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## An Updating of Withebait Farming (*Galaxias maculatus*) in Chile

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### 1. Introduction

The withebait *Galaxias maculatus* is an endemic species from Argentina and Chile (Cussac et al., 2004), and it is important in ecological webs in Argentinean and Chilean inland waters (Soto & Zúñiga, 1991; Soto et al., 1994; Modenutti et al., 1998). This species has a maximum size of 17 cm and its migratory juvenile stage is transparent or “crystalline” (Mardones et al., 2008). In Chile the juvenile stage is known as “puye” and the pigmented adult stage as “ao”. In Argentina in the larval stage is known as “puyen”, whereas in New Zealand is known as “withebait”, whereas the adults are known as “inanga” (McDowall, 1971).

This species inhabits rivers and littoral zones of lakes in Chile between Huasco river to Tierra del Fuego Island (28-53° S), *G. maculatus* predaes mainly microinvertebrates, its reproductive period is in southern spring and summer, and it is endangered due predation by exotic species (salmonids) and pollution of their habitats (Vila et al., 2006). Also this species has exposed to fisheries activities that in consequence generated a capture decreasing during the last decades, that would generate a collapse of fisheries, one tone of crystalline puye juveniles corresponds to approximately 3 million fish of 0.3 g individual body weight (Mardones et al., 2008). In this scenario there is legal management from Fisheries Subsecretary (SubPesca-Chile) and there are studies with the aim of *G. maculatus* farming that is developed by the Universidad Católica de Temuco.

### 2. Technical considerations for the establishment of whitebait cultivation

*Galaxias maculatus* develops part of its life cycles in freshwater, specifically in rivers and littoral zones of lakes as well as in estuarine environments (Vila et al., 2006). The first stages of its life cycle are carried out in freshwater and it comprises (a) conditioning of crystalline juveniles to obtain marine reproducers; (b) spawning; and (c) fertilization. The second stage is realized under brackish water conditions and comprises (a) incubation and (b) larval culture (Mitchell, 1989; Vega et al., 1996; Valdebenito & Vega, 2003; Mardones et al., 2008; Hicks et al., 2010).

The most important elements and details to consider for *G. maculatus* culture are: (a) selection of the species, (b) selection of the location and the water source for cultivation, (c)

appropriate cultivation systems and (d) selection and training of personnel (Mardones et al., 2008).

For reproduction it is necessary a broodstock proportions of one male: three females, and the average production of one female is approximately 600 fertile eggs (Valdebenito & Vega, 2003), and it is possible close the reproductive cycle in a period of 390 days (Tables 1 and 2; Mardones et al., 2008), and it is possible reach crystalline juveniles with commercial weight and size in 180 days after fertilization (Mitchell, 1989; Mardones et al., 2008).

ID	Stages	Day
1	Fertilization	1
2	Incubation & hatch	30
3	First feeding	40
4	Crystalline larvae	140
5	Maturation	180
6	Total duration	390

Table 1. Time of duration of the stages of the life cycle of *Galaxias maculatus* (Cf: Mardones et al., 2008)

Operation	% Survival	% Mortality	Number of inviduals
Number of males			103237
Number of females			309710
Fertilization*			185825893
Incubation**	50	50	148660714
Hatching	80	20	74330357
First nourishment	80	20	59464286
Postlarva	70	30	47571429
Crystalline larvae***			33300000

\* Fertilization: total number of eggs obtained from the spawning, 80 % being adequate for incubation.  
\*\* Incubation: number of incubated eggs.  
\*\*\* One kilogram of product = 3330 crystalline larvae approximately.

Table 2. Survival performance required to culture 10 tonnes of *Galaxias maculatus* (Cf: Mardones et al., 2008)

2.1 The selection of sites

This procedure is based in two aspects: the first is the determination the availability of optimal water quality and the topography of the terrain. Desirable locations have the following features: (a) close to sea level, (b) availability of water with different salinities including brackish water from 5-11 ppt and (c) coastal gravel or sand material, which will allow the installation of stakes in the sea or the sinking of deep sub-superficial wells for marine water (30 m) and deep wells for freshwater (100 m)(Mardones et al., 2008).

## 2.2 Overview of the production process

The production process begins with the getting of wild crystalline juveniles at the time of their migratory returns in September-October, that corresponding to southern spring (Allibone & Townsend, 1997; Valdebenito & Vega, 2003; Boy et al., 2006). They are transported to the culture facilities, where they are held up to six months to reach sexual maturity and as first step, the live specimens are placed in quarantine and given a prophylactic treatments to eliminate bacterial and parasite diseases brought from wild environment. Once mature broodstock are stripped by abdominal pressure and the eggs are fertilized and incubated over a period of 25-30 days (Valdebenito & Vega, 2003).

Once incubation occurs, the small larvae remain in the first feeding stage for 38-40 days, during which time they are fed ad libitum. Subsequently the larvae continue to a fattening stage where they remain for 140 days until they reach the weight and size of the commercial crystalline juveniles to be harvested and processed. A fraction of animals of this group is retained to be conditioned as reproducers for the next cycle. The total duration of the first production cycle late approximately 480 days, it begins from the purchase of the natural fry through conditioning as breeders until harvest and processing of 10 tonnes of crystalline juveniles (Valdebenito & Vega, 2003).

## 2.3 Purchase and transport of wild juveniles

Captures of wild juveniles are not constant as they are very dependent on the climate and other factors, fluctuating between 0.5 to 20 tonnes/season, this period requires approximately 90 days, and once, approximately 8 kg of juveniles are captured daily and the juveniles are placed in two tanks of 8 m<sup>3</sup> each one, until sufficient amounts are reached to realize the transport to the culture centre, where they will be conditioned (Valdebenito & Vega, 2003). The purchase price at this stage is approximately USD 20/kg (Mardones et al., 2008). The carrying density is 12 kg\*m<sup>-3</sup>, and it is used tanks capacity of 3 m<sup>3</sup> the mortality ranges from 0 to 5 % during transportation but is less when adults are captured (Valdebenito & Vega, 2003). The juveniles easily adapt to the captivity, and the total mortality from the arrival of the fish at the culture centre until pre-spawning is 60 %. The total capture of wild juveniles required to ensure sufficient reproducers in the first year considers: (a) each juvenile weighs approximately 0.3 g; (b) mortality due to transport is 0 %; (c) mortality up to pre-spawning is 60 %; (d) 30 % of the animals will not reproduce; and (e) the sex ratio at purchase is 1:1 as it is only possible to determine the sex once the reproducers are mature (Mardones et al., 2008). The table 3 shows the number and total biomass of juveniles that need to be purchased.

## 2.4 Broodstock conditioning

Once they arrived the juveniles are held in twelve 2 m<sup>3</sup> tanks for approximately 270 days until they become in optimal reproductive conditions (Valdebenito & Vega, 2003). In according to literature (Valdebenito & Vega, 2003; Mardones et al., 2008) the water quality parameters at this stage are 10-15° C, 0 ppm salinity, a density of 7 kg/m<sup>3</sup> and a daily exchange rate of 2. There are two main periods: (1) quarantine period and (2) period of sexual maturity.

Items	Ammount
Number of reproducers required for spawning	412946
Number of exemplary in a reproductive state	619420
Virginal reproducer (30 %)	884885
Mortality until pre-spawning (60 %)	2212213
Mortality (0 %)	2212213
Number of wild alevins to buy	2212213
Kilograms alevins	664

Table 3. Number of crystalline juvenile required. Cf: Mardones et al., (2008)

A single quarantine it is necessary when wild fish enter the culture center. This process consists of periodic application of prophylactic treatments from the day following entry to the hatchery until 14 days in order to eliminate potential ectoparasites and external bacteria. The prophylactic treatments are calculated based on saline and oxytetracycline baths and oxytetracycline oral administration (Table 4; Valdebenito & Vega, 2003; Mardones et al., 2008).

Treatment	Concentration	Application
Saline bath	6 g L <sup>-1</sup>	Two times per week until 40 day, bath of 30 min.
Oxytetracycline - bath	35 mg L <sup>-1</sup>	Day 2, bath of 30 min.
Oxytetracycline - oral	75 mg kg <sup>-1</sup>	Day 3-14 incorporated into feed.

Table 4. Types of prophylactic treatments applied and their specifications. Cf: Mardones et al., (2008)

The juveniles are fed with beef liver in the first week; 75 % liver and 25 % oil-free salmonid starter diet in the second week; 50 % liver and 50 % starter in the third week; 25 % liver and 75 % starter in the fourth week, and 100 % starter in the fifth week. Once quarantine period finished, the juveniles are conditioned for 140 days to become reproducers and during this period they are fed a pelletized freshwater salmon diet that provides good growth and disease resistance (Table 5). The diet ration is at 2 % body weight daily in 10 daily rations by automatic feeders, depending on the biomass and the growth of the fish (Table 6; Valdebenito & Vega, 2003; Mardones et al., 2008).

The broodstock are reared in 8 m<sup>3</sup> fibreglass tanks 3 m in diameter. They remain in these tanks during vitellogenesis and they are transferred avoiding excess manipulation, only during the spawning period for separation according to their maturity level (Valdebenito & Vega, 2003). The broodstock are spawned three times during their lifetime and so a program of annual replacement and genetic management is required in order to maintain the reproductive cycle. The broodstock are replaced from juvenile produced, as well as from those fish that are not sexually mature from the previous year (Mardones et al., 2008).

Sex can be differentiated by colour and abdomen size. The state of maturity, once reached, is classified by visual check of the external morphology of the specimens and abdominal pressure on a monthly, biweekly basis according to the descriptions of (Valdebenito & Vega, 2003). This maturity stage is determined weekly for the stages mature 2 and hatched 2 with

Diet	Total length (mm)	% Proteins	% Lipids	% Carbohydrates	Crude energy MJ kg <sup>-1</sup>
Starter 00	0.3 – 0.5	57.9	30.2	11.9	22.5
Starter	0.5 – 1.0	55.6	33.4	11.0	22.9
Alevins crumble 1	1.0 – 1.5	53.5	36.4	10.1	23.4
Alevins crumble 2	1.5 – 2.0	51.4	39.2	9.4	23.8

Table 5. Ongrowing diet sequence. Cf: Mardones et al., (2008)

Stage (day)	Unitary weight	Number of individuals	Biomass (kg)	Feed day (kg)	Feed total (kg)
Crystalline 1 – 60 days	0.3	2212213	664	13	796
Young 61 – 120 days	1	2212213	2212	44	2655
Pre-spawning reproduction 121-180 days	3	884885	2655	53	3186

Table 6. Characteristics of the fish and feed supplement quantities. Cf: Mardones et al., (2008)

the purpose of reducing the stress level of reproducers. During the post-spawning period for females and males are transferred to unoccupied tanks and a series of additional tanks are required for the juveniles obtained to replenish the broodstock (Mardones et al., 2008).

3. Spawning and fertilization

The maturity of the broodstock is not instant or simultaneous and the spawning period is extended over whole year, with a peak between May and September (Alliborne & Townsend, 1997; Valdebenito & Vega, 2003). The main period covers approximately 90 days, with a peak interval of 30 days (Valdebenito & Vega, 2003; Dantagnan et al., 2007). The spawning of captive individuals can be synchronized through management of the photo-thermal period manipulation, variations of the temperature or hormonal management. It must be remembered that, of the total, of the total reproducers surviving until the end of the conditioning stage 30% of these will be virginal and will be used in the next spawning.

After sex determination, males and females are separated into different tanks. Once they reach maturity stage 3, they are selected for spawning using abdominal massage (Mardones et al., 2008). The spawners are anaesthetized using MS222 (Boy et al., 2006) at a concentration of 0.3 mL\*L<sup>-1</sup>, and through slight abdominal pressure the gametes of females and males are released. Approximately 100 females can be spawned per hour. The mature reproducers have an average weight of 3 g, and once spawned they lose 29% of their initial weight (Valdebenito & Vega, 2003).

fertilization is achieved by the first extracting the semen from the males into a petri dish, and the obtained eggs are deposited in mono-layers in the incubation trays, which consist of a net of 0.2 mm mesh (Valdebenito & Vega, 2003). The eggs are fertilized using sperm diluted with water and left to rest for between 15 and 30 min, during which time they adhere to the base of the incubation trays, one male is used for every three females (Valdebenito & Vega, 2003; Dantagnan et al., 2007; Mardones et al., 2008). After this time,



the eggs are washed repeatedly with clean water to eliminate the excess semen and contaminating particles. It is known that each female produces an average of 600 eggs and that approximately 80% of the eggs are fertilized, and for incubation those lots with more than 60 % embryos are used, the remainder being eliminated (Valdebenito & Vega, 2003; ., 2008).

#### 4. Incubation and hatching

This stage extends from 45-120 days from the first incubated eggs to the last hatching, once the hydration process has occurred and the eggs are fertilized, the tare placed in an incubation room for approximately 25-30 days until the hatching of the larvae, with has a controlled environment and high humidity (Barile, 2003; Mardones et al, 2008). The trays are placed in modules of 10 U in hanging structure, and during this period (Barile, 2003; Mardones et al., 2008), they are protected from light specially sunlight because the natural ultraviolet radiation damages the DNA in the eggs (Battini et al., 2000). The optimal parameters for incubation have been found to be 10-12°C, 90% humidity and 10-16 g\*L<sup>-1</sup> salinity. In addition to providing adequate environment, the eggs require to be sprayed with water at salinity of 10-16 g\*L<sup>-1</sup> (Barile, 2003; Mardones et al., 2008). The number of daily sprays and the number of incubation trays to be used are mainly dependent on the number of females that are spawned daily and the duration of this stage, the optimal number of pulses is three, if 3200 females are spawned every day (Barile, 2003; Mardones et al., 2008). Nevertheless, the females do not mature in a synchronized form and so the total incubation period may extend for up 90 days from the first hatching of larvae, and typically, 96000 females can be spawned over 30 days (Barile, 2003; Valdebenito & Vega, 2003; Dantagnan et al., 2007;Mardones et al., 2008).

For the production of 10 tonnes of juveniles, a total of 185826000 eggs are required, using 3200 incubation trays, where they will remain until hatching, the hatching under natural conditions is a completely asynchronous process, covering a long period until are released (Dantagnan et al., 2007; Mardones et al., 2008). In captivity conditions, the hatching can be synchronized through temperature management (Barile, 2003; Mardones et al., 2008). Once the eggs are about to hatch, they are transferred to the hatching systems, which consist of small tanks with a capacity of 10 trays each, finally these are closed systems with salinity from 10 to 16 g\*L<sup>-1</sup> and controlled temperatures varying between 12 - 15°C (Mardones et al., 2008),

#### 5. Larviculture

In according to literature (Borquez et al., 2003; Dantagnan et al., 2007) larviculture extends for 270 days, from the time the first larvae are obtained until the harvest of the last juveniles, this stage has two significant period: (a) first feeding and (b) culture of postlarvae. One day after hatching, the small larvae are transported to fattening tanks, where they will remain for 180 days, the first 38-40 days corresponds to the stage of the first feeding and the remaining days to the culture of postlarvae, after which the crystalline juvenile are ready (Bórquez et al., 2003; Dantagnan et al., 2007; Mardones et al., 2008).

The larvae absorb the yolk sack between 5 and 8 dph (days post-hatching), if the larvae do not receive food immediately after this, high mortality takes place due starvation, the

highest mortality rate of about 20% being observed between 12 and 13 dph (Bórquez et al., 2003; Dantagnan et al., 2007; Mardones et al., 2008). The optimal conditions for larval development are  $13 \pm 1^{\circ}\text{C}$ ,  $16 \text{ g}\cdot\text{L}^{-1}$  salinity, a density of  $90 \text{ larvae} \cdot \text{L}^{-1}$  and a rate of exchange of  $1 \cdot \text{day}^{-1}$  (Barile et al., 2003). Larval densities are reduced from  $90 \text{ larvae} \cdot \text{L}^{-1}$  at 1-20 dph to  $20 \text{ larvae} \cdot \text{L}^{-1}$  at 20-40 dph. In according to literature (Bórquez et al., 2003; Dantagnan et al., 2007; Mardones et al., 2008) it is possible found high survival due low essential fatty acids (EPA and DHA) at salinities between 10-15 ppt, whereas an inverse situation was observed with freshwater (Dantagnan, 2003). At hatching, the larvae are able to accept small particles and they are fed daily, by hand, to satiation on rotifers enriched with fatty acids and *Artemia* nauplii. The feed sequence and quantities are specified in table 7 (Mardones et al., 2008).

Period (day)	Nourishment feed	Daily in rotifers (kg; $1\cdot 10^6$ rotifers = 0.15 g)	Daily ration (kg; $3 \cdot 10^9$ nauplius = 0.6 g)	Total amount (kg)
0 – 20	$40 \text{ rotifers} \cdot \text{ml}^{-1}$	4	0	79286
21 – 30	$(40 \text{ rotifers} + 4 \text{ nauplius}) \cdot \text{ml}^{-1}$	18	0.002	178417
31 – 40	$(30 \text{ rotifers} + 10 \text{ nauplius}) \cdot \text{ml}^{-1}$	13	0.006	133854
Total		35	0.008	391556

Table 7. Diet for the stage of first feeding. Cf: Mardones et al., (2008)

6. Postlarvae culture

After first feeding, the larvae pass on to the postlarval culture stage, the obtained larvae are fed only with inert diets for a period about 140 days, the optimum conditions in this phase are  $13^{\circ} \text{C}$ ,  $15\text{-}16 \text{ g}\cdot\text{L}^{-1}$ salinity, a density of  $10 \text{ kg} \cdot \text{m}^{-3}$ , an exchange ratio 0.33 of the tank capacity daily, for the postlarvae culture, a total of 33300000 individuals are used with an equivalent biomass of 10000 kg (Bórquez et al., 2003; Dantagnan et al., 2007; Mardones et al., 2008). It is necessary a density of  $10 \text{ kg} \cdot \text{m}^{-3}$ ,  $1000 \text{ m}^3$  of culture capacity, that representing 125 tanks of  $8 \text{ m}^3$  in use for 300 days, based on the quantitative data presented here, at the end of the larval culture phase 10 tonnes of junveniles will be produced (Mardones et al., 2008).

7. Harvest and commercialization

At the end of the larval culture period, the commercial product will be obtained as eel like that is called, crystalline juvenile with no, or only slight, coloration a body length of 4-6 cm and a body weight of 0.3 g, before harvesting, the fish are starved for 2 days and are placed live in tanks of clean water for depuration (Mardones, 2003; Dantagnan et al., 2007; Mardones et al., 2008). These specimens are slaughtered by asphyxia, washed in a solution of  $5 \text{ g}\cdot\text{kg}^{-1}$  chlorine and citric acid or ascorbic acid at  $5 \text{ g}\cdot\text{kg}^{-1}$ , ready for subsequent



transportation to the packaging plants (Mardones, 2003; Mardones et al., 2008). Currently, whitebait is sold fresh as well as frozen in Chile and New Zealand, at a price that fluctuates between 10 and 45 USD\*kg<sup>-1</sup>. It is known that, in Chile the product is sold fresh on the beach at price of 10 USD\*kg<sup>-1</sup> is required (Mardones, 2003; Mardones et al, 2008).

## 8. Discussion

Puye or whitebait culture has considerable aquaculture potential and the production technology and package outlined here already runs successfully (Dantagnan et al., 2007; Mardones et al., 2008). Nonetheless, there are several ways in which the system could be further optimized, the good quality domesticated broodstock is a key stage that would benefit from further work. Reproducers with a higher fecundity and better survival rates, including greater resistance to endo-parasitic infection, would be beneficial. These domestic broodstock could then more reliably support production of eggs and larvae at a lower cost.

Domestication and improvement of management and culture technologies must focus on the reduction in mortality that takes place up to the spawning stages in the broodstock as well as in the incubation and postlarval stages. For broodstock and juveniles produced entirely in captivity, it may not be necessary to use of intensive prophylaxis, diminishing fish manipulation, stress and mortality, that is a frequent procedure in aquaculture.

Development of technologies to increase the densities during the broodstock conditioning stage and to coordinate the spawning under controlled conditions would be useful. It is also necessary to increase the larviculture densities, improve the enrichment of live food and further automate all culture processes, including massive incubation and larval feeding.

While the results to date are very positive, the cost of production is high, bearing in mind that, at least a price relatively similar to that obtained by fresh product on the beach (USD 10) must be obtained. In the financial analyses conducted to date, the costs of training have not been considered, but for full development but for full development culture industry, the early training of staff is absolutely necessary.

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This book provides an understanding on a large variety of aquaculture related topics. The book is organized in four sections. The first section discusses fish nutrition second section is considers the application of genetic in aquaculture; section three takes a look at current techniques for controlling lipid oxidation and melanosis in Aquaculture products. The last section is focused on culture techniques and management, ,which is the larger part of the book. The book chapters are written by leading experts in their respective areas. Therefore, I am quite confident that this book will be equally useful for students and professionals in aquaculture and biotechnology.

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