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Research Article

Progress in farming of Chilean silverside *Basilichthys microlepidotus* Jenyns, 1841: an alternative for productive diversification

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ABSTRACT. This work describes the progress made in farming of Chilean silverside (*Basilichthys microlepidotus*), on the basis of broodstock captured in 2012 in the Mataquito River. A total of 179 individuals (adults and juveniles) were captured using a fishing rod fitted with a hook. The broodstock were transported to IFOP´s Experimental Center in Huihue-Chiloé for reproductive conditioning. Following a period of 18 months, the individuals distributed in different farming units reached the gonad maturation stage and spawned naturally. The eggs collected hatched after remaining 16 days in incubation under laboratory conditions. The group of newly hatched larvae displayed lengths around 8.0 ± 0.2 mm, reached lengths close to 18.5 ± 2.8 mm, 30 days of culture (post-hatching). Larvae harvesting took place in tanks with filtered and sterilized freshwater. The reabsorption of the vitelline vesicle was followed by digestive tract development, stage at which the larvae started to be fed with live food (*Artemia* nauplii), complemented with a commercial feed supplement. The growth of the larvae was described until 45 days post-hatching and following 266 days of culture, close to 45% of the larvae reached the juvenile stage (11.3 ± 2.6 cm). This paper deals with aspects related to larvae survival and the introduction of improvements to streamline larvae and juvenile production in order to upscale the culture of this species at the commercial level (aquaculture diversification), in addition to exploring the possibility of carrying out repopulation programs with juveniles.

Keywords: *Basilichthys microlepidotus*, Chilean silverside, broodstock, spawning, larvae, aquaculture.

Avances en el cultivo del pejerrey chileno *Basilichthys microlepidotus* Jenyns, 1841: una alternativa para la diversificación productiva

RESUMEN. Se describe los avances en el cultivo del pejerrey chileno (*Basilichthys microlepidotus*), a partir de la captura de reproductores realizada durante el 2012 en el Río Mataquito. Un total de 179 peces (adultos y juveniles) fueron capturados utilizando una caña de pescar provista de anzuelo. Los peces capturados fueron trasladados para su acondicionamiento reproductivo a las instalaciones ubicadas en el Centro Experimental que mantiene IFOP en Huihue-Chiloé. Luego de 18 meses los ejemplares distribuidos en diferentes unidades de cultivo alcanzaron el estado de madurez gonadal y desovaron de forma natural. Los huevos recolectados lograron eclosionar luego de permanecer 16 días en incubación en condiciones de laboratorio. El grupo de larvas recién eclosionadas presentó longitudes cercanas a los 8,0 ± 0,2 mm, alcanzando luego de 30 días (post-eclosión) de cultivo tallas de 18,5 ± 2,8 mm. El cultivo larval se desarrolló en estanques con suministro de agua dulce filtrada y esterilizada. Después de la reabsorción del saco vitelino se produjo el desarrollo del tracto digestivo, momento en que las larvas comenzaron a ser alimentadas con una dieta de alimento vivo (nauplius de *Artemia*), complementada con un suplemento alimenticio comercial. Se describió el crecimiento de las larvas hasta el día 45 post-eclosión, y luego de 266 días de cultivo, cerca del 45% de las larvas alcanzó el estadio juvenil (11,3 ± 2,6 cm). Se discuten aspectos relacionados con la sobrevivencia larval y la introducción de mejoras para optimizar la producción de larvas y juveniles que permitan escalar el cultivo a nivel comercial (diversificación acuícola), además de explorar la posibilidad de efectuar repoblamiento con juveniles.

Palabras clave: *Basilichthys microlepidotus*, pejerrey chileno, reproductores, desove, larvas, acuicultura.

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INTRODUCTION

To start farming a species, a group of broodstock must be captured in their natural environment and conditioned in captivity. The farming process generally begins with juvenile captures (Sadovy & Pet, 1998; Botero & Ospina, 2002; Papandroulakis *et al*., 2004; Belmonte *et al*., 2007; Grignon, 2010; Muñoz *et al*., 2012). Fish captured from the natural environment become future brood fish and can be used to perform studies related to their behavior, management, feed acceptance, survival and growth.

Fish farming in Chile has shown a dynamic development, reaching a production of 195,000 ton and exports over US$1,400 million accumulated up to May, 2015 (SUBPESCA, 2015). Nevertheless, this important development has only occurred in marine and exotic species. Farming of native fish is a recent subject-matter, and has mainly focused on research related to the capture of wild juveniles of species such as *Cilus gilberti* (corvina drum), *Eleginops maclovinus* (Patagonian blennie), *Merluccius microlepidotus* (southern hake), *Seriola lalandi* (yellowtail amberjack), *Seriolella violacea* (palm ruff), *Paralichthys adpersus* (Chilean flounder), *Oplegnathus insignis* (Pacific beakfish) and *Medialuna ancietae* (hatchet fish; Silva & Flores, 1989; Cortes *et al*., 2001; Bustos & Landaeta, 2005; BCG, 2007).

Native ichthyofauna of lakes and rivers in Chile is comprised by approximately 44 species, in respect of which culture studies have not been performed (Habit *et al*., 2006). One of these species, capable of reaching appropriate lengths and weights in culture conditions, which would increase their commercial interest and potential for aquaculture is the fresh water silverside (*Basilichthys microlepidotus*). Farming of this species would enable the development of repopulation aquaculture, mainly focusing on the recovery of natural populations presently depressed due to anthropic actions.

Chilean silverside (*B. microlepidotus*) mainly inhabits well oxygenated waters in rivers, small creeks, lagoons and lakes, preferably in low speed of water flow, transparent and with a minimum depth of 40 cm, and uses aquatic vegetation as shelter, where they usually co-exist with the introduced species *Cyprinus carpio* (common carp). Juvenile individuals of this freshwater species inhabit riverside or coastal environments that are used as nursery sites (Rojas, 2015). The distribution of *B. microlepidotus* extends from the Huasco River (28º30'S) to the Aconcagua River (32º20'S; Dyer 2000a). However, a recent study by Véliz *et al*. (2012) showed that *B. microlepidotus* and *B. australis* (from the Aconcagua River to Chiloé Island (42º18'S); Campos *et al*., 1984) form a monophyletic group in central Chile which should be called *B. microlepidotus*. This species has omnivore habits, and mainly feeds on diatoms, filamentous algae, and Chironomidae adult and larvae (Urzúa *et al*., 1977; Bahamondes *et al*., 1979; Rojas, 2015).

Freshwater silverside is a fish with white, firm and consistent flesh, constituting an important resource for farmers, riverbank communities and recreational fishers. Despite being an attractive aquatic resource and harvested for consumption purposes, it is not included in official landing statistics.

Wild *B. microlepidotus* juveniles tolerate well captivity and handling, accept commercial feed and have a relatively high survival rate, which makes it an attractive species for Chilean aquaculture. In order to evaluate this species as an alternative for aquaculture diversification, *B. microlepidotus* broodstock was harvested, and the conditioning, spawning and first larvae culture under laboratory conditions is described.

MATERIALS AND METHODS

Capture and transport of wild broodstock

In 2012, nine fishing campaigns were conducted to capture adult and juvenile individuals of Chilean silverside from the Mataquito River (Fig. 1). Fish were caught with hook and line by local recreational anglers and researchers from the Instituto de Fomento Pesquero (IFOP).

The captured fish were collected in a cage *in situ* during each sampling period (stocking densities under 10 kg m^{-3}) placed in a section of the Mataquito River close to where the fishing took place, in order to ensure the fish a constant water flow and supply of dissolved oxygen (8-12 mg L^{-1}). Once the capture was completed, silverside individuals were transported in densities below 6 kg m^{-3} for adults and 10 kg m^{-3} for juveniles, in fiberglass tanks with 500 L capacity, conditioned to enable monitoring several water physical and chemical parameters (*e.g.*, temperature, dissolved oxygen concentrations, oxygen saturation, pH, among others), and carried to farming facilities (Water Recirculation System) located in the Experimental Center at IFOP in Huihue-Chiloé. Prior to and during carriage fish were fasted (~12-24 h) in order to minimize the negative effects associated with stress as a result of being captured and transported to the farming facilities.

Morphometric relationships of broodstock

Length structure

Before entering the maintenance system, the fish were weighed with a precision of 0.1 g (total weight, TW;

Figure 1. Capture sites for Chilean silverside distributed along the Mataquito River.

pce-bsh 10000 digital scale) and measured (total length, TL; Spenafish ichthyometer) from the tip of the mouth up to the longest lobe of the caudal fin (generally measured with the two lobes compressed along the middle line), in order to establish the group's length structure, length-weight relationship, and condition factor (K) of the broodstock. The differences in length frequency distribution between males and females using a non-parametric Kruskal-Wallis test (Gotelli & Ellison, 2004).

Length-weight relationship

The length-weight relationship in the broodstock was derived using a linear regression (potential function), by estimating "a" and "b" values of the equation $TW = aTL^b$ (Froese, 2006), where TW is the total weight in grams, TL is the total length in centimeters, "b" is the allometric coefficient, and "a" is a constant (Smyly, 1955). This relationship was calculated using the total number of Chilean silverside individuals, separately for males and females. To detect eventual differences in the length-weight relationship between sexes, a co-variance analysis was made, and to determine if the allometric coefficient, "b", differed from theoretical value 3 that identifies isometric growth at weight, an chi-square test (χ^2) was applied.

Condition factor

The condition factor was estimated by Fulton index (K) that expresses the volumetric relationship in terms of weight, in accordance to the mathematical expression by King (1995). Such factor may indicate the nutritional status of organisms (Collins & Anderson, 1995) under cultivation, and it is useful to numerically compare and quantify the status of the fish (degree of wellbeing and robustness), which makes it possible to determine the conditions in which the best yields can be obtained (Nikolsky, 1963). The condition factor was established in accordance with equation, $K = 100 (TW\ TL^{-3})$, where TW is the total weight in grams (g) and TL is the total length in centimeters (cm). The complete statistical analyses were performed with the use of the Statistica 7.0 statistical package.

Reception and maintenance of broodstock

The captured fish were transferred to a maintenance system comprised by six fiberglass tanks with 2 m diameter and 2,000 L capacity, connected to a water re-circulation system (WRS; 20% water replacement per day) comprised by a heat pump with 5 HP, connected to a pipe network (PVC 50 mm) with a sand filter of 0.11 m^3 and a filter flow of 6.6 $m^3\ h^{-1}$. It is also fitted with UV disinfection equipment, two 500 L tanks that act as bio-filter, a Skimmer (dissolved organic matter elimination) and a 2 kW titanium immersion heater.

Throughout the period, the fish were fed with salmon feed (Ewos brand). This diet is specific for "Recirculation Boost 20/1300" systems, caliber 3 mm. *Spirulina* powder was used as vegetable protein (Mater de Solarium Biotechnology), which has the particular feature of adhering to the walls of feed pellets, and thus constitutes a microalgae with a high nutritional value for broodstock. Dissolved oxygen levels (mg L^{-1}) and water temperature (ºC) in the tanks were recorded daily with the use of an oxygen and temperature automatic monitoring and control system (PT 4; Point Four by Pentair).

Reproductive conditioning

The total number of broodstock were distributed according to length (<15, 15-20, >20 cm), in five conditioning tanks (G1, G2, G3, G4 and G5). The broodstock was maintained in a water re-circulation system, at low densities (up to 6 kg m^{-3}) and a dissolved oxygen concentration from 8-12 mg L^{-1}. Water physical and chemical variables were monitored daily, as well as ammonium concentrations (NH_4) from excretion, urine and decomposition non-consumed feed and phosphates

(PO_4) as a result of biological activity of fish and overfeeding with balanced food. Broodstock were measured (TL) and weighed (TW) on a monthly basis in order to record the evolution of fish and eventual changes in length *vs* weight relationship of the different groups maintained in captivity. Controlled fish were previously anaesthetized with a concentration of 0.15 mL L^{-1} of benzocaine (BZ-20) during 3-5 min.

Spawning and management of eggs

The eggs obtained in spontaneous spawning in each culture unit were collected from the bottom with the use of a brush attached to a siphon. Eggs collected in the shape of clusters were separated manually in order to be taken to incubation and larval culture facilities. The eggs were placed in a cylinder-conical incubator with 10 L capacity, previously cleaned with filtered water at 10 µm and treated with UV light.

To estimate the total number of eggs three 1 mL samples were taken and subsequently were counted with the use of a Leica S6D magnifier, with a lighting system including a Leica KL 300. The value obtained was averaged and extrapolated to the total volume of collected eggs. Subsequently, the eggs were placed in a maintenance tank to determine the number of viable eggs by decantation. The eggs accumulated at the bottom were selected to be introduced into the incubators, while those on the surface were eliminated.

Incubation and hatching

The incubator system is connected through recirculation with a fiberglass water accumulating tank with 2,000 L capacity. Each incubator is comprised by an acrylic cylinder tube with 10 L capacity, allowing an around 40,000 eggs/incubator maximum incubation density. The flow of water circulating upwards maintains the movement of eggs, in order to verify that healthy eggs, on account of their weight, remain within the third lower part of the incubator. After verifying the hatching of the eggs, the larvae were recounted to determine the hatching percentage. Subsequently, the larvae were transferred to larvae culture tanks. The incubation process took place at a temperature from 18-20°C.

Larval culture

Fiberglass cylinder tanks with 2,000 L capacity were used. The tanks are fitted with a 1 mm mesh at the central lower part to avoid larvae escapement. The culture capacity of each tank must not exceed 15 kg m^{-3}, which accounts for 50,000 larvae in every 2,000 L tank.

Up to a culture period of 14 days, the water replacement was 30% of the total volume, as long as the water quality conditions remained at levels that are appropriate to ensure the survival of fingerlings. Starting on day 14, a continuous water flow was used ensuring a daily replacement three times the tank volume. During this process, daily records were made of water physical and chemical parameters such as temperature, oxygen concentration, pH, and alkalinity and nitrogenized compounds. Waste or mortalities were removed with a siphon.

Larvae and juvenile feeding

During early life stages, Chilean silverside were fed with a diet based on living food (*Artemia* nauplii), complemented by balanced artificial feed (Microvit Hi protein) with a caliber of around 100 µm. Silverside juveniles (~0.8-19.6 cm) were fed with artificial feed (Pellet micro) of different calibers (0.2, 0.8, 1.3, 2.0 and 3.0 mm), especially formulated for recirculation systems.

RESULTS

Capture and assessment of broodstock

In 2012, a total of 179 individuals of *B. microlepidotus* were captured in a nine-month period. The group of future broodstock showed differences in length and weight. It was also established that the sex ratio between males and females was close to 1:1, after verifying the sex to all individuals (Table 1). Captured individuals were separated and distributed in five tanks (G1, G2, G3, G4 and G5) according to criteria such as length and culture start date (Table 2).

Morphometric relationships of broodstock

In the analysis by sex, 179 individuals were considered, of which 95 were male and 84 female. *B. microlepidotus* individuals showed a bimodal length-frequency distribution between the sexes (Fig. 2), nevertheless, statistically significant differences were not observed (K-W test: $F_{[1,140]} = 2.093$; $P > 0.05$) at length for males and females.

The total length and total weight relationship of male and female *B. microlepidotus* captured individuals showed that over 90% (R-squared) of weight variations are explained by length, and by a coefficient value allometry $b > 3$, indicating that individuals of larger size have increased their weight in highest proportion than its length, showing positive allometric growth (Fig. 3). The applied covariance analysis, using length as independent variable and weight as a co-variable, showed statistically significant differences between sexes ($P < 0.05$, Table 3), where females are more robust than males.

Table 1. Descriptive statistics of length structure (total length, TL) and weight (total weight, TW) of males and females (broodstock) from Mataquito River. SD: standard deviation.

		Total length (cm)				Total weight (g)			
Sex	Nº individuals	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
Males	95 (53.1%)	14.0	31.0	21.1	4.6	15.0	219.5	72.6	48.1
Females	84 (46.9%)	11.0	30.5	21.1	5.1	6.5	205.0	77.2	56.0
Total fishes	179 (100.0%)	11.0	31.0	21.1	4.8	6.5	219.5	74.6	51.6

Table 2. Variable records separated by Chilean silverside broodstock group. Total mean length (±SD). *Registration of each fishes group from admission to 01/13/2015.

	Fishes group				
Variables	G-1	G-2	G-3	G-4	G-5
Date farming start	27/04/2012	09/06/2012	22/07/2012	18/10/2012	16/12/2012
Number of fishes	26	36	52	30	35
Total length (cm)	26.5 ± 4.1	19.4 ± 3.0	11.3 ± 2.5	10.3 ± 1.7	24.3 ± 4.3
Pond biomass (kg m^{-3})	3.2	4.5	4.0	2.0	4.9
Water flow (L min^{-1})(*)	12.0	12.0	12.0	12.0	12.0
Food (g day^{-1}) (*)	52.0	50.0	45.0	30.0	48.0
Food (% body weight)(*)	0.8	0.6	0.6	0.8	0.5
Growth rate (cm $month^{-1}$)(*)	0.4	0.4	0.5	1.1	0.4
Weight increase (g $month^{-1}$)(*)	8.3	8.3	6.0	8.3	10.3

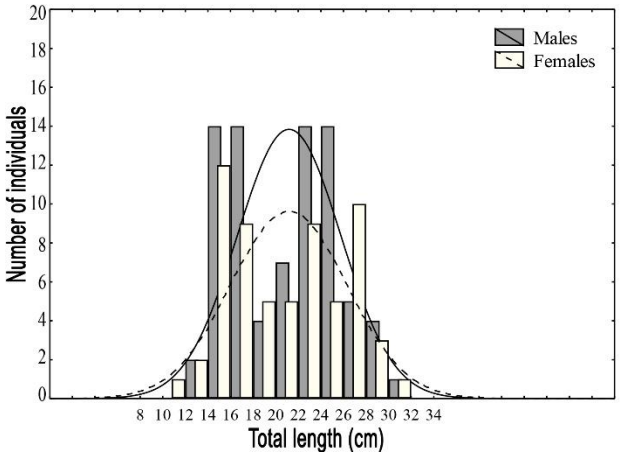

Figure 2. Chilean silverside broodstock. Size-frequency distribution by sex.

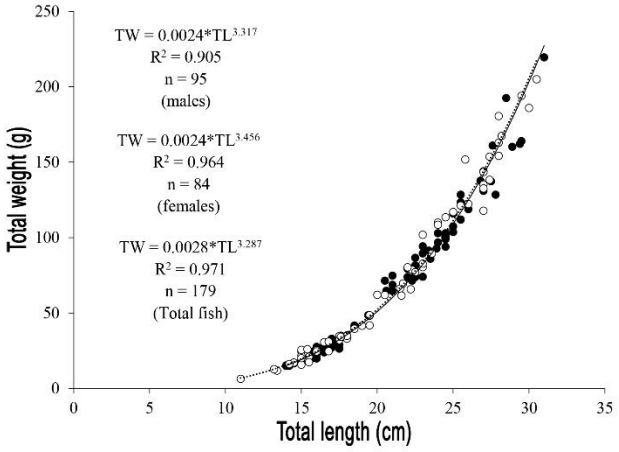

Figure 3. Linear relationships between total length (TL) and total weight (TW) of males (black circles) and female's broodstock (white circles) captured in the Mataquito River.

Condition factor values (K) calculated for Chilean silverside broodstock (separated by sex) tend to increase with length. In general, females displayed slightly higher K values than males (Fig. 4), except for one group of female silverside captured in March, 2012 that displayed clearly higher K values than males. These variations in the condition factor not represent statistically significant differences (ANOVA test: $F_{[1,139]} = 1.157$; $P > 0.05$) between both groups.

Reproductive maintenance and conditioning

After being transferred from the capture zone to the farming facilities in Chiloé, the fish were distributed in five tanks (G1, G2, G3, G4 and G5) and maintained throughout the entire period under conditions similar to the physical-chemical parameters recorded in the Mataquito River during a one-year period (Table 4).

Table 3. Results of ANCOVA test using length as independent variable and weight as co-variable on the basis of the sex of Chilean silverside individuals. Where; the slope (a), the intercept (b) and correlation coefficient (*r*) come from the linear equations of male and female broodstock. **P* < 0.05.

Species	Sex	a	b	*r*	*F* (ANCOVA)	*P* (ANCOVA)
B. microlepidotus	Males	3.41	2.80	0.95	5.086	0.026*
	Females	3.55	2.95	0.96		

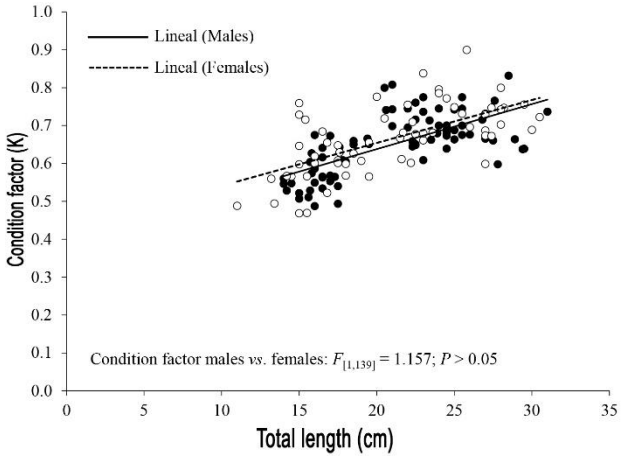

Figure 4. Variations in condition factor for male (black circles) and females broodstock group (white circles) of Chilean silverside from Mataquito River.

The fish started to be fed two days after arriving at the farming facility, in order to allow the broodstock to adapt to captivity conditions. The fish were fed rations twice a day "*ad libitum*" (one in the morning and another in the afternoon), using a ration equal to 2% of the mean weight of the fish as a point of reference. The amount of feed was gradually adjusted on the basis of the amount of feed remaining at the bottom of the tank, after they were fed. In general, depending on the date of introduction of the broodstock to the system, the fish displayed differences in the amount of feed (based on weight of feed) accepted, on the basis of mean body weight (g) recorded in each group. The fish distributed in the five culture units accepted in average close to 0.7% of the body weight of each broodstock fish present in each tank (Table 2).

After 33 months since the introduction of the first broodstock to the farming system (21.1 ± 4.8 cm and 74.6 ± 51.6 g), the fish kept in the tanks showed in average an increase in length and total weight close to 31.3 ± 2.9 cm and 296.3 ± 53.6 g, respectively. During the period in which the broodstock remained in captivity, the progress in culture was reflected in a growth rate close to 0.7 cm month^{-1} and an increase in weight around 8.7 g month^{-1} (Fig. 5).

Spawning

After 18 months of culture under temperature (~15-22°C) and dissolved oxygen (~7.0-12.0 mg L^{-1}) conditions similar to those recorded (within an annual cycle) in the Mataquito River, the fish spawned spontaneously after reaching reproductive maturity (~23-24 cm TL). A total of 7,032 eggs (2.0 ± 0.1 mm in diameter) were collected from the different broodstock tanks (G1 = 1,300 eggs), (G2 = 1,516 eggs), (G3 = 1,416 eggs), (G4 = 1,700 eggs) and (G5 = 1,100 eggs). The collected eggs were transferred to two hatcheries in order to be cultured in laboratory conditions maintaining an average water temperature (± SD) of 19.5 ± 1.2°C.

Incubation and larvae culture

Following 16 days of incubation (at a temperature of ~19.5 ± 1.2ºC) a total of 3,258 larvae were obtained (8.0 ± 0.1 mm), accounting for 45% of hatching. Subsequently, the early stages of silverside were taken to a larval culture tank (at a temperature of ~20.3 ± 1.2ºC) with a capacity of 2,000 L. Following 30 days post-hatching (PH), lengths close to 18.5 ± 2.8 mm were recorded. Subsequently, following 140 days of culture, the number of larvae dropped to 2,879 individuals, accounting for a survival close to 90%. During the first three days of culture (at a temperature of ~19.5 ± 1.2ºC; pH 6.6-7.4) the fresh water flow was maintained uninterrupted, and partial water renewal began on day four of post-hatching, with 20% of the total culture volume, until reaching 60% starting from day 20. From the initial number of larvae (3,258 ind) up to 527 days in culture (2,335 ind), the group showed in percentage terms a survival close to 69.6% (Fig. 6).

Larval feeding

Immediately following larvae hatching (~6.0 mm), early life stages of silverside began to be fed with live feed, based on *Artemia* nauplii (~4.4 mm) and a commercial food supplement of 4.0 mm caliber (Tropical Mikrovit Hi Protein). Throughout the larval culture process, this type of diet was very palatable to the larvae, evidencing that the early life stages of silverside are capable of directly consuming prey with

Table 4. Physical and chemical parameters as mean values (±SD) recorded in the re-circulation system during Chilean silverside farming. Temperature (Temp.), dissolved oxygen (O_2), oxygen saturation (% Sat.), ammonium (NH_4), and phosphate (PO_4).

Parameters in water recirculation system (WRS)								
							Daily water exchange	
Temp. (°C)	O_2 (mg L^{-1})	% Sat.	Salinity	pH	NH_4 (mg L^{-1})	PO_4 (mg L^{-1})	Vol. used	% Exchange daily
17.8 ± 3.8	10.4 ± 3.2	111.1 ± 29.7	1.6 ± 1.7	6.6 ± 1.6	0.5 ± 0.2	2.4 ± 0.9	16,000 L	20%

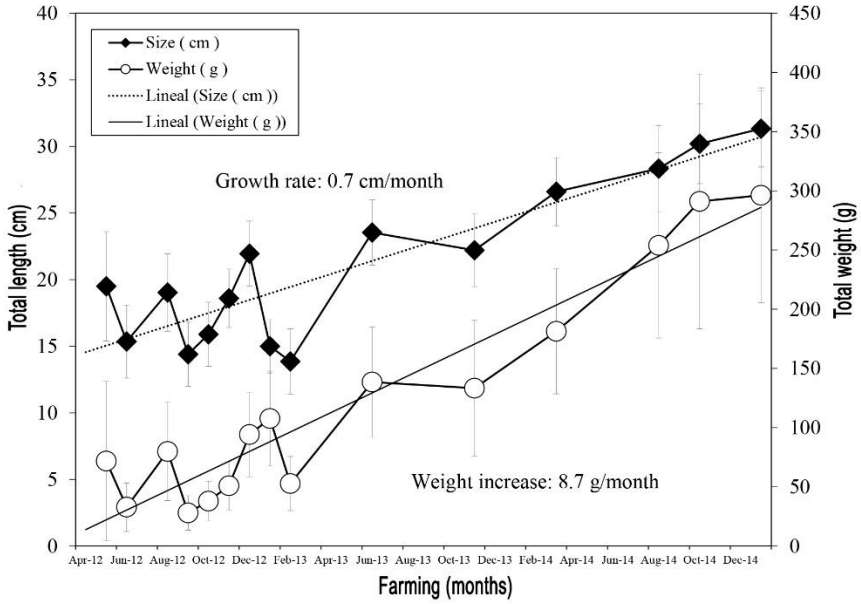

Figure 5. Growth in captivity of Chilean silverside broodstock (*B. microlepidotus*).

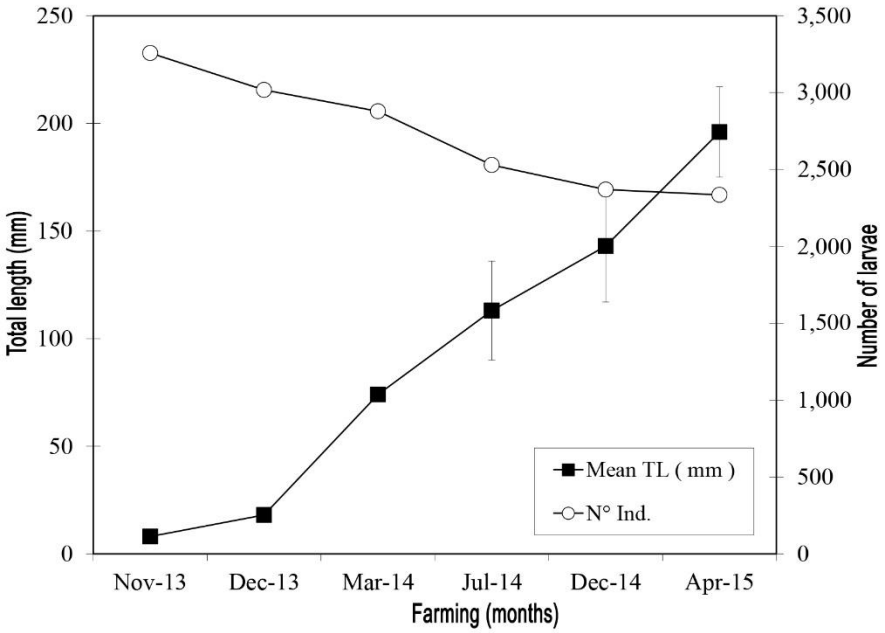

Figure 6. Evolution in growth and survival of Chilean silverside larvae born in captivity. The bars show standard deviation values.

Table 5. Daily feed rations provided to Chilean silverside larvae culture.

Farming days	*Artemia* nauplius (g)	No. rations	Balanced food (g)	Nº rations
1	25	1	-	-
2	50	1	-	-
3	50	1	15	1
4	50	1	15	2
5	50	1	15	4
6	25	1	15	4
7	25	1	20	1
8	30	2	20	1
9	30	3	20	1
10	35	3	20	1
11	35	3	25	1
12	35	3	25	2
13	40	3	25	3
14	40	3	25	3
15	45	3	28	3
16	45	3	30	3
17	50	3	30	3
18	50	3	48	5
19	50	3	70	5
20	55	1	70	6
21	60	1	85	6
22	65	1	90	6
23	70	1	90	6
24	75	1	90	6
25	80	1	90	6
26	90	1	135	9
27	100	1	150	10

Table 6. Characteristics and composition of feed (micro-pellet) provided to larvae and juveniles Chilean silverside.

Diet	Caliber (mm)	Fish weight range (g)	Protein (%)	Lípids (%)	Gross energy (kcal kg^{-1})	Quantity (Nº pellet kg^{-1})	Particle size (mm)
Micro-pellet	0.2	<0.2-0.5	54.0	16.0	21.0	$4.0x10^6$	0.5
	0.8	0.5-1.5	54.0	16.0	21.0	$2.6x10^6$	0.7
	2.0	1.5-6.0	53.0	20.0	22.1	$1.0x10^6$	1.1

a high degree of mobility. During the first 27 days of larval culture, feed rations (*Artemia* nauplii and *Spirulina*) and daily feeding frequencies were adjusted in order to obtain better yields (Table 5). After the first 27 days of culture, and up to 527 days of culture, a balanced diet was provided to fish larvae, which slightly varied in terms of characteristics and nutritional composition (Table 6).

Larval development

Following 16 days of incubation (~19.5 ± 1.2ºC) egg and early larval stages of Chilean silverside were determined and this closed the life cycle of this species under laboratory conditions (Fig. 7). During the incubition and development of larvae, the following four phases were identified:

Embryonic phase (0-15 days; from fertilization to hatching): Non-floating eggs, round with 2.0 ± 0.1 mm diameter, presence of lipid drops at the beginning, which turn into a single drop after six days. Prior to hatching, the inner part of the eggs is completely occupied by an embryo (rolled up on it) and shows different degrees of dark pigmentation (Figs. 8a-8e).

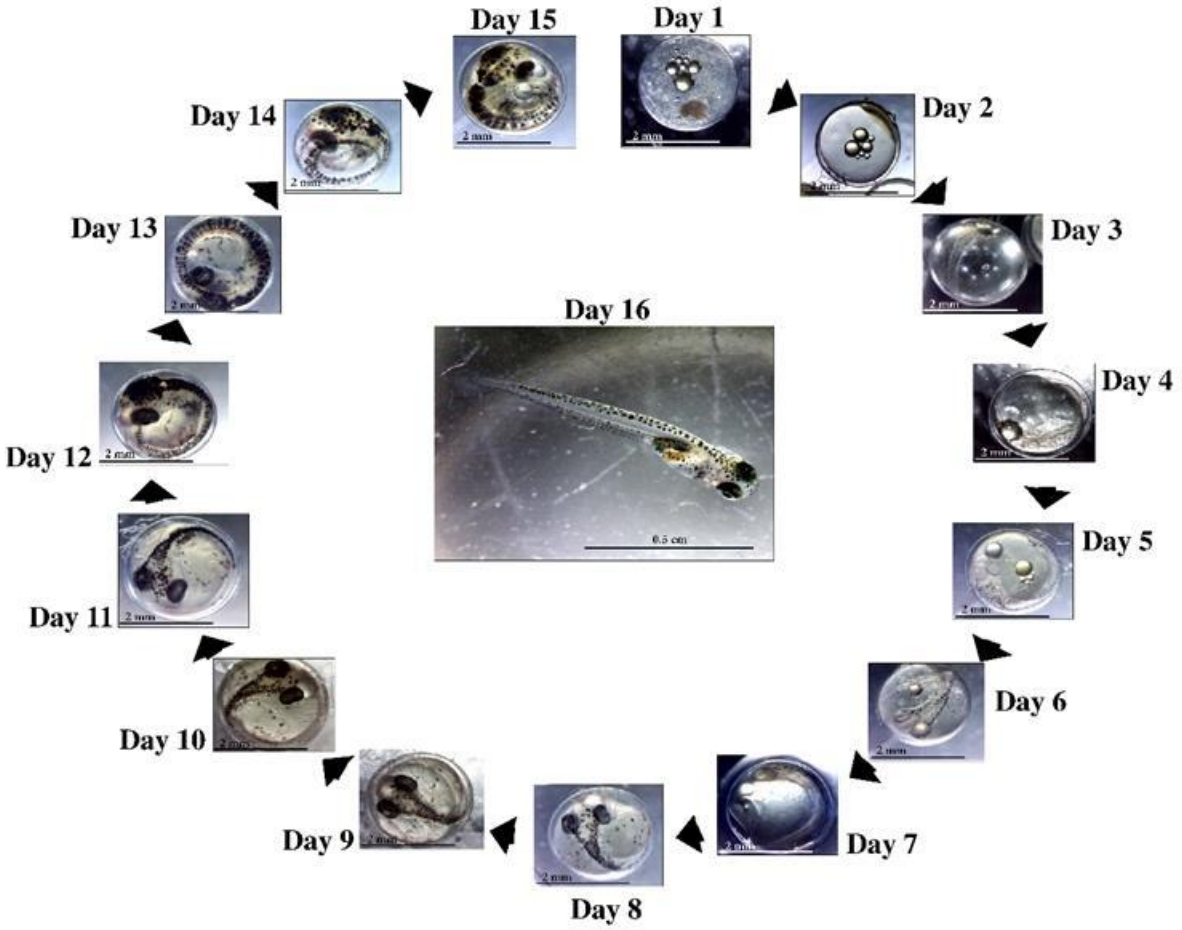

Figure 7. Development of eggs and early larvae stages of Chilean silverside cultivated under laboratory conditions.

Larval phase with yolk-sac (1-3 days post-hatching; from hatching, up to complete yolk-sac absorption, which occurs approximately during the third day): After hatching, fish larvae have dark eyes and noticeably pigmented bodies with an approximate length of 1-2 cm. Larvae begin to absorb the small yolk-sac and the body pigmentation increases (Fig. 8f). Exogenous feeding with live prey begins during the first day. During this phase, early stages of silverside are capable of directly ingesting *Artemia* nauplii.

Larval phase (4-45 days post-hatching; from the absorption of the yolk-sac to juvenile stage): The absorption of the yolk-sac is completed during this stage, and larvae fish of approximately 3-5 cm length had lost their pigmentation, which is replaced by a grey color covering the entire dorsal region (Fig. 8g). During this phase, larvae fish are actively fed on the basis of a diet comprised by *Artemia* nauplii and micro-formulated feed, with the aim of avoiding malformations and/or diseases as a result of nutritional deficiencies produced during the first stages of development of this species.

Juveniles (6-10 cm): The size of the mouth and mobility of the jaws enables the fish to catch larger-sized prey (Fig. 8h), leading to a progressive change in diet toward formulated feed for salmon species. During this development stage, the diet of juvenile silverside, in addition to pellets, is based on a microalgae spirulina feed supplement (vegetable protein) with a high nutritional value.

DISCUSSION

Farming of native fish generally begins with the capture of juveniles in the natural environment (Sadovy & Pet, 1998; Lecaillon, 2004; Roo *et al*., 2005; Vermond, 2007; Grignon, 2010) on the basis of guidelines that orient this process and the subsequent confinement of captured individuals, with the use of friendly systems that provide an appropriate wellbeing to the fish in their new environment (De Tolla *et al*., 1995; AFS, 2004). Nevertheless, from the time of capture of wild juveniles, until they are able to spawn under controlled cul-

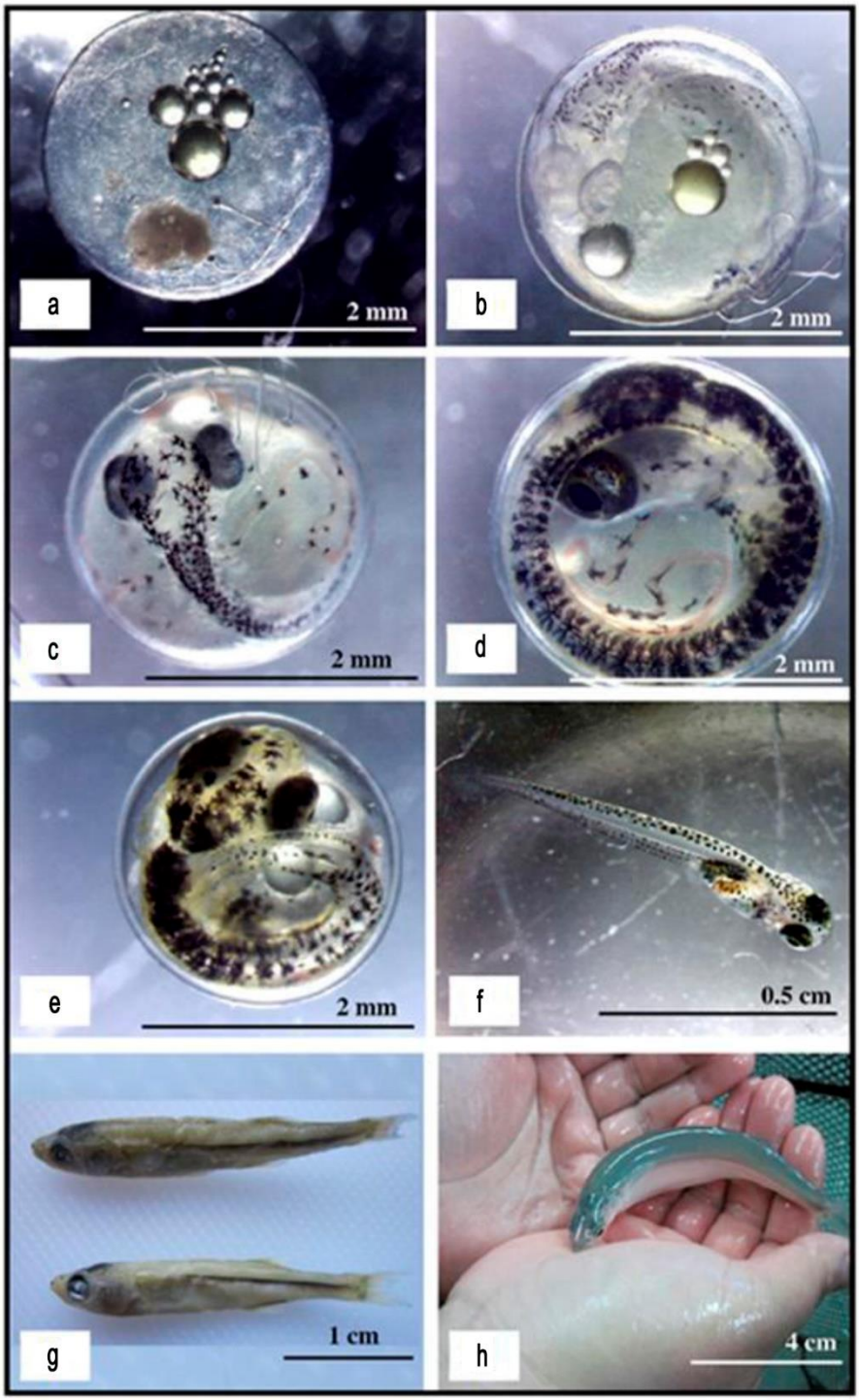

Figure 8. Development stages of *Basilichthys microlepidotus*. a-e) Embryonic stage, f) larvae stage with yolk-sac, g-h) larval phase and juvenile.

ture systems, a certain time period could elapse, which cannot be clearly established.

In the specific case of Chilean silverside broodstock, spawning occurred spontaneously, a condition that is typical of a variety of species (pelagic and reef fish) as with other native species like *Paralichthys microps*, *P. adspersus*, *Seriollela violacea* (Centrolophidae) and *Seriola lalandi* (Carangidae) (Wilson, 2009; Silva & Oliva, 2010) and species from other latitudes like *Cromileptes altivelis*, *Epinephelus tauvina*,

E. merra, *E. fuscoguttatus*, *E. coioides*, *Plectropomus leopardus* (Serranidae), *Lutjanus guttatus*, *L. sebae*, *L. argentimaculatus* (Lutjanidae) (Sugama *et al*., 2004; Jagadis *et al*., 2007; Herrera-Ulloa *et al*., 2009; Mathew, 2010). In these species, the fertilized eggs are collected and arranged in incubators.

For spontaneous spawning is recommended cylindrical tanks (6 m in diameter), with capacity above 60 ton and a depth between 2.0 and 2.5 m (Sugama *et al*., 2004), dimensions and form allows broodstock to swim around the tanks during spawning. In this study, fiberglass tanks of 2,000 L capacity were used that allowed the broodstock spawning spontaneously after acclimatized and conditioned reproductively, in these culture systems.

The diet of broodstock plays an important role in embryonic and larval development during embryogenesis stages and the first days of larval culture, as reported by Wilson (2009) with relation to *Paralichthys adspersus* broodstock and the effect of parental larval diet. On the other hand, natural products do not always provide the appropriate levels of nutrients required for broodstock, leading to the need for vitamin supplementation (Alvarez-Lajonchere, 2007) and learning about the basic nutritional requirements and mechanisms to satisfy such requirements with practical diets in order to encourage a profitable and sustainable production of these fish (Gatlin, 2000).

About diet of the silverside in natural environments, Ringuelet (1942) concluded that in specimens of up to three months old, the first food is unicellular algae, reducing its importance in the diet up to 20% to be gradually replaced in the juveniles by copepods and cladocerans, which are the main food item in adults (Ringuelet *et al*., 1980; Escalante, 1985; Grossman, 1994).

Chilean silverside captured in the natural environment were provided a specific commercial diet for recirculation systems; complemented with powder spirulina in order to cover vegetable origin protein requirements. Considering the results obtained with the broodstock group from the Mataquito River, we believe that it is necessary to explore and develop a specific diet that will improve the performance of broodstook in the short term, and by this increase the number of viable eggs and larvae. In this regard, and despite not having a specific diet, silverside fed with pellets formulated for salmonids showed appropriate weight and length rates in order to develop experimental farming of this freshwater species.

In their natural environment, early life stages of Chilean silverside prefer feeding on phytoplankton and micro-crustaceans, while the Argentinian silverside (*Odontesthes bonariensis*) mainly feeds on zooplankton, practically from the fingerling stage until reaching the usual commercial sizes. This characteristic places silverside among the few farmed fish species that feed on organisms situated at the lower levels of the trophic pyramid (Reartes, 1995). This condition offers an advantage from the economic point of view by avoiding the need for infrastructure to farm microalgae used as feed for fish larvae. Instead, the early stages of Chilean silverside were directly fed live feed based on *Artemia* nauplii, and commercial feed supplement, which demonstrated good results in terms of palatability.

The results obtained in the hatching system (57.0%) with respect to the total incubated eggs, and larvae survival (79.9% up to day 15 post-hatching) and juveniles (45.0% with a mean weight of 11.3 cm), allows us to make a positive assessment of the progress made up to date in the farming of this Chilean freshwater native species. To this regard, considering the low mortalities not only in juveniles captured in the natural environment, but also in those born in captivity, we believe that a method to gradually improve the reproductive success of this species, is through early conditioning of the first generation of juvenile Chilean silverside born in captivity conditions

This first study on conditioning of broodstock, incubation and larval culture of *B. microlepidotus*, opens important perspectives for future farming activities, in addition to exploring the possibility of developing re-population aquaculture with juveniles. This aspect is especially relevant considering the significant number of interested recreational fishers and coastal artisanal fishers that depend on the exploitation of this aquatic resource, which is currently in a vulnerable state (Habit *et al*., 2006), despite the regulations applied by the fisheries authority.

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