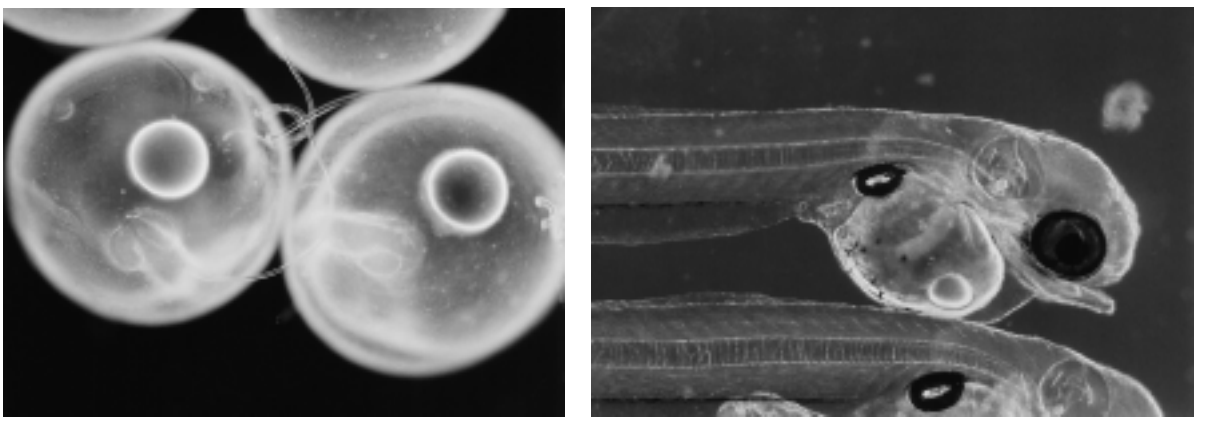

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FIGURE 1. Eggs and larvae of *Chirostoma estor estor* with the evidence of the oil drop and sticky threads.

are relatively large and well developed when exogenous feeding begins, while fishes with very small eggs, such as marine fishes, have an incomplete digestive tract at this stage.

Pátzcuaro silverside eggs are small (0.9 to 1.2 mm in diameter) and have from 6 to 8 adherent threads. Hatching occurs after 7 to 8 days at 25°C and its total length at this stage is 4.5 to 5 mm. The yolk sac disappears on the 3rd post-hatching day (Campos-Mendoza, 2000; Barriga-Tovar, 2000). There is a notable early eye development in larvae that is reflected in their ability to capture prey from the moment of hatching. The length of the larval period has not yet been defined nor is there any basic knowledge on this early stage about the structural and functional status of the digestive system and metabolism.

As already mentioned, and in contrast with that observed in some other fresh water fish species, *C. estor* eggs have a very small yolk sac that contains one to several oil globules, which function as an energy source which is consumed during larval development. Some remnants can still be observed after the 10th post-hatching day if the animals are well fed (Fig. 1). Another interesting characteristic of the eggs is a well-developed gallbladder with a pale to intense green colour that is easily observed from two days pre-hatching. This is remarkable because it does not appear before 8 days after hatching in other species. The green colour of the gallbladder is an unequivocal sign of larval starvation and so it is an effective indicator of the necessity to provide more food or use a higher feeding frequency.

First feeding

The most critical stage in fish culture is massive production of larvae because it is here where high mortalities often occur. This phenomenon seems to be caused by unsuitable nutrition (Jones and Houde, 1986) after the crucial transition period between the reabsortion of yolk-sac and the first feeding of larvae (Watanabe and Kiron, 1994). This has also been the case with Mexican silversides which have up to now had low survival rates, similar to other marine fishes.

Marine fish larviculture is still almost completely based on live feed, that besides requiring space and infrastructure, is also costly (Kolkovski *et al.,* 1993). Because of this, research is in progress to reduce or even eliminate the live feed period and replace it with artificial feed. Nevertheless, fish growth and survival is lower than that obtained with living feed and one of the explanations for this seems to be an immature digestive

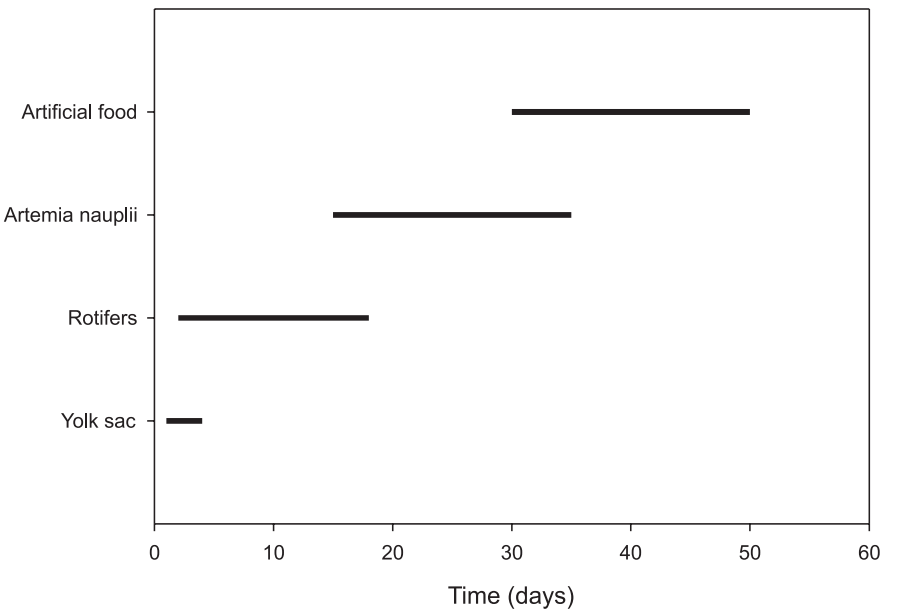

FIGURE 2. Feeding program for Pátzcuaro silverside (*Chirostoma estor estor*) up to weaning.

[2] The transition period between live and artificial feeds, known as "weaning", is a critical stage in larviculture. Weaning in marine fishes can take some weeks while freshwater fishes can be weaned in early stages and sometimes since the moment of opening their mouths (Cahu and Zambonino-Infante, 1995).

system (Hoffer and Nassir-Uddin, 1985) that hampers the digestion of artificial feed[2] . Our work with the "pez blanco" suggests that this species behaves in some aspects as a marine fish and this also seems to be the case in the weaning time, as it cannot be achieved before 1-3 months (Martínez-Palacios *et al*., 2004).

In order to establish a more reliable and reproducible feeding base line than that obtained with the zooplankton collected from the lakes, and taking into account their predatory behavior and mouth size, the massive culture of living feed was established. We first began with a live feed scheme that started with the rotifer *Brachionus rubens* but Campos-Mendoza (2000) found that only the neonates could be used because of the mouth size of the larvae. By culturing at salinities between 5 and 10 it becomes possible to use the rotifer *Brachionus plicatilis¸* whose sizes range from 90 to 120 µm in length, eliminating the necessity of sorting. The rotifers are fed with *Chlorella spp* cultured at salinities of 5-10 (Martínez-Palacios *et al.,* 2003). The experience gained from this work has enabled us to establish a successful feeding program that has greatly diminished the high mortality of *Chirostoma estor estor* in the early stages. Larvae are fed with rotifers for the first fifteen days, followed by *Artemia franciscana* nauplii until the 30th day, when the weaning starts with the substitution of artificial feed[3] (Fig.2). However, recent results indicate that *C. estor* larvae can be fed with rotifers for the first 30 days without affecting growth and survival[4] and this allows elimination of the use of *Artemia*, which is one of the most expensive consumables (Hernández-González, *pers. com*.).

Weaning

The weaning of *C. estor estor* starts after the 25th post-hatching day by gradually substituting the nauplii of *Artemia* for flakes of artificial feed[5] (Forcada-Arens, 2002). The species do not eagerly eat artificial feed until several weeks of weaning. Size is an important characteristic of artificial feed because the silverside rejects particles as big or bigger than its mouth size. Sinking rate needs to be low as larvae will not eat feed from the bottom. Feed also needs to be offered very frequently as their digestive tract has a low feed storage capability due to the absence of a real stomach and a short intestine requiring consequently frequent feeding, as is common with zooplanktophagous organisms.

Buccal and pharyngeal structures of larvae and juvenile C*hirostoma estor estor*

Aspects of the buccal anatomy of this species have been examined in order to elucidate their feeding habits. Fresh observations of the dissected jaws, branchial arches and pharyngeal teeth were performed on

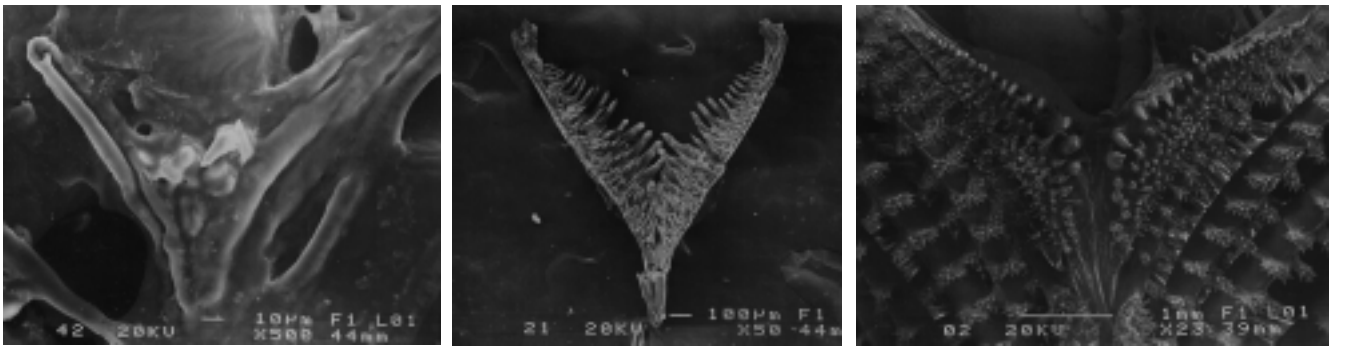

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FIGURE 3. Pharyngeal teeth of *Chirostoma estor estor:* (a)10 days old; (b) 80 days old; (c) 1 year old (Scanning electron microscopy).

[3] Rotifers were cultured in 200-l conical tanks at 30 and were fed with *Chlorella vulgaris* at a rate of 10-20 x 10^6cells/ml. The larvae were fed with approximately 25 rotifers/ml. For the first 15 days, *C. vulgaris* was added daily to the tank systems, maintaining a density of approximately 1 x 10^6 cells/ml in order to feed the rotifers. From day 15 larvae were fed on recently hatched, decysted *Artemia franciscana* (Lavens *et al*., 1986). A commercially prepared flake feed was then offered from day 25 of the experiments.

[4] Larvae at twelve days post-hatching were cultured at 20.5°C at a population density of 10 larvae/l during 30 days. Larvae were fed only with rotifers.

[5] Commercially prepared vitamin enriched 47% protein flake feed. Flakes were ground by hand to obtain fine particles of less than 250 µm appropriate for the larvae.

larvae, juveniles and adults specimens cultured in our laboratory. The resultant dissected structures were further prepared by drying and copper metallization for electron microscopic photography. The details of a 10 post-hatching day larvae pharyngeal lower jaw can be observed in Fig. 3a, showing only two monocuspid pharyngeal teeth. However, the teeth become numerous from the 80th post-hatching day (Fig. 3b). The first branchial arches are very elongated, which is characteristic of filter feeding fish, such as the genus *Coregonus* (Lagler *et al*., 1977). By the age of 90 days these long gill rakers start to be highly ornamented with small spines (Fig. 4). Full complexity is reached when the organism is one year old when ornamentations and bunches of teeth are located alternately in the branchial arches (Fig. 3c), forming a complex filtration system.

In summary, *Chirostoma estor estor* has a small terminal mouth with inconspicuous monocuspid mandibular teeth (Fig. 5), which could be associated with a diet of small particles (Trewavas, 1983); the species has clear pharyngeal structures with fine unicuspid teeth for grinding small particles, and ornamented branchial arches with bundles of small teeth along them, conforming a complex sieve. All of this is designed for consumption of small particles. Juvenile and adult fish have a filtration system typical of a zooplanktophagous species. Nevertheless the adult fish occasionally feed on small fish and crustaceans such as astacids.

Digestive tract and digestion capacity

The gut length to body length ratio of juveniles and adults of *C.estor estor* is 1:0.3, which is characteristic of carnivorous species. The species is stomachless (Ross *et al.,* in press) and this correlates with the low pH found in the adult gut with values of 6.5 in the anterior section and 8 in the mid and posterior section (Graham, 2001).

According to Pedersen *et al.* (1987), the digestive capacity is directly related to the availability of diges-

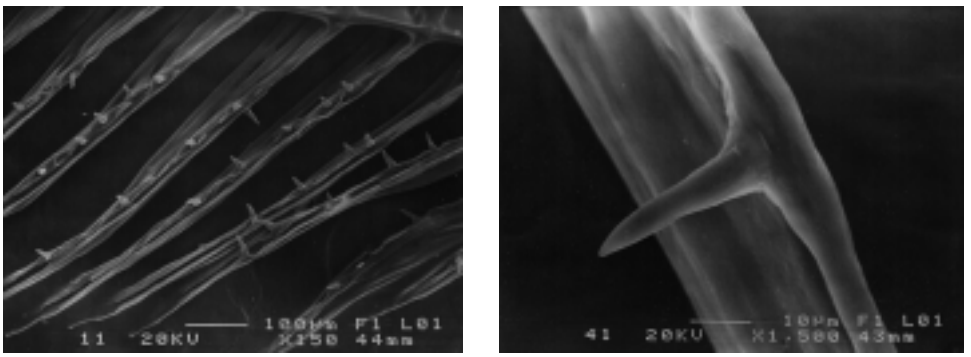

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FIGURE 4. Gill rackers of *Chirostoma estor estor*: (a) 90 days old; (b) gill racker detail (Scanning electron microscopy).

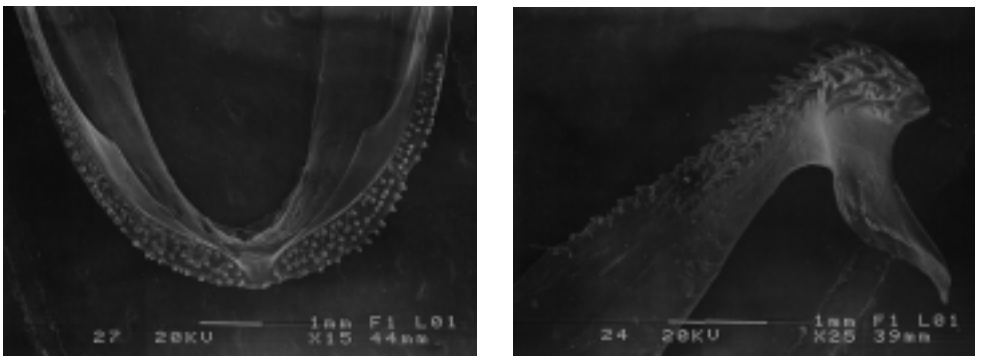

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FIGURE 5. Detail of the Jaw with monocuspid teeth of one year old *Chirostoma estor estor* (Scanning electron microscopy).

tive enzymes, necessary for the extra cellular breaking of food. Thus, enzyme analysis can be used as a tool to determine digestive capacity of larvae in early stages of life cycle; the peak of enzymatic activity being indicative of physiological readiness of larvae to digest exogenous food (Gawlicka *et al.*, 2000). We have detected trypsin-like enzymatic activities in *Chirostoma estor* larvae, which indicates that the species are able to digest protein at early stages (Ríos-Durán, 2000). However, it is still necessary to determine the quantity and quality of these enzymes and the function of other important digestive enzymes, in order to know the real digestive capacity of the species The digestive enzymes of this silverside analyzed to date act in a wide pH range (between 2 and 10). Trypsin-like enzymes, which act at basic pH, are the most important proteases in this species and exhibit a high activity throughout the gut Although the pH along the gut is between neutral and alkaline, there is still low activity of digestive enzymes at low pH[6] (Graham, 2001).

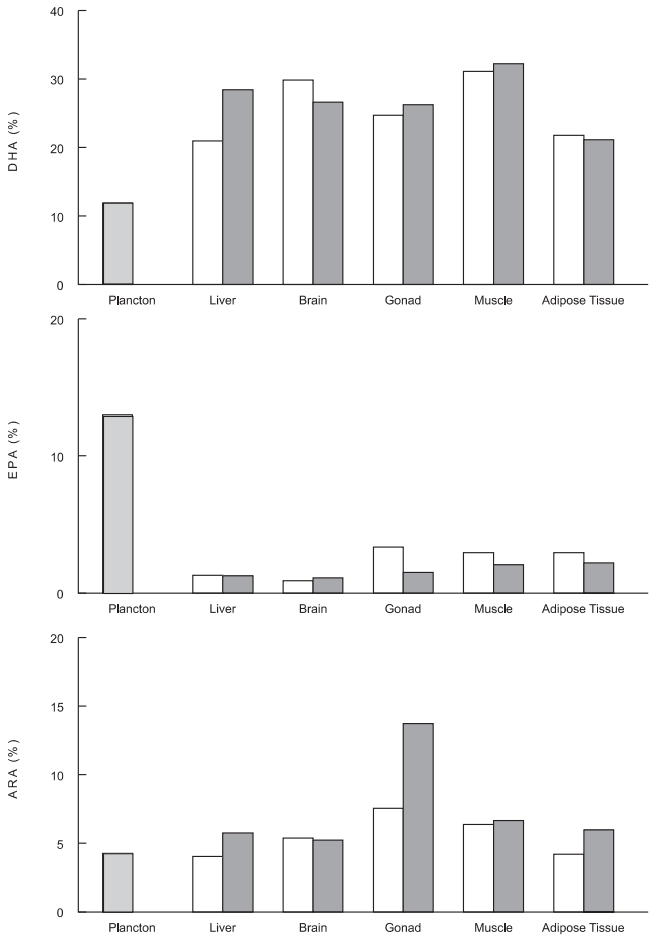

FIGURE 6. Proportion of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) in plankton and selected tissues of female (light bars) and males (dark bars) of *Chirostoma estor*.

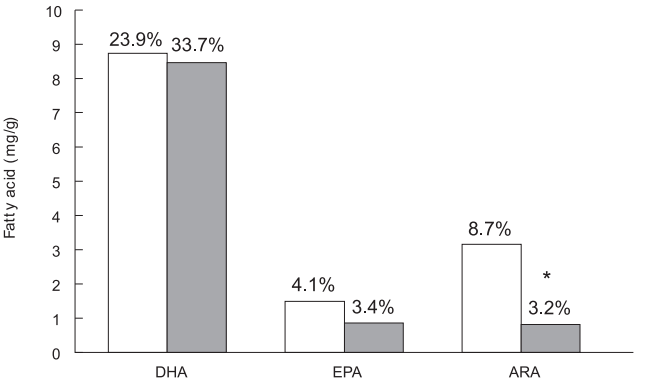

FIGURE 7. Concentration and proportion (above each bar) of DHA, EPA and ARA in recently fertilized eggs of *Chirostoma estor* obtained from wild (light bars) or pond-reared (dark bars) spawners. *indicates significant differences in concentration between both groups.

Fatty acid requirements

The establishment of nutritional requirements for essential fatty acids (EFA) is a primary concern for culture of commercial species. Most freshwater fish, in contrast to marine species, have the ability to elongate and desaturate 18-carbon fatty acids (18:2n-6, linoleic acid and 18:3n-3, linolenic acid) to highly unsaturated fatty acids (HUFA) of 20 carbons (20:4n-6, arachidonic acid or ARA and 20:5n-3, eicosapentaenoic acid or EPA) and 22 carbons (22:6n-3, docosahexaenoic acid or DHA). Thus, it is generally assumed that fatty acids of 18 carbons are EFA for freshwater species, whereas ARA, EPA and DHA are the EFA for marine species (Sargent *et al*., 1995). However, the nutritional habits of fish also determine the EFA requirements: herbivorous species obtain fatty acids mainly of 18 carbons from the diet and thus must convert them to ARA, EPA and DHA, whereas carnivorous species obtain these fatty acids directly from food and have a low ability for desaturation and elongation of 18 carbons fatty acids (Sargent *et al*., 1999). Since *C. estor* has several fea-

[6] The presence of pepsin in fish larvae is usually considered to be an indicator of a functional stomach (Moyano *et al.,* 1996), which happens at the same time as stomach differentiation, and allows a better degradation and utilization of the diet components (Zambonino-infante and Cahu, 1994). When the stomach of the larvae is not completely developed and consequently there is an absence of pepsin, this is compensated by a high alkaline proteolytic activity in the gut (Walford and Lam, 1993; Moyano *et al.*, 1996).

tures similar to marine Atherinopsids and has been considered as a carnivore species, EFA requirements could be more typical of a marine carnivore than of a freshwater fish. If this is so, ARA, EPA and DHA should be included in the diet and thus for its culture, the specific requirements of these fatty acids need to be established.

Wild adults had high levels of DHA (20 to 32% of total fatty acids) but very low levels of EPA (1 to 3%) in different tissues analyzed (Fig. 6). The proportion of fatty acids in tissues contrasts with the fatty acid composition of plankton sampled from Pátzcuaro Lake (12% DHA and 13% of EPA), which is the most important food supply of *C. estor* (Martínez-Palacios *et al*., 2003). One possibility is that this species selectively accumulates fatty acids according to its needs, and the other is that this species has the capability of converting EPA or other omega 3 fatty acids to DHA. The capacity for DHA synthesis is further supported by the presence of DHA in larvae fed a diet of rotifers that have very-low DHA levels but high levels of alfa-linolenic acid (18:3n-3) as discussed below. It is interesting that a freshwater species derived from marine ancestors (Barbour, 1973), which in general do not have the ability of HUFA synthesis (Sargent *et al*., 1995), seems to have developed the capability of synthesizing DHA from EPA or 18:3n-3.

Eggs from wild organisms had a higher content of 20:4n-6 (ARA) and a similar content of DHA compared to eggs from pond-reared fish (Fig. 7). As in adults, eggs had a very high proportion of DHA (24-34%) and low proportion of EPA (3-4%) indicating the importance of DHA for early larval development (Sargent, 1995). EPA levels in eggs, although very low, are nevertheless higher than in tissues, and therefore it is suggested that a certain selective incorporation of EPA into eggs could be occurring in accordance to its specific role in early larval development (Sargent *et al*., 1995). The greatest difference between eggs from wild and pond-reared organisms was the low levels of ARA in the later, which suggest a lack of ARA in the diet of pond-reared brood stock. In addition, in more advanced stages fed on *Artemia*, very low levels of DHA were also found, reflecting the absence of this fatty acid in the diet (Aparicio-Simón, 2004).

An experiment was made in order to observe larval performance fed rotifers enriched with different oils after 20 days[7] (Valencia-Betancourt *et al.*, 2004). Signifi-

TABLE 1.

Survival and growth of *Chirostoma estor* larvae fed with different rotifers diets.

	C. vulgaris	**Yeast**	**Corn oil**	**Cod liver oil**	**Salt-Creek**
Initial dry weight (g)	0.07±0.006	0.08±0.003	0.08±0.003	0.08±0.01	0.07±0.005
Final dry Weight (g)	1.72±0.41^{a}	1.1±0.09^{c}	1.05±0.07^{c}	1.61±0.42^{ab}	1.35±0.12^{bc}
Initial length (mm)	5.07±0.12	5.01±0.07	5.04±0.05	5.03±0.06	5.02±0.06
Final length (mm)	14.1±0.8^{a}	9.75±0.1^{c}	9.60±0.8^{c}	11.0±0.9^{b}	9.91±0.6^{c}
Survival (%)	83.3±10.4	66.7±2.9	66.7±7.6	75.0±5.0	68.3±2.9

Means (± standard deviation) with different letters are significantly different.

TABLE 2.

Selected fatty acid composition (%) of the five rotifer diets fed to *Chirostoma estor* larvae.

	C. vulgaris	Yeast	Corn oil	Cod liver oil	Salt- Creek
18:2(n-6)	24.5	3.7	32.3	16.4	3.2
18:3(n-3)	20.8	1.3	2.5	2.7	1.6
20:4(n-6)	0.5	1.4	1.2	1.3	2.4
20:5(n-3)	0.8	1.5	1.1	7.0	6.5
22:6(n-3)	0.5	3.0	1.5	6.3	26.9

cantly higher larval dry weight and length was obtained with rotifers fed on microalgae or yeast enriched with cod liver oil, compared to rotifers fed on yeast only or enriched with corn oil (Table 1). Intermediate values of dry weight were observed for the enrichment with the commercial high HUFA emulsion, although larval length with this treatment was as low as yeast alone or with cod liver oils, despite the high content of HUFA present in this diet (Table 2) and in the larvae fed this diet (Table 3). The same trend was observed for survival, although no significant differences were obtained[8]. These results suggest that very high concentrations of HUFA are probably not needed, considering the data of general performance of larvae.

CULTURE OF *Chirostoma estor estor*

Reproduction

Artificial fertilization of *C. estor estor* is managed using carbamide solution (Horvát *et al*., 1984) to avoid activation of the sticky threads (Fig. 1). This achieves a more efficient fertilization, because the clusters of eggs are smaller which considerably reduces mortality by asphyxiation. After fertilization, freshwater is added triggering the thread mechanism and the eggs are then collected using nylon bundles to avoid agglomeration, and possible consequently fungal infections[9]. The fertilized eggs are then placed in incubators with brackish water

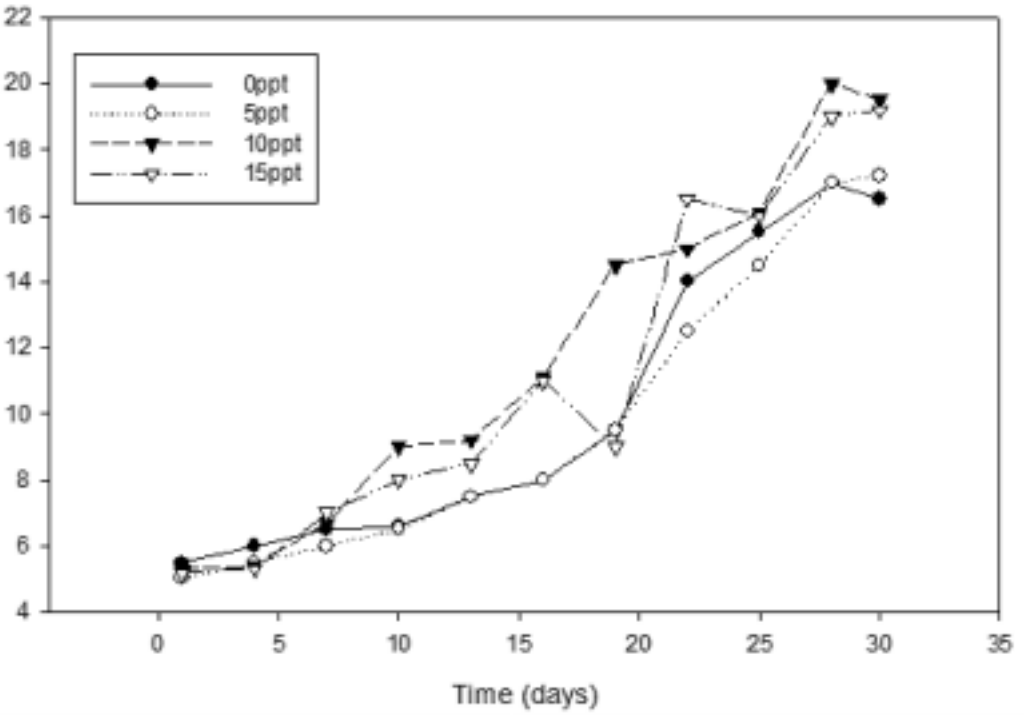

FIGURE 8. Larval growth (total length) of *C. estor estor* at different salinities.

[7] Larvae were obtained from wild animals (Martínez-Palacios *et al*., 2004) and cultivated in 6-L tanks at a density of 20 larvae/tank. Three days after hatching, larvae were fed rotifers (*Brachionus plicatilis*) divided into five treatments (3 replicates each): 1) rotifers fed *Chlorella vulgaris* (C), 2) rotifers fed yeast (Y), 3) rotifers fed yeast enriched with corn oil (YC), 4) rotifers fed yeast enriched with cod liver oil (YL), 5) rotifers fed yeast enriched with a commercial emulsion with high HUFA content (Salt-Creek, Ratio-HUFA, (YS)).

[8] As observed in other fish species (Lavens *et al*., 1994), yeast can substitute for microalgae in the rotifer diet, provided an adequate enrichment medium is supplied, in this case cod liver oil. The better performance with the diet of rotifers fed with *C. vulgaris* could be related to other nutrients supplied by microalgae. In any case and considering the high levels of linoleic (18:2n-6) and alfa-linolenic acid (18:3n-3) in rotifers fed on microalgae, supplementation of HUFA does not seem to be necessary if enough of their precursors are present. This is further supported by higher levels of DHA in larvae fed the rotifers with *C. vulgaris* than in those with rotifers fed yeast alone or with corn oil enrichment (Tables 2 and 3). However, the lower levels of DHA and EPA obtained with *C. vulgaris* compared to enrichment with cod liver oil or Salt Creek emulsion indicates that synthesis of these fatty acids is limited.

TABLE 3.

Selected fatty acid composition (%) of *Chirostoma estor* larvae at the beginning and at the end of the experiment.

	Initial		*C. vulgaris*		Yeast		Corn oil		Cod liver oil		Salt- Creek	
	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL
18:2(n-6)	2.2	1.0	2.2	2.3	2.9	2.7	12.4	12.6	9.8	6.0	3.3	1.6
18:3(n-3)	1.2	0.4	1.4	0.4	0.7	0.2	1.9	0.5	1.7	0.4	2.3	0.8
20:4(n-6)	3.4	5.2	1.4	4.3	2.1	3.7	1.6	4.6	2.0	3.6	2.7	3.9
20:5(n-3)	1.0	0.8	0.6	0.4	0.5	0.3	0.9	0.4	1.2	1.2	1.7	1.6
22:6(n-3)	10.8	28.3	6.3	8.0	4.4	4.7	3.8	6.1	8.8	15.0	12.0	21.3

NL: Neutral lipids, PL: polar lipids

at 10 and a temperature of 25°C, where hatching occurs after seven days (Martínez-Palacios *et al*., 2002). One day before hatching, when the eggs are totally eyed, salinity is reduced to 5, which allows a massive hatching (between 85 to 90%) with mortalities of only 10 to 15% and no fungal infection (Martínez-Palacios *et al.,* 2004).

Temperature and salinity studies

Studies of larval growth at different temperatures (Martínez-Palacios *et al*., 2002) showed that best growth and survival was obtained at 25°C. This temperature is now used routinely for massive production and manipulation of the species.

Trials with eggs and larvae of *C. estor estor* at different salinities (Martínez- Palacios *et al.*, 2004) showed that best growth (total length) and survival of larvae was at 15, much better than 0 (Fig. 8). Eggs develop best at 10, although the hatching is not efficient if salinity is not reduced to 5 or 0 before hatching. This may be related to the inhibition action of the movements of the fish embryo due to the high osmotic impact on the perivitelline environment (Martínez-Palacios *et al.*, 2004)[10]. An additional benefit is the total inhibition of fungal infection in at salinities higher than 10[11]. Based on these results, the best strategy for the culture of the species is to incubate the eggs at a salinity of 10, maintaining them at this salinity until the eyes appear, and thereafter immediately reduce to 5 until most of the fish hatch. Later, it is necessary to increase the salinity to 10 in order to reduce the infection risk and to avoid the mortality of rotifers *B. plicatilis* (Martínez-Palacios *et al.*, 2004). This protocol has enabled reduction of mortality almost to zero during the incubation and development of larvae and juveniles of the species.

Population densities

Population density is one of the most critical factors in larviculture having strong influence on social interactions such as aggression (Kaiser *et al.,* 1995; Sakakura and Tsukamoto, 1999), hierarchy (Schreck, 1981) and cannibalism (Katavic *et al*., 1989; Moore and Prange, 1994), which cause variation on size, survival and growth in fishes (Sheik-Eldin *et al.*, 1997). *C. estor estor* larvae can be grown for 30 days at a density up to 40 larvae/L at 24.8°C with almost no effect on growth (dry weight) or survival[12] (Hernández-González, *pers. com.*).

[9] Currently, the brood stock is maintained in circular tanks, where reproduction occurs naturally and we do not require artificial fertilization or the nylon bundles for the collection of the fertilized eggs.

[10] These results reveal the euryhaline character of the species, which is clearly derived from its marine and estuarine ancestors, from which the Pátzcuaro silverside recently evolved (Barbour, 1973).

[11] As mentioned previously, the genus *Chirostoma* seems to have a marine origin, based on the fact that the majority of the members of the family Atherinopsidae appear in marine environments and many of them are euryhaline (Berra, 1981; Nelson, 1994). This suggest that *Chirostoma*'s species could becapable of adapting to saline environments. If this were so, the culture of this species in saline waters would bring several benefits, since it might reduce the intensity of the ectoparasitism such as *Saprolegnia sp.* It would also have metabolic benefits with reduction in the energy spent in osmoregulation. If the cost of the osmoregulation is low, then the proportion of metabolic energy available for growth and reproduction can be higher (Prunet and Bornancin, 1989).

Closing the life cycle of *C. estor estor* in captivity

For the first time, our research has led to culture of Mexican silversides in closed systems. The species achieve maturity within the first year, when their total length is between 12 and 15 cm. In 2003 we obtained the first spawns in captivity and since then larvae have been produced consistently. *C.estor estor* is a frequent spawner, laying and maturing a regular number of eggs, from 500 to 3,000, depending upon the size of the adult female. There is a synchronization of spawning with the lunar cycles as natural spawns occurs mainly close to full moon periods (Campos-Mendoza, *pers. com.*). Manipulation of photoperiod has showed that 24 hour continuous illumination or a 18L:6D light cycle produces a high spawning frequency suggesting a strong influence of photoperiod on the spawning event (Campos-Mendoza, *pers. com.*).

CULTURE PERSPECTIVES

The growing knowledge base on *C. estor* features has enabled us to understand and resolve many problems faced by our predecessors (Lara, 1974; Armijo and Sasso, 1976; Rojas and Mares, 1988). Among the most remarkable findings is the apparent retention of some marine characteristics, including euryhalinity; late weaning and their zooplanktophagous preferences, resembling marine rather than freshwater fishes.

Currently, we are starting commercial semi-intensive culture of the species and are investigating optimization of farming systems. Considerable further work is needed due to the relative sensitivity of the species and present studies focus on nutritional requirements (lipids, protein/energy ratio, essential amino acids requirements, vitamin C), improved weaning through better knowledge of key digestive enzymes, improving the artificial feed preparation, digestibility studies and stress responses.

[12] Eggs were obtained from artificial fertilization using gametes from mature *C.estor estor* obtained from Lake Pátzcuaro. The eggs were held at 10 at 25°C for the first 5 days and subsequently the salinity was slowly reduced to 5 over 48 h to stimulate massive hatching from day 7 (Martínez-Palacios *et al*., 2002). Larvae were held at 24.8°C and fed on the feed program used at our laboratory.

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