

# Reproduction of landlocked *Aplochiton zebra* Jenyns (Pisces, Galaxiidae)

Lattuca ME, Brown D, Castiñeira L, Renzi M, Luizon C, Urbanski J, Cussac V. Reproduction of landlocked *Aplochiton zebra* Jenyns (Pisces, Galaxiidae).

Ecology of Freshwater Fish 2008. © 2008 The Authors. Journal compilation © 2008 Blackwell Munksgaard

**Abstract** – Life-history studies comparing landlocked versus diadromous populations reveal factors associated with life-history evolution under different scenarios. We describe several aspects of spawning and development of landlocked *Aplochiton zebra* in Patagonia (Argentina) based on the presence of eggs, the gonadal development, the analysis of otolith daily growth increments validated by tetracycline marking experiments, and the analysis of standard length frequencies. Among Patagonian galaxiids, *A. zebra* showed intermediate size and age at first maturity and high fecundity, with vitellogenic oocytes covered with short chorionic filaments. Free embryos of *A. zebra* were larger than those of *Galaxias maculatus* and *Galaxias platei*. This information is particularly significant for conservational purposes when we consider the reduced distribution of landlocked populations of the species and the risks imposed by salmonid introduction.

**M. E. Lattuca<sup>1</sup>, D. Brown<sup>2</sup>,  
L. Castiñeira<sup>3</sup>, M. Renzi<sup>2</sup>, C. Luizon<sup>4</sup>,  
J. Urbanski<sup>3</sup>, V. Cussac<sup>1,5</sup>**

<sup>1</sup>Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, <sup>2</sup>Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Mar del Plata, <sup>3</sup>Centro de Ecología Aplicada del Neuquén (CEAN), Junín de los Andes, <sup>4</sup>Secretaría de Desarrollo Sustentable y Ambiente de Tierra del Fuego, Ushuaia, <sup>5</sup>Universidad Nacional del Comahue, Bariloche, Argentina

Contribution INIDEP n° 1496.

**Key words:** Patagonia; otolith daily growth increments; spawning season; chorionic filaments; embryonic development; free embryos

María Eugenia Lattuca, Bernardo Houssay 200, (9410) Ushuaia, Tierra del Fuego, Argentina; e-mail: elattuca@gmail.com

Accepted for publication December 14, 2007

## Introduction

The family Galaxiidae (*sensu* Nelson 1994) is found throughout the southern cool-temperate zone in the Southern Hemisphere (McDowall 2000, 2002). Several species in this family display alternative life histories wherein some populations are diadromous while others are landlocked (Campos 1970a,b; Pollard 1971; Ferriz 1987; McDowall et al. 1994; Peredo & Sobarzo 1994; Barriga et al. 2002, 2007; Cussac et al. 2004; Chapman et al. 2006). McDowall (1980) proposed that evolution of anadromy in this group implies an adaptive ability for escaping cold Pleistocene winters. Particularly, the distribution of *Aplochiton* species shows a latitudinal range, south of 36°41'S, where both landlocked and diadromous populations are present (McDowall 1971; Cussac et al. 2004). Although the distributional area of *Aplochiton zebra* is large, its captures are very infrequent and when successful, they are very scarce (Lattuca et al. 2007).

Three genera of galaxiids occur in South America: *Galaxias*, *Brachygalaxias* and *Aplochiton*. Life-history information is available for some, but not all, South American species (Cussac et al. 2004). The

embryonic development of *Galaxias maculatus* (Benzie 1968b), *Galaxias platei* Steindachner (Ortubay & Wegrzyn 1991), *Brachygalaxias bullocki* (Regan) (Campos 1972) and *Aplochiton taeniatus* Jenyns (Campos 1969), as well as their spawning conditions (Benzie 1968a; Campos 1969, 1972, 1984; Ferriz 1987; Ortubay & Wegrzyn 1991), have been previously described.

Available data suggest that, in addition to differences in migration, diadromous and landlocked forms differ in other life-history aspects. For example, the diadromous forms of *G. maculatus* have larger eggs, free embryos and metamorphic larvae than landlocked populations (Cussac et al. 2004). Breeding season also varies between landlocked and diadromous populations and within these groups along the geographic range (Boy et al. 2007). Landlocked populations must endure the first winter in oligotrophic lakes, which exacerbates first feeding demand and increases the probability of winter starvation in relation to diadromous populations. Precise data about *A. zebra* reproduction are not available. Smitt (1901) mentioned that this species seems to spawn in March and Eigenmann (1928) observed specimens laying

eggs in the same month in a small outlet stream from Riñihue Lake.

Occurrence of multiple examples of life-history variation in landlocked versus diadromous populations (Chapman et al. 2006; Barriga et al. 2007; Boy et al. 2007) provides a unique opportunity for comparative studies that will allow us to address the evolution of life-history characteristics in different environments. The aim of this study is to describe the main aspects of spawning and embryonic development of landlocked *A. zebra*, whose life-history traits are poorly known and whose distribution has been severely reduced, probably by salmonid introduction (McDowall et al. 2001; Cussac et al. 2004).

**Materials and methods**

Fieldwork was carried out into the frame of a bigger fish survey (Milano et al. 2006; Ruzzante et al. 2006) along Patagonia. Successful captures were carried out during 2000–2004 in only five of the sampled freshwater Patagonian lakes of Pacific drainage (Fig. 1): Lake Rivadavia (42°37'S, 71°41'W, 527 m

a.s.l.), Lake Futalaufquen (42°49'S, 71°41'W, 518 m a.s.l.), Lake Puelo (42°10'S, 71°40'W, 150 m a.s.l.), Lake Foyel (41°44'S, 71°25'W, 800 m a.s.l.) and Lake Lácar (water-mill-type trap at 'Estación de Captura y Reproducción de Peces del Arroyo Pocahullo', San Martín de los Andes, Provincia del Neuquén, 40°09'S, 71°21'W, 625 m a.s.l.).

Samples were taken using different fishing gears: seine (25-m long, 1.5-m high and 3-mm stretched mesh size), gill (30-, 40-, 60-, 100-, 120- and 140-mm stretched mesh distance, each 10-m long and 2-m high), ichthyoplankton (one 50.5-cm diameter, 260-cm length, 1500-µm mesh size; and the other 32-cm diameter, 105-cm length, 270-µm mesh size) and dip nets, electrofishing and baited traps. Seine netting covered, in five to six operations, ca. 300 m of shoreline from the shallower littoral zone up to 1.5 m depth. Gillnets were placed in two ways, perpendicular to the shore line and parallel to the coast at 10, 20, 30 and 50 m deep including benthic and pelagic arrangements following Vigliano et al. (1999). Ichthyoplankton nets were trawled from an inflatable motorboat at surface level and at two different depths in the limnetic

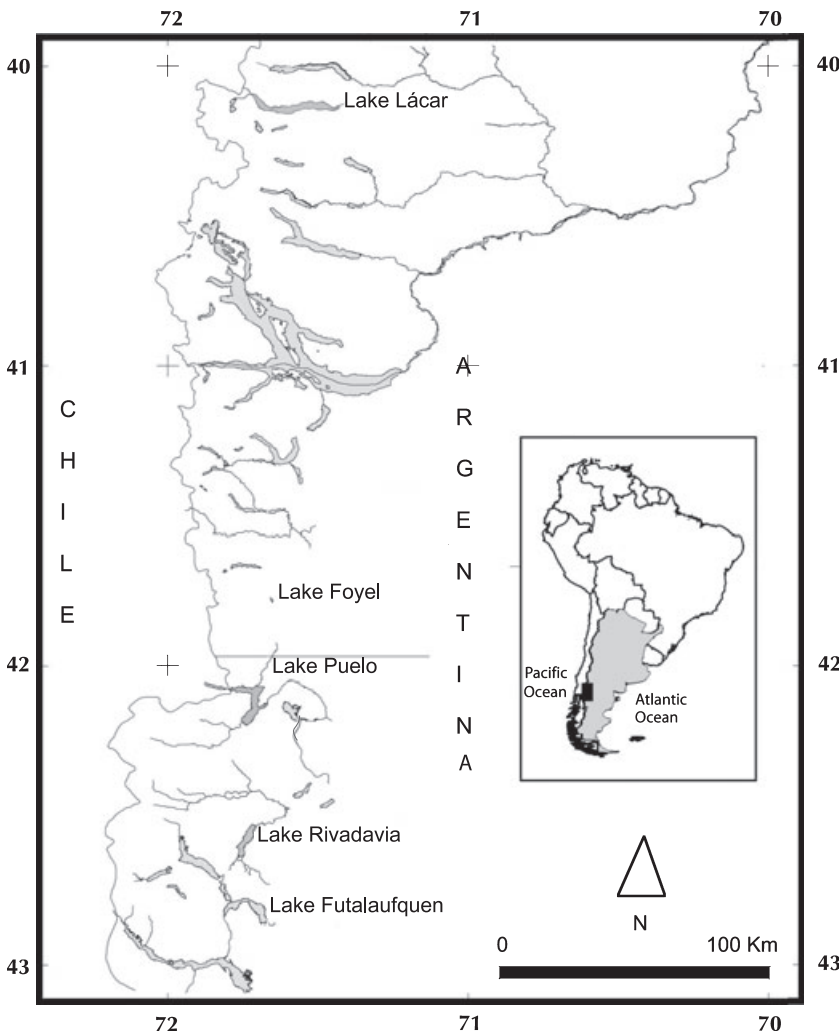


Fig. 1. Geographic locations of lakes. Approximate location desired in the text: below line 79.

zone along a fairly stable transect. Fish starting their reproductive migration up Pocahullo stream at Lake Lácar were detoured by a barrier made of canes and driven through a channel to a water-mill-type trap. This trap consists of three baskets which take the fish out of the channel and slide them through trays into lateral pools. In these pools, fish were daily classified by sex, species and degree of maturity. Dip nets were used to collect fish from the lateral pools of the trap. Baited traps were placed on the lakebed at different depths ranging from 1 to 50 m.

Ontogenetic periods were defined according to Balon (1990). Individuals were fixed in 4% formaldehyde (for gonadal study), in 96% ethanol (for otolith study), or carried alive (for age validation) to the laboratory. They were measured with a vernier calliper ( $\pm 0.1$  mm) and weighed ( $\pm 2$  mg). To assess adulthood, gonad development was examined with a stereomicroscope, considering the presence of vitellogenic follicles or running sperm. The adult gonads were dissected, weighed and preserved in 4% formaldehyde for histological analysis. Slides for microscopy were obtained by embedding small pieces of dehydrated gonads in paraffin or paraffin-wax, cut ( $7 \mu\text{m}$ ) and stained with Haematoxylin and Eosin (Martoja & Martoja-Pierson 1970). Fecundity was estimated in two ways: (i) as the product of the number of vitellogenic follicles in a piece of ovary and the ratio between the weight of both ovaries and the weight of the piece (Kipling & Frost 1969) and (ii) from direct counting of eggs deposited by one female in captivity.

Age determination through enumeration of daily growth increments visible in otolith microstructure is commonly applied (Campana & Nielson 1985). This requires validation of the periodicity of growth increments to estimate true age (Beamish & McFarlane 1983). The use of tetracycline chlorhydrate (TCC) is a common procedure for validation (Campana & Nielson 1982). To attempt the validation, 10 *A. zebra* juveniles [37–41 mm standard length (SL)] from Lake Puelo, were marked with TCC following Vigliola (1997). The concentration and time of immersion that would mark the otoliths but not kill the fish were determined following Tzeng & Yu (1989), Sogard (1991), Szedlmayer & Able (1992). Therefore, marking was carried out by placing the fish in a  $200 \text{ mg}\cdot\text{l}^{-1}$  TCC solution in a 30-l aquarium with constant aeration for 2 h. Following the treatment, fish were placed immediately in two net cages ( $0.35 \text{ m}^3$ ) at 5 m deep in a protected bay and were, after 10 days, sacrificed and preserved in alcohol 96%. *Sagittae* otoliths of TCC-treated fish and of juveniles captured in Lake Rivadavia in February ( $N = 28$ , 34–58 mm SL) and May 2000 ( $N = 28$ , 49–61 mm SL) and in Lake Puelo in February 2002 ( $N = 16$ , 35–41 mm SL), were removed under a stereomicroscope, placed on a glass slide and covered with a drop of transparent

thermoplastic glue named Pro-texx<sup>®</sup> (Learner Laboratories, Pittsburgh, PA, USA). Fluorescent marks in the otoliths of the TCC-treated fish were examined under UV illumination in an epifluorescence microscope Olympus IX70, and photographed with an SC35 Olympus camera (Olympus Optical Co. Ltd., Tokyo, Japan). The number (between the first increment clearly discernible and the otolith margin), width and radius of each daily increment and total radius of *sagittae* otolith were recorded under transmitted light, using an Axioscope Zeiss microscope at 200–400 $\times$  magnification with an associated Sony CCD camera and a Kontron image analysis system (Kontron Elektronik GmbH, Eching, Germany). Birth dates were estimated as the capture date minus the number of otolith growth increments, which were counted between the first increment clearly discernible, an intense band around the nucleus, and the otolith margin. It was assumed that this intense band around the nucleus is the ‘hatch ring’.

The SL frequencies of individuals captured in Lake Puelo ( $N = 109$ , 35–160 mm SL) were analysed by Bhattacharya’s polymodal analysis and then fitted to the von Bertalanffy model, using the FAO-ICLARM Stock Assessment Tools (Gayanilo et al. 1994).

To assess the environmental condition necessary for *A. zebra* adults to perform upstream spawning movements, Pocahullo stream temperature and Lake Lácar water level (2001–2004) were analysed. Water temperature was recorded with a thermograph Kohari 168/24, Tokyo, Japan, and lake water level data came from the records of the Subsecretaría de Recursos Hídricos, Ministerio de Planificación Federal, Inversión Pública y Servicios, Provincia del Neuquén. Natural photoperiod (<http://www.cra.gov.ar/dta/ais/aip/docs/20.pdf>) was estimated on the basis of data corresponding to the closest airport (110 km, San Carlos de Bariloche).

Embryonic development was observed on eggs collected, during July–September 2003, from the concrete wall of the outlet channel of the salmonid trap in the Pocahullo stream at Lake Lácar, and maintained in dishes protected from light and at the same temperature as that recorded in the stream. During the first egg collection in August 2003, water temperature (thermograph Kohari 168/24, Tokyo, Japan), conductivity (Hanna, HI 8733, Hanna Instruments Inc., Woonsocket, RI, USA), water velocity and oxygen concentration (Iijima ID-100 MLSS, Iijima Electronics Corp., Gama-gori, Japan) were recorded. Later stages of development were observed in newly collected eggs. The chronology of the events described was referred to as the accumulated thermal units (ATU, the sum of mean daily temperatures). Photographs were taken with a stereomicroscope and an attached Minolta X-300 camera (Minolta Camera Co. Ltd., Osaka, Japan).

After hatching, free embryos ( $N = 10$ ) were photographed, measured and their myotomes counted, using

an image analysis software (Image-Pro Plus<sup>®</sup>, version 4.0, Media Cybernetics, Silver Spring, MD, USA). Total length (TL) and mean yolk sac diameter were recorded on individuals fixed in 4% formaldehyde. Number and position of melanophores were also recorded.

## Results

### Spawning season

Juvenile captures occurred in the lake's littoral zones in summer (December, February and March), autumn

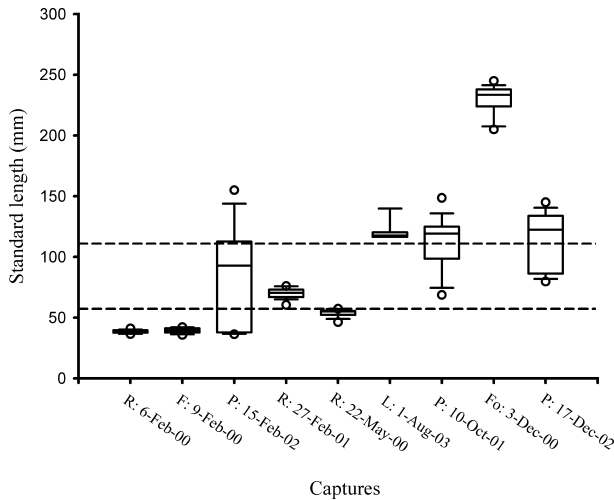


Fig. 2. Size structure of *Aplochiton zebra* captured in different lakes and dates. Median, quartiles and 5th and 95th percentiles are indicated. Horizontal lines indicate size at first winter (59 mm SL) and minimum adult size (116 mm SL). R: Lake Rivadavia, F: Futalaufquen, P: Puelo, L: Lácar and Fo: Foyel. Approximate location desired in the text: below line 173.

Table 1. Captures of *Aplochiton zebra* in five freshwater lakes.

Lake	Date of capture	Fishing gear	Life period	N	Range (mm SL)
Foyel	12/01	Gill net (40 mm†)	Adult	10	205–245
Futalaufquen	02/00	Seine net	Juvenile	54	35–42
Lácar	07/03	Salmonid trap	Adult	2	118–142
	08/03	Salmonid trap	Adult	4	116–120
	07/04	Salmonid trap	Adult	3	126–137
	08/04	Salmonid trap	Adult	2	130–134
Puelo	10/01	Gill net (30 mm†)	Adult	2	150–160
		Seine net	Juvenile	13	60–113
	02/02	Seine net	Adult	21	118–159
			Juvenile	38	32–115
			Adult	12	116–160
	12/02	Seine net	Juvenile	14	78–112
			Adult	23	117–150
03/04	Seine net	Juvenile	20	36–52	
		Adult	27	120–160	
		Adult	3	36–38	
Rivadavia	02/00	Baited traps	Juvenile	98	33–42
		Seine net	Juvenile	57	44–59
	05/00	Seine net	Juvenile	15	36–76
			Juvenile	15	36–76

Approximate location desired in the text: below line 173.

†Stretched mesh size.

(May) and spring (October), whilst adult individuals were captured in the lake's littoral zones in February, August (at the Pocahullo stream), October and December (Fig. 2). Captures also indicated the presence of mature adults and eggs adhered to the substrate during July–August in the mouth of Pocahullo stream at Lake Lácar (Table 1).

A winter spawning season was observed in Lake Lácar on the basis of: (i) the presence of eggs, collected at Pocahullo stream (Lake Lácar) in July 30 (2003) and 31 (2004) and August 10 (2003) and 13 (2004) and (ii) the gonadal status of fish caught by the salmonid trap at Pocahullo stream in winter 2003–2004 (seven males and four females, see below). Neither vitellogenic females nor running ripe males were observed in spring, summer and autumn, in any of the lakes. Adults captured in Lake Puelo in spring (October 2001) were not ripe.

After the TCC immersion of juveniles captured in Lake Rivadavia, a high mortality rate (seven of 10 fish) was registered in the cages, probably as a result of fungi proliferation. The fish that survived showed clearly defined fluorochrome marks and the number of increments deposited between the TCC mark and the otolith margin indicated that such a daily basis held true for the cage maintenance period (Fig. 3). Birth dates were estimated as the capture date minus the number of otolith growth increments. Growth ring counts in otoliths of juveniles caught in February (78–89,  $N = 28$ ) and May 2000 (143–182,  $N = 13$ ) allowed us to establish birth dates in November–early January.

Ring counts in otoliths of juveniles caught in Lake Puelo during February 2002 (86–105,  $N = 16$ )

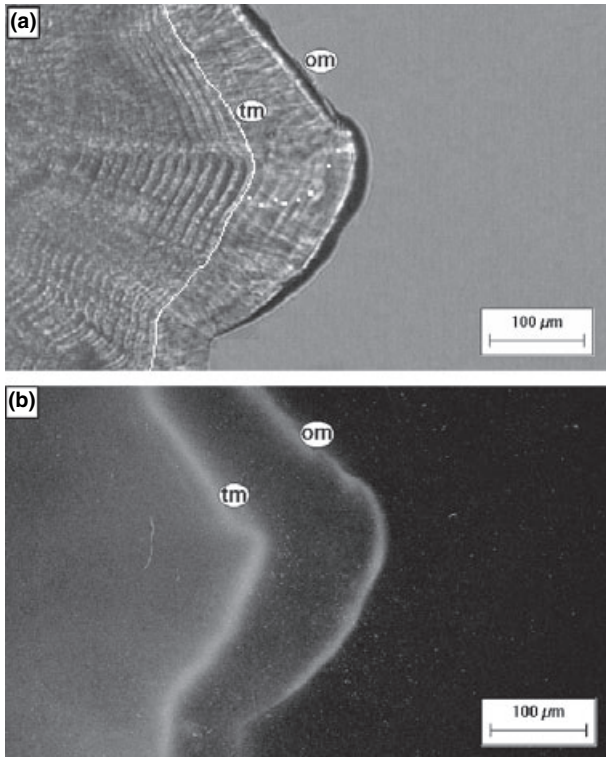


Fig. 3. Sagitta marked with tetracycline chlorhydrate (TCC). (a) Microscope image under normal light and (b) under fluorescent light. Symbols indicate: tm: TCC mark (white line), om: otolith margin and daily growth increments (white dots). Approximate location desired in the text: below line 189.

allowed us to establish birth dates in November. The analysis of SL frequencies of individuals showed two (in October 2001 and December 2002) and three (in February 2002) length modes for each sampling date; indicating that the littoral captures consisted of two (in October 2001 and in December 2002) and three (in February 2002) different cohorts (Fig. 4). Confidence intervals (95%) for the parameters of the von Bertalanffy growth function fitted to SL versus age data [ $N = 109$ ,  $r^2 = 0.93$ ,  $L_\infty$  (mm) = 144.75–167.11,

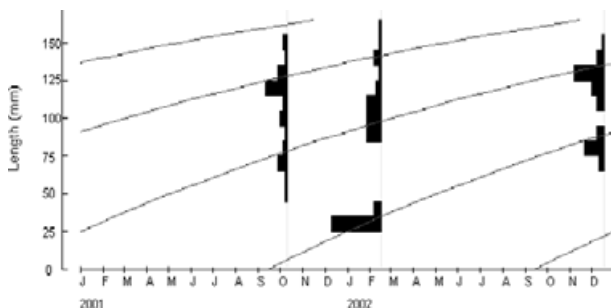


Fig. 4. Standard length frequencies plot and von Bertalanffy growth model superimposed. Approximate location desired in the text: below line 200.

$k = 0.49\text{--}0.76$ ,  $t_0$  (years) =  $-0.19$  to  $0.03$ ] allowed us to establish that the size (10.8 mm SL) corresponding to November (birth dates) is included into the range of size at hatching recorded in the laboratory (8.0–12.7 mm TL for individuals of Lake Lácar, see below).

#### Acquisition of gonadal maturity

At Lake Lácar, adult size was recorded only for those individuals captured during 2003 and 2004. The smallest male with spermatozoa was 116.4 mm SL and the smallest vitellogenic female was 129.8 mm SL, 2 and 3 years old, respectively. Histological analyses confirmed the sexual maturity of these individuals (see below).

#### Sexual dimorphism

The only difference between females and males was found in their genital papilla. Both sexes possess a urogenital papilla, immediately anterior to the anal fin origin. This papilla is formed by an epidermal basis and an epithelial protrusion and is more conspicuous in males (Fig. 5).

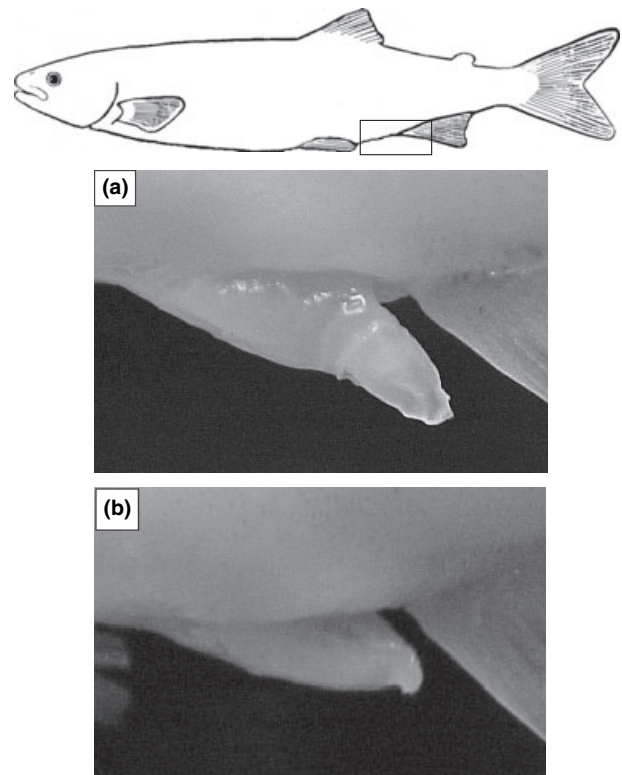


Fig. 5. Urogenital papilla of *Aplochiton zebra* adults. (a) female, (b) male. Approximate location desired in the text: below line 210.



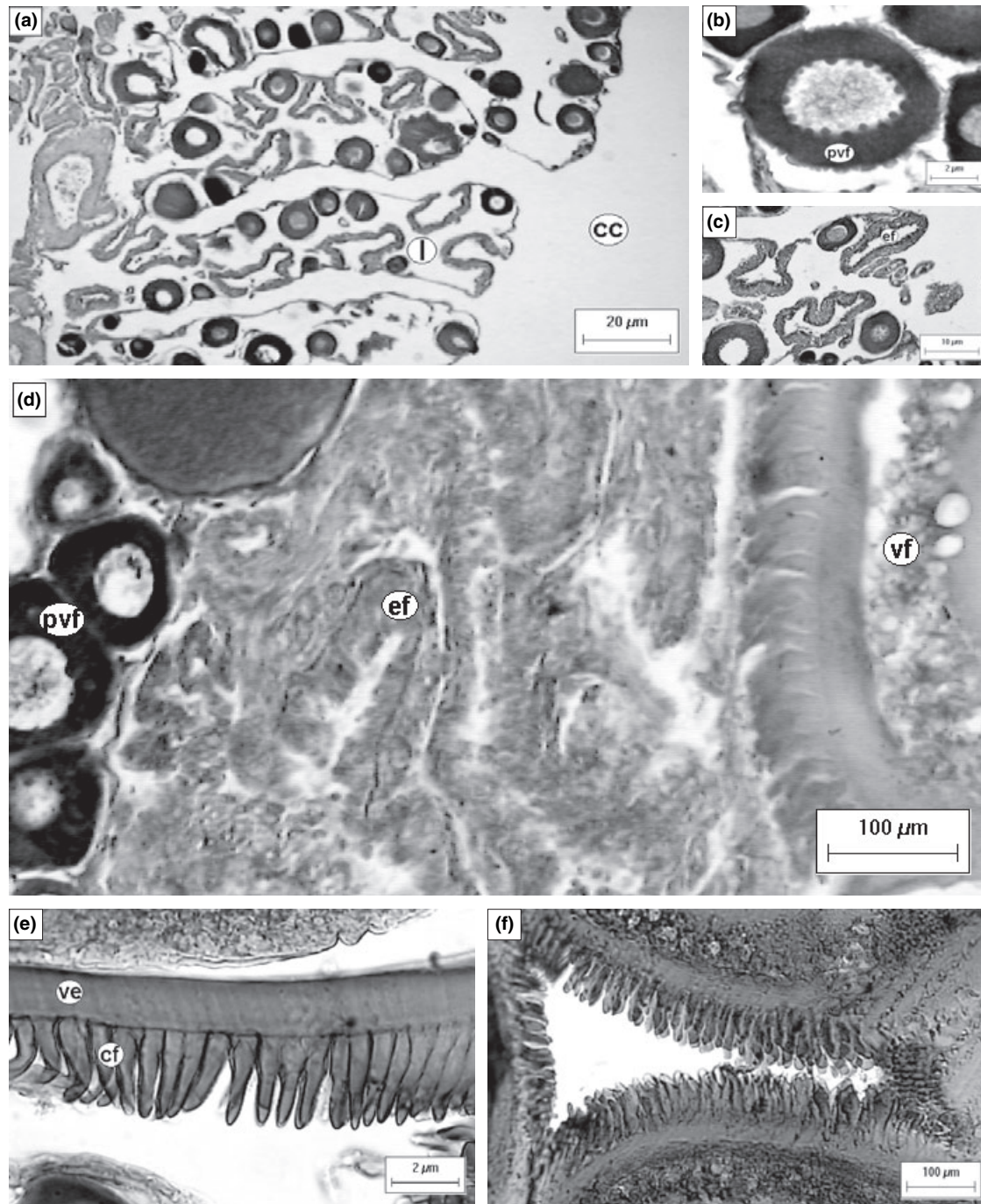


Fig. 6. Female gonads of *Aplochiton zebra*. (a) lamellar structure of the ovary, (b) previtellogenic follicle, (c) empty follicles, (d) previtellogenic, vitellogenic and empty follicles (e) vitelline envelope with chorionic filaments and (f) ovulated oocytes. cc: coelomic cavity, cf: chorionic filaments, ef: empty follicle, l: lamellae, pvf: previtellogenic follicle, ve: vitelline envelope, vf: vitellogenic follicle. Approximate location desired in the text: below line 222.

### Female gonads

As in most Salmoniformes, the ovary is a compact paired organ (Nagahama 1983), with its lamellae in contact with the peritoneal cavity (Hoar 1969; McDowall 1969) (Fig. 6). During the breeding season, histological observations showed ovaries with follicles

at different developmental stages (Fig. 6). The ovaries contained mainly vitellogenic follicles, small basophilic follicles and also empty follicles, which confirmed the spent female condition. Histological observations ( $N = 25$ ) also revealed the presence of short filaments ( $2.57\text{-}\mu\text{m}$  long) arising from the chorionic surface of vitellogenic oocytes (Fig. 6).

Fecundity was rather high, estimation from direct counting was 5700 eggs per female ( $N = 1$ , 142 mm SL) and the mean number of eggs per female estimated following Kipling & Frost (1969) was 6400 ( $N = 3$ , 129–134 mm SL).

#### Male gonads

Seminal tubules showed a cystic organisation (Nagahama 1983). Running ripe males had an enormous

quantity of spermatozoa in the lobular lumen (Fig. 7). Flagella could only be observed under phase contrast microscope.

#### Environmental conditions

*Aplochiton* individuals were recorded at the Pocahullo stream (Lake Lácar system) from July 29 to August 13 in 2003 and 2004, in a wide range of stream water temperatures ( $5.8 \pm 2.8$  °C) but in a

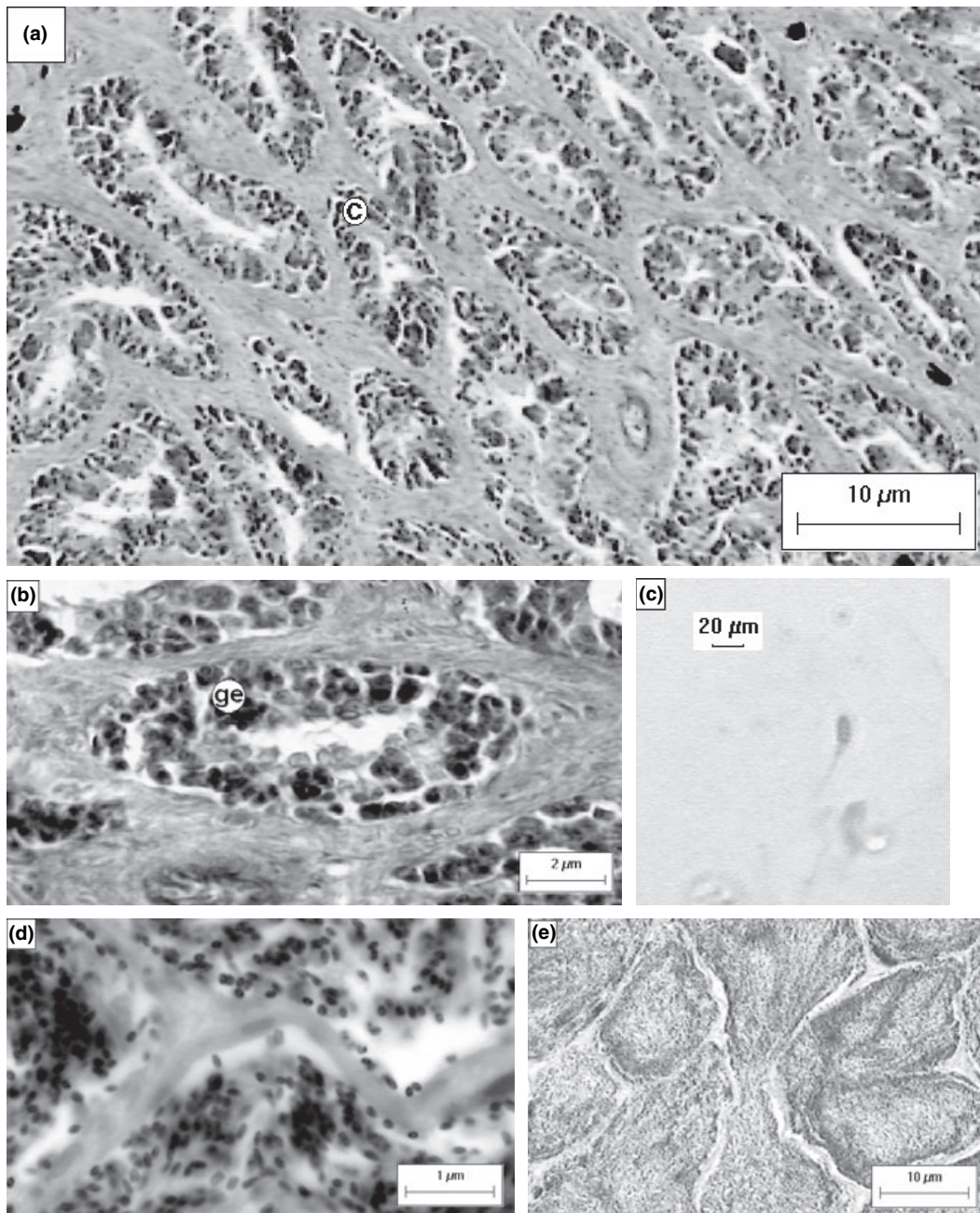


Fig. 7. Male gonads of *Aplochiton zebra*. (a) cysts in a spent testicle, (b) germinative epithelium of a cyst, (c) spermatozoa in a tubular lumen, (d) wall of a cyst and (e) cysts in a running ripe testicle. c: cyst, ge: germinative epithelium. Approximate location desired in the text: below line 226.



narrow range of lake water levels ( $640.64 \pm 0.25$  m a.s.l.) and narrow photoperiod range (10 h 4 min to 10 h 36 min).

#### Eggs and embryonic development

During the first egg collection, water temperature was 4 °C, conductivity was 79  $\mu$ S, water velocity was 0.58  $\text{m}\cdot\text{s}^{-1}$  and oxygen availability was 13.5  $\text{mg}\cdot\text{l}^{-1}$ . During hatching water conditions remained constant.

Eggs were adhered to the concrete wall of the outlet channel of the salmonid trap, from surface to ca. 60-cm depth. Patches of adhered eggs seemed to be associated with some disturbances of the wall surface, in this case a vertical pipe and a smoothed corner. Patches consisted of a single layer of eggs with a sinusoidal profile.

The collected eggs of *A. zebra* (1.45–1.80 mm diameter), already under cleavage, were pale translucent amber and had a strongly adherent vitelline envelope. The perivitelline space was 0.20- to 0.29-mm thick and the yolk mass (0.77–1.18 mm diameter) had several oil droplets.

The epiboly of the blastoderm begins at 8 ATU, forming a small yolk plug between 8 and 18 ATU. Neurulation could be observed since 11–18 ATU. The final events related to the structure of the future free embryo could be observed between 64 and 70 ATU, when the embryos showed great motility. The first signs of pigmentation are apparent in the eyes from 59 to 80 ATU, and at 76 ATU otoliths can be seen inside the otic capsules. At 131 ATU, the embryo is coiled over the yolk sac in one and a half coils. The neural folds are closed and some melanophores appear in the ventral surface and below the yolk sac. At 100 ATU, pectoral fin buds begin to show just behind the head region on the top of the yolk sac. At 114 ATU, the median fin-fold extends from the lower edge of the yolk sac to the mid-dorsal region. At 125 ATU, it starts to expand in the caudal region forming a caudal fin and the head appears much more erect than at the earlier stages. At 141 ATU, the development of the mouth and the gill slits can be first seen. The melanophores in the ventral surface and below the yolk sac appear much more reticulate. At 149 ATU, the head is highly lifted and the embryos appear much larger and seem to have reached their final proportions (Fig. 8). At 206 ATU, hatching takes place, just over 40–42 days after development began, at a daily temperature between 4.5 and 8 °C.

#### Newly hatched embryos

The mean size at hatching was 10.8 mm TL (8.0–12.7 mm TL, Fig. 8). The yolk sac (diameter 1.04–1.27 mm) showed a single oil droplet positioned in its

anterior end. The number of myotomes ranged from 68 to 71. Pigmentation at hatching (13–20 melanophores) was restricted to the ventral surface of the embryo, including the yolk sac. Pigmentation consisted of two parasagittal rows of melanophores, some melanophores just before the anus, and postanal melanophores positioned in a single row. Free embryos started to swim 24 h after hatching, being noticeably attracted to light.

#### Discussion

Sexual dimorphism in *A. zebra* is not conspicuous; but sexes could be distinguished by differences in the morphology of the urogenital papilla, as Humphries (1987) observed in *Galaxias truttaceus* Valenciennes, in *Galaxias auratus* Johnston and in *Galaxias brevipinnis* Günther. Among Patagonian galaxiids, *A. taeniatus* (Campos 1969) and *B. bullocki* (Campos 1972) can also be distinguished by the shape of the urogenital papilla.

Ovaries of *A. zebra* could be classified among the ‘synchronous group’ proposed by Nagahama (1983). This type of ovaries occurs in those species that generally spawn once a year and have a relatively short breeding season. Histological analyses revealed the presence of filaments located on the chorionic surface of vitellogenic oocytes. Other species [e.g. *Oryzias latipes* (Temminck and Schlegel) and *Odonesthes bonariensis* (Valenciennes), Iwamatsu & Keino 1978; Iwamatsu 1992; Iwamatsu et al. 1993; Guandalini et al. 1994] have an adhesive coat of filaments surrounding the eggs. These structures serve primarily to attach the eggs to the substratum (Kuchnow & Scott 1977; Busson-Mabillot 1977). In the case of *A. zebra*, the adhesion to the substratum could be essential considering that the eggs were found heavily attached to the concrete walls, along which the water of the stream runs.

Alternative life-history styles have been observed for diadromous and landlocked populations of other galaxiid, *G. maculatus*. Barriga et al. (2007) indicated a spring-summer spawning season for a riverine landlocked population of *G. maculatus* (River Caleufu – Argentina). This coincides with data from lake populations of the species (Cussac et al. 1992; Barriga et al. 2002) and with diadromous species from Tierra del Fuego (Boy et al. 2007), but not with other diadromous populations from Chile (Campos 1970a; Peredo & Sobarzo 1994) and New Zealand (McDowall et al. 1994). Lacustrine landlocked populations of *G. maculatus* in western Australia have fostered a protracted breeding and larval recruitment period in comparison to limited recruitment in the riverine environments (Chapman et al. 2006). In this context, *A. zebra* has a spawning season that varies between



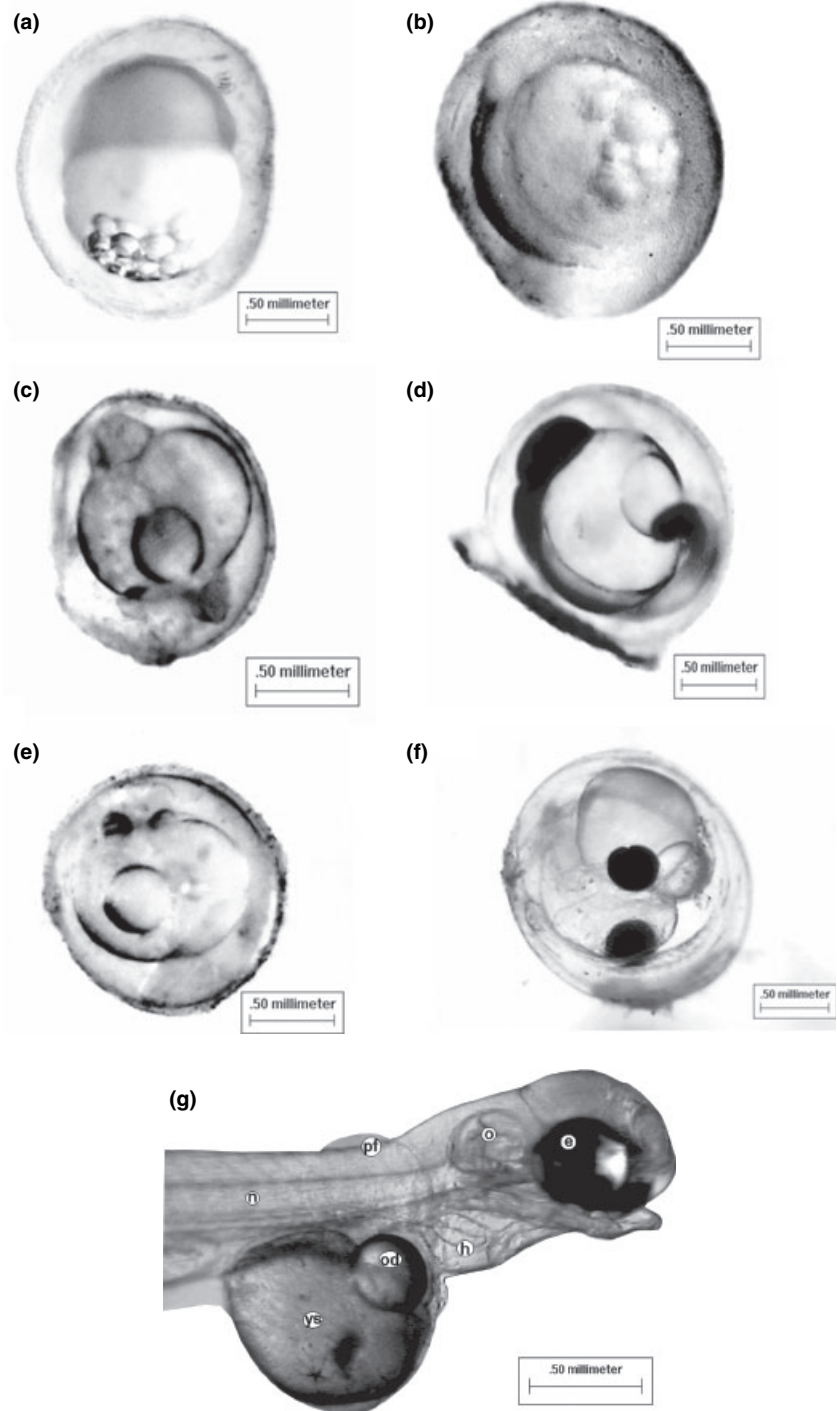


Fig. 8. Embryonic development of *Aplocheilichthys zebra*. (a) gastrulation, (b) neurulation, (c) optic vesicles in formation, (d) tail development, (e) embryo with strongly pigmented eyes, coiled over the yolk sac, (f) embryo previous to eclosion and (g) newly hatched embryo of 12 mm TL. e: eye, h: heart, n: nothocord, o: otolith, od: oil droplet, ys: yolk sac. Approximate location desired in the text: below line 270.

autumn (Lake Riñihue, 39°50'S, Eigenmann 1928; Smitt 1901) and spring; July–August (Lake Lácar, 40°09'S), September–October (Lake Puelo, 42°10'S) and October–November (Lake Rivadavia, 42°37'S). The narrow range of photoperiod observed for spawning dates in Lake Lácar, suggests a reproductive chronology based on photoperiod rather than temperature, like other temperate fishes seem to have (Matthews 1998; Wootton 1998). However, due to

the fragmentary data, future work must be conducted to test whether this variation is correlated with latitude or simply responds to inter annual variability in spawning conditions.

In the present study, a daily increment formation was detected in sagittae otoliths. The few rings observed inside the 'hatch ring' can be attributed to prehatch growth. Previous studies also demonstrated a prehatch growth in *Galaxias* species (Cussac et al.

1992; McDowall et al. 1994). Uncertainty of counts and pre-hatch growth could contribute to errors in ages determined from ring counts. Even recognising that these are potential errors of 5–10 or even more days, the data still provide strong indications of spawning season (McDowall et al. 1994).

Adult females reached 126.5–142 mm SL and males 116.4–132.1 mm SL. Adult *A. taeniatus* collected by Campos (1969) from Lake Llanquihue were 84–115 mm SL, females also being slightly larger (and older) than males. Values for the smallest running ripe male and spawning female of *G. maculatus* and *G. platei* in northern Patagonian lakes were provided by Barriga et al. (2002) and Milano (2003) and adult female sizes of other South American Galaxiidae are shown in Cussac et al. (2004). Among them, *A. zebra* showed intermediate size and age at first maturity. Fecundity (eggs per female), egg diameter and yolk mass were rather high in comparison with other galaxiids present in Patagonia (Cussac et al. 2004).

Embryonic development of *A. zebra* was described for the first time. As most of galaxiid fishes (Benzie 1968a; Campos 1969, 1972, 1984; McDowall 1968, 1984), *A. zebra* lay their eggs on the substrate. Embryonic development of *A. zebra* did not differ from most of the teleosts with small eggs (Matkovic et al. 1985; Cussac et al. 1985; Blaxter 1969; Balon 1990; Cussac & Ortubay 2002) and particularly from other Galaxiidae (Benzie 1968b; Campos 1969, 1972; Ortubay & Wegrzyn 1991), taking 40–42 days at a mean daily temperature between 4.5 and 8 °C.

Newly hatched embryos of *A. zebra* (8.0–12.7 mm TL) were larger than those of landlocked *G. maculatus* (4.2–5.1 mm TL, Cussac et al. 1992; Barriga et al. 2002) and *G. platei* (4.7–7.1 mm TL, Ortubay & Wegrzyn 1991). The size of newly hatched embryos of *A. zebra* and the latitudinal variation suggested by the spawning season could be a sign of adaptation for maximising body size at first winter (in the sense of Shuter & Post 1990) and also play an important role in competitive interactions with landlocked *G. maculatus*. In fact, landlocked populations of both species are mutually exclusive with the sole exception of Lake Lácar, in the north of the distributional range of both species (Cussac et al. 2004). Presence data of *A. zebra* and particularly the absence of small juveniles in the littoral (Fig. 4) suggest that the existence of intralacustrine movements. Ontogenetic niche shifts have already been reported for *G. maculatus*, *Galaxias gracilis* McDowall and *G. platei* (Cussac et al. 1992; Rowe & Chisnall 1996; Barriga et al. 2002).

Considering the extremely reduced distribution and abundance of landlocked populations and the risks imposed by salmonid introduction (Cussac et al. 2004; McDowall et al. 2001), conservation of this species

must not only imply the preservation of adult habitat, but also that of the spawning and juvenile rearing habitat. In this sense, information about reproduction and early life history of *A. zebra* takes a special significance.

## Acknowledgements

Authors thank Administración de Parques Nacionales, for giving permission to collect native fishes within P. N. Lanín, P. N. Los Alerces and P. N. Lago Puelo. Authors are also grateful to Jorgelina Boretto and Jorge Kuroda for their technical assistance. This work was supported by grants from Universidad Nacional del Comahue, Agencia Nacional de Promoción de Ciencia y Tecnología (ANPCYT) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

## References

- Balon, E.K. 1990. Epigenesis of an epigeneticist: the development of some alternative concepts on the early ontogeny and evolution of fishes. *Guelph Ichthyology Reviews* 1: 1–48.
- Barriga, J.P., Battini, M.A., Macchi, P.J., Milano, D. & Cussac, V.E. 2002. Spatial and temporal distribution of landlocked *Galaxias maculatus* and *Galaxias platei* (Pisces: Galaxiidae) in a lake in South American Andes. *New Zealand Journal of Marine and Freshwater Research* 36: 345–359.
- Barriga, J.P., Battini, M.A. & Cussac, V.E. 2007. Annual dynamics variation of a landlocked *Galaxias maculatus* (Jenyns 1842) population in a Northern Patagonian river: occurrence of juvenile upstream migration. *Journal of Applied Ichthyology* 23: 128–135.
- Beamish, R.J. & McFarlane, G.A. 1983. Validation of age determination estimates: the forgotten requirement. NOAA National Marine Fisheries Service Technical Report 8: 29–34.
- Benzie, V. 1968a. Some ecological aspects of the spawning behaviour and early development of the common whitebait, *Galaxias maculatus attenuatus* (Jenyns). *Proceedings of the New Zealand Ecology Society* 15: 31–39.
- Benzie, V. 1968b. Stages in the normal development of *Galaxias maculatus attenuatus* (Jenyns). *New Zealand Journal of Marine and Freshwater Research* 2: 606–627.
- Blaxter, J.H.S. 1969. Development: eggs and larvae. In: Hoar, W.S. & Randall, D.J., eds. *Fish physiology*, vol. III, eggs and larvae. New York: Academic Press, pp. 178–252.
- Boy, C.C., Morriconi, E. & Calvo, J. 2007. Reproduction in puyen, *Galaxias maculatus* (Pisces: Galaxiidae), in the southernmost extreme of distribution. *Journal of Applied Ichthyology* 23: 547–554.
- Busson-Mabillot, S. 1977. Un type particulier de secretion exocrine: celui de l'appareil adhésif de l'oeuf d'un poisson teleostéen. *Biologie Cellulaire* 30: 233–244.
- Campana, S.E. & Nielson, J.D. 1982. Daily growth increments in otoliths of starry flounder (*Platichthys stellatus*) and the influence of some environmental variables in their production. *Canadian Journal of Fisheries and Aquatic Sciences* 39: 937–942.

- Campana, S.E. & Nielson, J.D. 1985. Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* 44: 1014–1032.
- Campos, H. 1969. Reproducción del *Aplochiton taeniatus* Jenyns. *Boletín del Museo Nacional de Historia Natural* 29: 207–222.
- Campos, H. 1970a. *Galaxias maculatus* (Jenyns) en Chile, con especial referencia a su reproducción. *Boletín del Museo Nacional de Historia Natural* 31: 5–20.
- Campos, H. 1970b. Introducción de especies exóticas y su relación con los peces de agua dulce de Chile. *Boletín del Museo Nacional de Historia Natural* 162: 3–9.
- Campos, H. 1972. Breeding season and early development of *Brachygalaxias bullocki* (Osteichthyes: Galaxiidae). *Texas Journal of Science* 23: 531–544.
- Campos, H. 1984. Gondwana and neotropical galaxioid fish biogeography. In: Zaret, T.M., ed. *Evolutionary ecology of neotropical freshwater fishes*. The Hague: Dr. W. Junk, pp. 113–125.
- Chapman, A., Morgan, D.L., Beatty, S.J. & Gill, H.S. 2006. Variation in life history of land-locked lacustrine and riverine populations of *Galaxias maculatus* (Jenyns 1842) in Western Australia. *Environmental Biology of Fishes* 77: 21–37.
- Cussac, V. & Ortubay, S. 2002. Gametogenesis and development of *Gymnocharacinus bergi* (Pisces, Characidae): reproductive mode relative to environmental stability. *Environmental Biology of Fishes* 63: 289–297.
- Cussac, V.E., Matkovic, M. & Maggese, M.C. 1985. Desarrollo embrionario de *Rhamdia sapo* (Valenciennes, 1840) Eigenmann y Eigenmann, 1988 (Pisces, Pimelodidae). II. Organogénesis media, organogénesis tardía y eclosión. *Revista Brasileira de Biología* 45: 149–160.
- Cussac, V., Cervellini, P.M. & Battini, M.A. 1992. Intralacustrine movements of *Galaxias maculatus* (Galaxiidae) and *Odontesthes microlepidotus* (Atherinidae) during their early life history. *Environmental Biology of Fishes* 35: 141–148.
- Cussac, V., Ortubay, S., Iglesias, G., Milano, D., Lattuca, M.E., Barriga, J.P., Battini, M. & Gross, M. 2004. The role of biological traits in the distribution of South American galaxiid fishes. *Journal of Biogeography* 31: 103–121.
- Eigenmann, C.H. 1928. The fresh-water fishes of Chile. *Memoirs of Natural Academy of Sciences* 22: 1–80.
- Ferriz, R.A. 1987. Biología del puyen *Galaxias maculatus* (Jenyns) (Teleostomi, Galaxiidae) en un embalse norpatagónico. Ciclo de vida, ciclo gonadal y fecundidad. *Revista del Museo Argentino de Ciencias Naturales “B. Rivadavia”* 6: 29–38.
- Gayanilo, F.C. Jr, Sparre, P. & Pauly, D. 1994. The FAO-ICLARM stock assessment tools (FISAT II) user’s guide. *FAO Computerized Information Series (Fisheries)* 7: 1–126.
- Guandalini, E., Mantovani, A., Fazzi, P., Gelosi, E., Colombari, P.T., Mazzini, M. & Fausto, A.M. 1994. Histological study on the oocyte filaments of the silverside *Odontesthes bonariensis*. *Journal of Fish Biology* 44: 673–682.
- Hoar, W.S. 1969. Reproduction. In: Hoar, W.S. & Randall, D.J., eds. *Fish physiology*, Vol. III. New York: Academic Press, pp. 1–72.
- Humphries, P. 1987. External sexing of galaxiid fish. *Victorian Naturalist* 104: 185–186.
- Iwamatsu, T. 1992. Morphology of filaments on the chorion of oocytes and eggs in the medaka. *Zoological Sciences* 9: 589–599.
- Iwamatsu, T. & Keino, H. 1978. Scanning electron microscopic study on the surface change of eggs of the teleost, *Oryzias latipes*, at the time of fertilization. *Development, Growth and Differentiation* 20: 237–250.
- Iwamatsu, T., Nakashima, S. & Onitake, K. 1993. Spiral patterns in the micropylar wall and filaments on the chorion in eggs of the medaka, *Oryzias latipes*. *Journal of Experimental Zoology* 267: 225–232.
- Kipling, C. & Frost, W.E. 1969. Variation in the fecundity of pike *Esox lucius* L. in Windermere. *Journal of Fish Biology* 1: 221–237.
- Kuchnow, K.P. & Scott, J.R. 1977. Ultrastructure of the chorion and its micropyle apparatus in the mature *Fundulus heteroclitus* (Walbaum) ovum. *Journal of Fish Biology* 10: 197–201.
- Lattuca, M.E., Ortubay, S., Battini, M.A., Barriga, J.P. & Cussac, V.E. 2007. Presumptive environmental effects on body shape of *Aplochiton zebra* (Pisces, Galaxiidae) in Northern Patagonian lakes. *Journal of Applied Ichthyology* 23: 25–33.
- Martoja, R. & Martoja-Pierson, M. 1970. *Técnicas de histología animal*, 1st edn. Barcelona, España: Editorial Toray-Mason, 350 pp.
- Matkovic, M., Cussac, V.E., Cukier, M., Guerrero, G.A. & Maggese, M.C. 1985. Desarrollo embrionario de *Rhamdia sapo* (Valenciennes, 1840) Eigenmann y Eigenmann, 1988 (Pisces, Pimelodidae). I. Segmentación, morfogénesis y organogénesis temprana. *Revista Brasileira de Biología* 45: 39–50.
- Matthews, W.J. 1998. *Patterns in freshwater fish ecology*, 2nd edn. Chapman & Hall: Norwell, MA, 756 pp.
- McDowall, R.M. 1968. *Galaxias maculatus* (Jenyns), the New Zealand whitebait. *New Zealand Fisheries Research Bulletin* 2: 1–84.
- McDowall, R.M. 1969. Relationships of Galaxioid fishes with a further discussion of Salmoniform classification. *Copeia* 1969: 796–824.
- McDowall, R.M. 1971. Fishes of the family Aplochitonidae. *Journal of the Royal Society of New Zealand* 1: 31–52.
- McDowall, R.M. 1980. Freshwater fishes and plate tectonics in the southwest Pacific. *Palaeogeography, Palaeoclimatology and Palaeoecology* 31: 337–351.
- McDowall, R.M. 1984. Southern hemisphere freshwaters Salmoniforms: development and relationships. *American Society of Ichthyologists and Herpetologists Special Publication* 1: 150–153.
- McDowall, R.M. 2000. Biogeography of the southern cool-temperate galaxioid fishes: evidence from metazoan macro-parasite faunas. *Journal of Biogeography* 27: 1221–1229.
- McDowall, R.M. 2002. Accumulating evidence for a dispersal biography of southern cool temperate fishes. *Journal of Biogeography* 29: 207–219.
- McDowall, R.M., Mitchell, C.P. & Brothers, E.B. 1994. Age at migration from the sea of juvenile *Galaxias* in New Zealand (Pisces: Galaxiidae). *Bulletin of Marine Science* 54: 385–402.
- McDowall, R.M., Allibone, R.M. & Chadderton, W.L. 2001. Issues for the conservation and management of Falkland

- Islands fresh water fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems* 11: 473–486.
- Milano, D. 2003. *Biología de Galaxias platei* (Pisces, Galaxiidae): especiaciones relativas a su distribución. Ph.D. thesis. Bariloche, Argentina: Universidad Nacional del Comahue, 131 pp.
- Milano, D., Ruzzante, D.E., Cussac, V.E., Macchi, P.J., Ferriz, R.A., Barriga, J.P., Aigo, J.C., Lattuca, M.E. & Walde, S.J. 2006. Latitudinal and ecological correlates of morphological variation in *Galaxias platei* (Pisces, Galaxiidae) in Patagonia. *Biological Journal of the Linnean Society* 87: 69–82.
- Nagahama, Y. 1983. The functional morphology of teleost gonads. In: Hoar, W.S., Randall, D.J. & Donaldson, E.M., eds. *Fish physiology, reproduction*, vol IX, part A, endocrine tissues and hormones, New York, NY, USA: Academic Press, pp. 223–275.
- Nelson, J.S. 1994. *Fishes of the world*, 3rd edn. New York, NY, USA: J. Wiley and Sons, 600 pp.
- Ortubay, S. & Wegrzyn, D. 1991. Fecundación artificial y desarrollo embrionario de *Galaxias platei* Steindachner (Salmoniformes, Galaxiidae). *Medio Ambiente* 11: 84–89.
- Peredo, S. & Sobarzo, C. 1994. Actividad gonádica estacional de *Galaxias maculatus* (Jenyns, 1842) en el Río Cautín, IX Región, Chile. *Boletín de la Sociedad de Biología de Concepción* 65: 65–70.
- Pollard, D.A. 1971. The biology of a landlocked form of the normally catadromous salmoniform fish *Galaxias maculatus* (Jenyns). I. Life cycle and origin. *Australian Journal of Marine and Freshwater Research* 22: 91–123.
- Rowe, D.K. & Chisnall, B.L. 1996. Ontogenetic habitat shifts by *Galaxias gracilis* (Galaxiidae) between the littoral and limnetic zones of Lake Kanono, New Zealand. *Environmental Biology of Fishes* 46: 255–264.
- Ruzzante, D.E., Walde, S.J., Cussac, V.E., Dalebout, M.L., Seibert, J., Ortubay, S. & Habit, E. 2006. Phylogeography of the Percichthyidae (Pisces) in Patagonia: roles of orogeny, glaciation, and volcanism. *Molecular Ecology* 15: 2949–2968.
- Shuter, B.J. & Post, J.R. 1990. Climate, population viability, and the zoogeography of temperate fishes. *Transactions of the American Fisheries Society* 119: 314–336.
- Smitt, F.A. 1901. Poissons d'eau douce de la Patagonie recueillis par E. Nordenskiöld 1898-99. Bihang K. Svenska Vetenskapsakademiens Handlingar 26: 1–31.
- Sogard, S.M. 1991. Interpretation of otolith microstructure in juvenile winter flounder (*Pseudopleuronectes americanus*); ontogenetic development, daily increment validation, and somatic growth relationships. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 1862–1871.
- Szedlmayer, S.T. & Able, K.W. 1992. Validation studies of daily increment formation for larval and juvenile summer flounder, *Paralichthys dentatus*. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 1856–1862.
- Tzeng, W.-N. & Yu, S.-Y. 1989. Validation of daily growth increments in otoliths of milkfish larvae by oxytetracycline labeling. *Transactions of the American Fisheries Society* 118: 168–174.
- Vigliano, P., Macchi, P., Alonso, M., Denegri, A., Milano, D., Lippolt, G. & Padilla, G. 1999. Un diseño modificado y procedimiento de calado de redes agalleras para estudios cuali-cuantitativos de peces por estratos de profundidad en lagos araucanos. *Natura Neotropicalis* 30: 1–11.
- Vigliola, L. 1997. Validation of daily increment formation in otoliths for three *Diplodus* species in the Mediterranean Sea. *Journal of Fish Biology* 51: 349–360.
- Wootton, R.J. 1998. *Ecology of teleost fishes*, 2nd edn. London: Kluwer Academic Publications, 386 pp.