

## Early morphological variation and induction of phenotypic plasticity in Patagonian pejerrey

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The aim of this work was to study two aspects of phenotypic plasticity in the Patagonian pejerrey *Odontesthes hatcheri* (Teleostei: Atherinopsidae) the dependence of the early morphology on developmental time and temperature, and the induction of morphological changes by controlled feeding in juveniles. Newly hatched free embryos, incubated at two different temperatures (13 and 18°C), and juveniles were used for the study and induction of phenotypic plasticity. Body and head shapes were analyzed with geometric morphometrics and linear measurements. Our results showed that shape variation at hatching was related to the bending of the embryo head on the yolk sac, increasing the head-trunk angle due to progressive straightening of the embryo. The head-trunk angle was related with temperature at incubation, with embryos incubated at higher temperature being more bent. Embryos that hatched earlier had bigger yolk sacs than those that hatched later. In juveniles, controlled feeding experiments added new morphological variation to that of wild juveniles. In all comparisons, the slenderness of the head, the size of premaxilla and jaw, and the position of the eye showed an enlarged variation due to controlled feeding. These results will contribute to comprehending the complexity of the morphological variation of *O. hatcheri*.

O objetivo deste trabalho foi estudar a variação morfológica e plasticidade fenotípica do peixe-rei da Patagônia *Odontesthes hatcheri* (Teleostei: Atherinopsidae), a dependência da morfologia inicial no tempo de desenvolvimento e temperatura, e a indução de alterações morfológicas pela alimentação controlada em juvenis. Embriões recém-nascidos, incubados a duas temperaturas diferentes (13 e 18°C) e juvenis foram utilizados para o estudo de indução de plasticidade fenotípica. Formas do corpo e cabeça foram analisadas com técnicas de morfometria geométrica e medições lineares. Os nossos resultados mostraram que a variação da forma no nascimento foi relacionada com a curvatura da cabeça do embrião no saco vitelino, aumentando o ângulo de cabeça-tronco devido ao endireitamento progressivo do embrião. O ângulo da cabeça-tronco relacionou-se com a temperatura de incubação, com os embriões incubados na temperatura elevada sendo mais curvados. Os embriões que eclodiram mais cedo tinham sacos vitelinos maiores do que aqueles que eclodiram tardiamente. Em juvenis, os experimentos de alimentação controlada adicionaram nova variação morfológica àquela dos juvenis selvagens. Em todas as comparações, a espessura da cabeça, o tamanho da pré-maxila e mandíbula, e a posição do olho mostraram uma maior variação devido à alimentação controlada. Estes resultados irão contribuir para a compreensão da complexidade da variação morfológica de *O. hatcheri*.

**Key words:** Atherinopsidae, Development, Feeding, Morphometry, *Odontesthes hatcheri*.

### Introduction

There is abundant evidence of phenotypic plasticity in fishes (Balon, 2004). It is considered as the ability of an organism to react to environmental input with a change in form, state, movement, or rate of activity (West-Eberhard, 2003), or as the property of individual genotypes to produce different phenotypes when exposed to different environmental conditions (Pigliucci, 2001; Pigliucci *et al.*, 2006; Pfennig *et al.*, 2010).

Induced phenotypic plasticity has been studied extensively (Grünbaum *et al.*, 2007). During embryonic development, water temperature is the most important environmental factor that influences fish (Chambers & Leggett, 1987; Blaxter, 1992).

Temperature modulates the amount of time required to complete embryonic development, within a specific range for each species (Kunz, 2004; Kamler, 2008), and has effects on the morphology, physiology, and behavior of fish during development (Martell *et al.*, 2005). Environmental effects other than temperature can also act along the ontogeny, inducing a certain morphology. For example, morphological reversion in the head of *Micropterus salmoides floridanus* (= *Micropterus floridanus*) could have been experimentally related to food quality (Wintzer & Motta, 2005). Also, diet induced body and head shape variation has been observed in *Cichlasoma managuense* (= *Parachromis managuensis*) and *Lepomis humilis* (Meyer, 1987; Hegrenes, 2001).

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The distribution area of the Patagonian pejerrey *Odontesthes hatcheri* (Eigenmann), Patagonia, was signed by old and new processes that shaped the landscape and fauna: a Gondwanan heritage, the Andes uplifting, Pleistocene ice, volcanic activity, introduction of exotic fishes, and climate change (Pascual *et al.*, 2007). Larvae and juveniles of *O. hatcheri* perform ontogenetic habitat and diet shifts in Patagonian lakes. After hatching in the littoral zone, free embryos (*sensu* Balon, 1999) migrate to the limnetic zone where the exogenous feeding begins. Later, these larvae return to the littoral zone (Cussac *et al.*, 1992). They feed mainly on both nauplii of Cyclopoida and the rotifer *Pompholix sulcata* in the limnetic and in the littoral zones, up to their juvenile period, when their diet changes (Cervellini *et al.*, 1993). Larval Patagonian pejerrey showed marked shape changes after few days of fasting (Battini *et al.*, 1995). In adults, relationships between body shape and environmental factors such as total phosphorus, coastline development, and altitude (Conte-Grand, 2012), as well as between cephalic shape and mean depth, content of Chlorophyll-a, and mean summer air temperature were found (Crichigno, 2012).

The aim of this work was to study two aspects of phenotypic plasticity in the Patagonian pejerrey *Odontesthes hatcheri*; the dependence of the early morphology on developmental time and temperature, and the induction of morphological changes by controlled feeding in juveniles.

## Material and Methods

**Morphology of newly hatched sibling free embryos.** Adult *Odontesthes hatcheri* were captured using gillnets (15, 30, and 40 mm bar mesh) in a shallow lake in the Patagonian plateau, Carrilafquen Chica (41°12'S 69°25'W, see Reissig *et al.*, 2006 for details). Individuals were anaesthetized with benzocaine solution (0.05 g . L<sup>-1</sup>). Oocytes and sperm of four parental couples (PC) were obtained by stripping, and dry fertilization (Barnabé, 1990) was then performed. At the laboratory (Centro de Salmonicultura Bariloche, Universidad Nacional del Comahue), the eggs corresponding to two PC were incubated into small baskets into a 200 L aquarium with aeration and a daily 20% water exchange, at 18°C, and those corresponding to the other two at 13°C, always maintaining a 0.5% NaCl level. Both temperatures are included within the summer (breeding season) range of surface water temperature in lakes and reservoirs where the species is present. Water supply came from Gutierrez River, 4 km downstream an oligotrophic lake (Gutierrez Lake, 41°09'59"S 71°24'35"W, Quirós, 1988).

When the eggs began to hatch, and during three consecutive days, newly hatched free embryos were anaesthetized with benzocaine (0.05 g . L<sup>-1</sup>) and photographed (NIKON D70) under stereomicroscope (Leica Wild M3C). Two images of each individual were recorded, left side and cephalic dorsal view, taking care to minimize parallax error. In this way, newly hatched free embryos of different ages (AH = 1, 2, and 3, Table 1), in terms of days after fertilization (DAF), were obtained.

Ten landmarks were digitized on images, on the fish's left side: (1) anterior tip of the premaxilla, (2) anterior tip of dentary,

(3) anus, (4) dorsal profile of the body at the posterior end of the operculum, (5) angle of the first gill arch, between epiphyal and ceratobranchial, (6, 7, 8, and 9) dorsal, ventral, anterior, and posterior edge of the eye, and (10) limit between diencephalon and telencephalon (Fig. 1).

Body shape was quantified using the Geometric Morphometric Analysis (GMA) approach of thin-plate splines (TPS; Bookstein, 1991; Rohlf & Marcus, 1993; Parsons *et al.*, 2003; Adams *et al.*, 2004). Images were first scaled and rotated to a common size and orientation using a generalized Procrustes superimposition approach (Bookstein, 1991; Adams *et al.*, 2004). The mean (or consensus) body shape for each population was estimated and quantified as partial warp scores using tpsRelw (Morphometrics at Sunny Stony Brook, 2012). The description of shape variation was performed visualizing the deformation grids and direction vectors, at the extreme of each Relative Warp (RW) axis from left to right and from bottom to top. Discriminant Analysis (DA, SPSS Inc.) was performed employing the Partial Warps (PW), including uniform and non-uniform coordinates (weight matrix), in order to discriminate free embryos according to AH, PC and incubation temperature (Table 1).

Linear measures of the dorsal view images were taken with Image Pro-plus. These measures were (Fig. 2): mouth width (MW), head length (HL), yolk sac width (YW), yolk sac length (YL), eye diameter (ED), and total length (TL). The residuals of the double logarithmic regression of linear measurements on TL were obtained in order to avoid size dependence and used to perform DA among ages.

**Cephalic morphological variation of juveniles.** Juveniles were captured with seine net in the same lake (Carrilafquen Chica) and transported to the laboratory in 1% NaCl. One subset (N = 22) was anaesthetized with benzocaine (0.05 g . L<sup>-1</sup>) and photographed (left side). Captured fish were separated into two groups and put in 150 L aquaria, at room temperature (mean 12.4°C, ranging from 10 to 15°C). One group was fed wild zooplankton (coming from Lake Los Juncos, 41°03'S 71°00'W) and the other was fed *Tubifex* sp. Both groups were fed *ad libitum* twice a day. After 60 days, individuals were photographed again. Individuals fed with *Tubifex* sp. were photographed also at day 240.

In all cases, 11 landmarks were digitized with TpsDig v2.10 software (Morphometrics at Sunny Stony Brook, 2012): (1) anterior dorsal tip of premaxilla, (2) anterior ventral tip of premaxilla, (3) anterior tip of dentary, (4) posterior ventral tip of premaxilla, (5) posterior ventral tip of maxillary, (6) ventral contact point between symplectic and preopercle, (7) upper tip of pelvic fin base, and (8, 9, 10, and 11) upper, lower, anterior and posterior edge of the eye. Identity of landmarks was confirmed using an X-ray image (Fig. 3).

The following analyses and comparisons were performed: a) variation of head shape among just captured individuals (N = 22); b) comparison of head shape among individuals fed with zooplankton for 60 days, individuals fed with *Tubifex* sp. for 60 days, and just captured individuals of similar size (N =

**Table 1.** Data (lateral and dorsal views) obtained for newly hatched *Odontesthes hatcheri* embryos of Lake Carrilafquen. Temperature (T = 13 and 18°C), parental couples (PC 1 to 4), days after fertilization (DAF), and age of newly hatched embryos (AH 1 to 3) are indicated. Comparisons include the effect of AH, DAF, PC, the combined effect of temperature and PC (TPC) at AH 1 and AH 2, and the combined effect of PC and DAF (PCDAF) at AH 2. N = number of individuals.

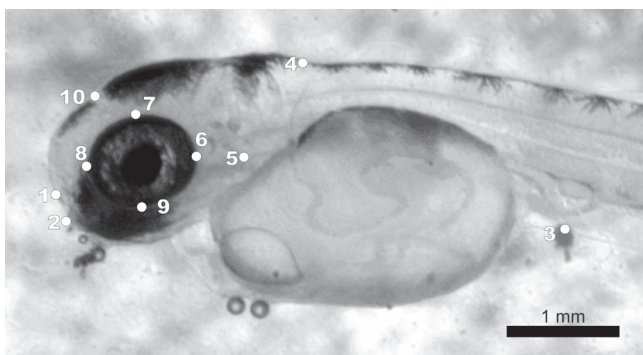
Temperature (°C)	Parental couples	DAF	AH	Lateral view (N)	Dorsal view (N)	Comparisons	
13	1	-	1	-	-	-	
		22	2	33	-	-	PCAH
		-	3	-	-	-	TPCDAF
	2	22	1	16	-	-	PCAH
		-	2	-	-	-	TPCDAF
		-	3	-	-	-	-
18	3	15	1	24	12	AH	
		16	2	31	28	AH	PCDAF
		17	3	16	13	AH	-
	4	-	1	-	-	-	-
		18	2	8	-	-	PCDAF
		-	3	-	-	-	-

64); and c) comparison of head shape between individuals fed with *Tubifex* sp., at 60 and 240 days, and just captured individuals of similar size (N = 71). The size ranges of the compared groups showed overlap (Table 2).

## Results

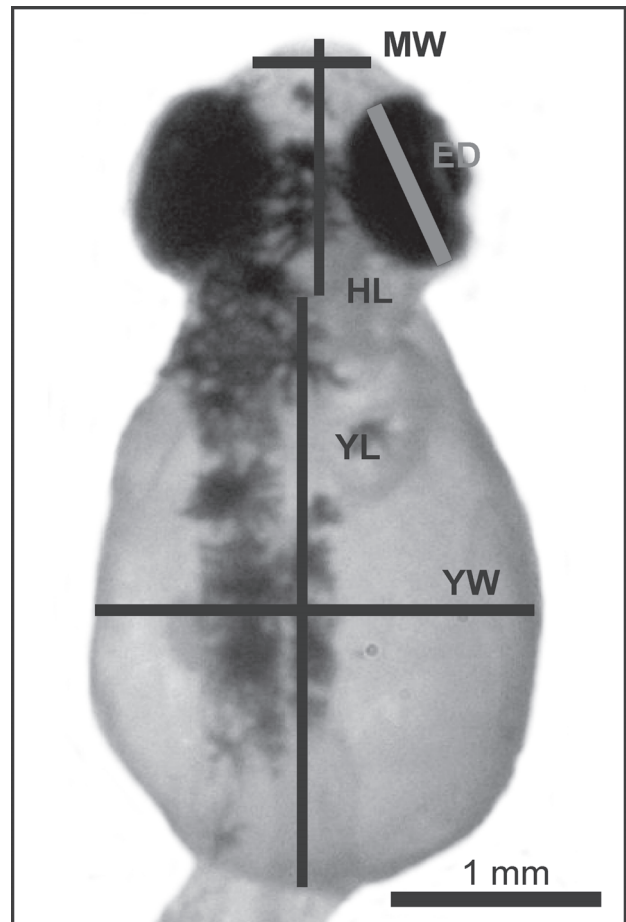
**Newly hatched free embryos, lateral view.** The first two RWs explained 75.29% of variation (N = 128, RW1 = 61.07%, and RW2 = 14.22%) and deformation grids showed the bending of the embryo over the yolk sac.

DA among AH (at 18°C and within the same PC, Table 1) showed one significant Discriminant Function (DF) that correctly classified 88.7% of cases and explained 82.4% of variation (DF1, N = 71, Wilks' lambda = 0.285,  $P < 0.001$ ). Deformation grids showed how the head of the embryo, initially curved over the yolk sac, lifts dorsalwards and straightens itself out (Fig. 4).



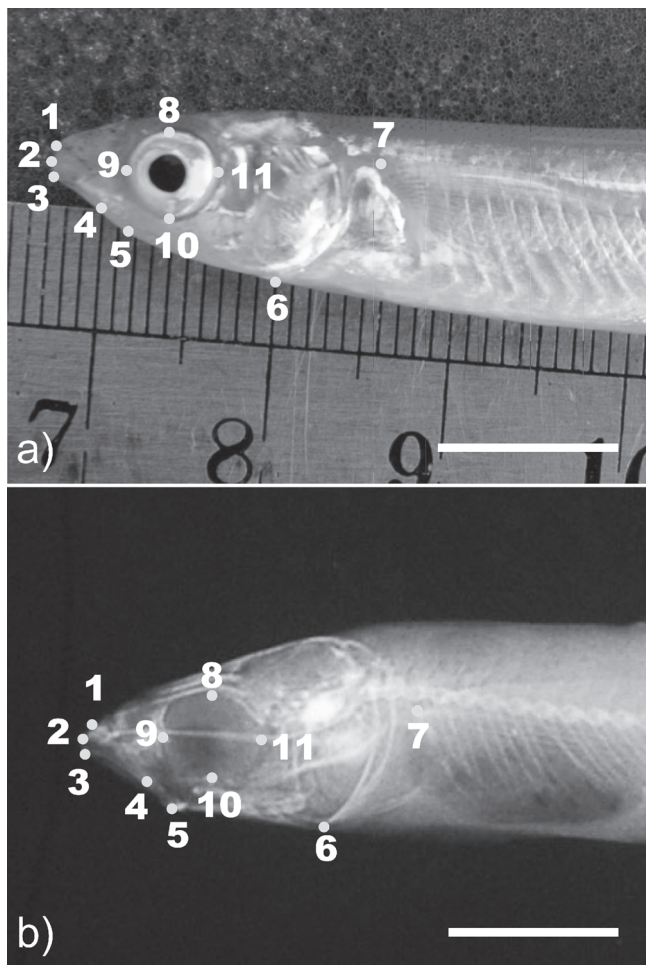
**Fig. 1.** Homologous landmarks on the left lateral view of a newly hatched free embryo: (1) anterior tip of premaxilla, (2) anterior tip of dentary, (3) anus, (4) dorsal profile of the body at the posterior end of operculum, (5) angle of the first gill arch, between epiphyal and ceratobranchial, (6, 7, 8, and 9) dorsal, ventral, anterior and posterior edge of eye, and (10) limit between diencephalon and telencephalon.

DA between PC with different AH and the same DAF and temperature (PCAH, Table 1) showed a significant DF (DF1, N = 49, Wilks' lambda = 0.239,  $P < 0.001$ ) that correctly classified 100% of cases and explained 100% of variation.



**Fig. 2.** Dorsal view of newly hatched free embryo. Mouth width (MW), head length (HL), yolk sac width (YW), yolk sac length (YL), eye diameter (ED).

DA between PC with different DAF and the same temperature and AH (PCDAF, Table 1) failed to show a significant DF (DF1, N = 39, Wilks' lambda = 0.405, P = 0.051). However, considering the combined effect of temperature, PC, and DAF at AH 1 (comparison TPCDAF in Table 1), DA showed one significant DF that correctly classified 97.5% of cases and explained 100% of variation (DF1, N = 40, Wilks' lambda = 0.249, P < 0.001). In the same way, combined effects of temperature, PC, and DAF at AH 2 (comparison TPCDAF in Table 1), showed one significant DF that correctly classified 92.2 % of cases and explained 100% of variation (DF1, N = 64, Wilks' lambda = 0.344, P < 0.001). Deformation grids from both comparisons showed that the individuals incubated at 18°C had their heads curved over the yolk sac, unlike individuals incubated at 13°C (Fig. 5).



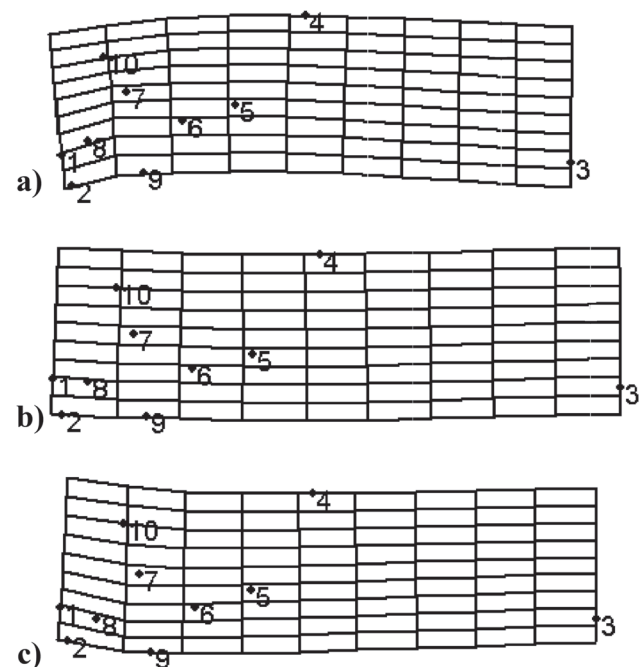
**Fig. 3.** Homologous landmarks on the left lateral view of the head of a just captured juvenile (a) and (b) X-ray image of skeletal structures: (1) anterior dorsal tip of premaxilla, (2) anterior ventral tip of premaxilla, (3) anterior tip of dentary, (4) posterior ventral tip of premaxilla, (5) posterior ventral tip of maxillary, (6) ventral contact point between symplectic and preoperculum, (7) upper tip of pelvic fin base, and (8, 9, 10, and 11) upper, lower, anterior and posterior edge of eye. Bar = 10 mm.

**Table 2.** Feeding, size, and time of captivity of juvenile *Odontesthes hatcheri*. Performed analyses (Relative warps, RW), comparisons (Discriminant analysis, DA), and number of individuals (N) are indicated.

Feeding	Size mean and range (mm)	Time of captivity (days)	Analyses and Comparisons (N)		
			RW (22)	DA (64)	DA (71)
No feed	49 (32-75)	0	(22)	(22)	(22)
Zooplankton	47 (40-63)	60		(13)	
<i>Tubifex</i> sp.	46 (32-66)	60		(29)	(31)
<i>Tubifex</i> sp.	56 (36-84)	240			(18)

**Newly hatched free embryos, linear measures in dorsal view.** DA among AH (at 18°C and within the same PC, Table 1) showed one significant DF that correctly classified 54.7% and explained 98.4% of variation (DF1, Wilks' lambda = 0.598, P < 0.001). The two main measurements included in the DF were the length and width of the yolk sac. AH 1 individuals had the widest and shortest yolk sacs (Fig. 6).

**Juvenile cephalic morphological variation and feeding experiment.** Recently captured individuals: the first two RWs explained 52.21% of variation (N = 22; RW1 = 32.63%; and RW2 = 19.58%). Deformation grids in RW1 show individuals with elongated head, longer premaxilla, longer lower jaw, and slightly lower eyes in the negative extreme. The opposite morphology



**Fig. 4.** Deformations grids for newly hatched embryos, with different DAF (15, 16, and 17), incubated at the same temperature (18°C): a) AH 1, b) AH 2, and c) AH 3 (see comparison AH in Table 1).

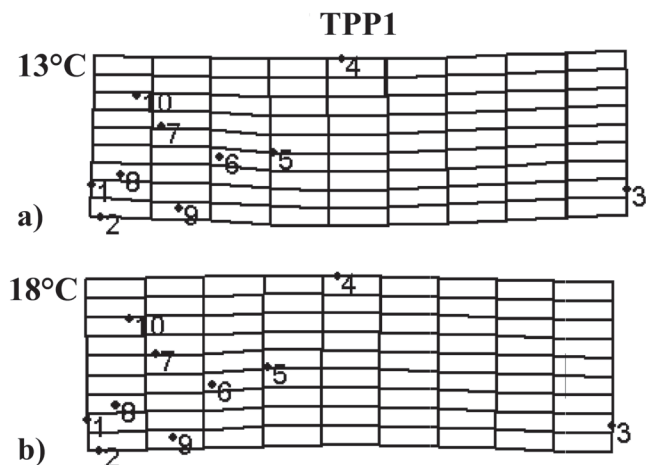
was observed in the positive extreme. In RW2, individuals with longer head, longer premaxilla, longer lower jaw, and bigger eyes, shifted forward and downward, were observed in the negative extreme. The opposite morphology was observed in the positive extreme.

Feeding with zooplankton and *Tubifex* sp. for 60 days: DA (N = 64) among just caught individuals, individuals fed with zooplankton and individuals fed with *Tubifex* sp. showed two significant DFs, correctly classifying 98.4% of cases and explaining 100% of variation (DF1, Wilks' lambda = 0.062,  $P < 0.001$ , and DF2, Wilks' lambda = 0.339,  $P < 0.001$ , Fig. 7). Deformation grids showed, for individuals fed with zooplankton compared to just caught individuals, higher mouth (anterior tips of premaxilla and dentary), and more anterior isthmus. Individuals fed with *Tubifex* sp., when compared to just caught individuals, had bigger eyes in a higher and more posterior position, wider anterior portion of premaxilla, lower mouth (anterior tips of premaxilla and dentary), more anterior isthmus, and more posterior pectoral fin base.

Feeding with *Tubifex* sp.: DA (N = 71) among times (0, 60, and 240 days) correctly classified 94.4% of cases and explained 100% of variation (DF1, Wilks' lambda = 0.083,  $P < 0.001$ , and DF2, Wilks' lambda = 0.339,  $P < 0.001$ ). DF1 separated just captured individuals from treated individuals and DF2 separated 60 days of feeding from 240 days of feeding groups (Fig. 8). The morphological variations along time showed an increase of eye size, an elongation of premaxilla and lower jaw, and an upward and forward movement of the isthmus.

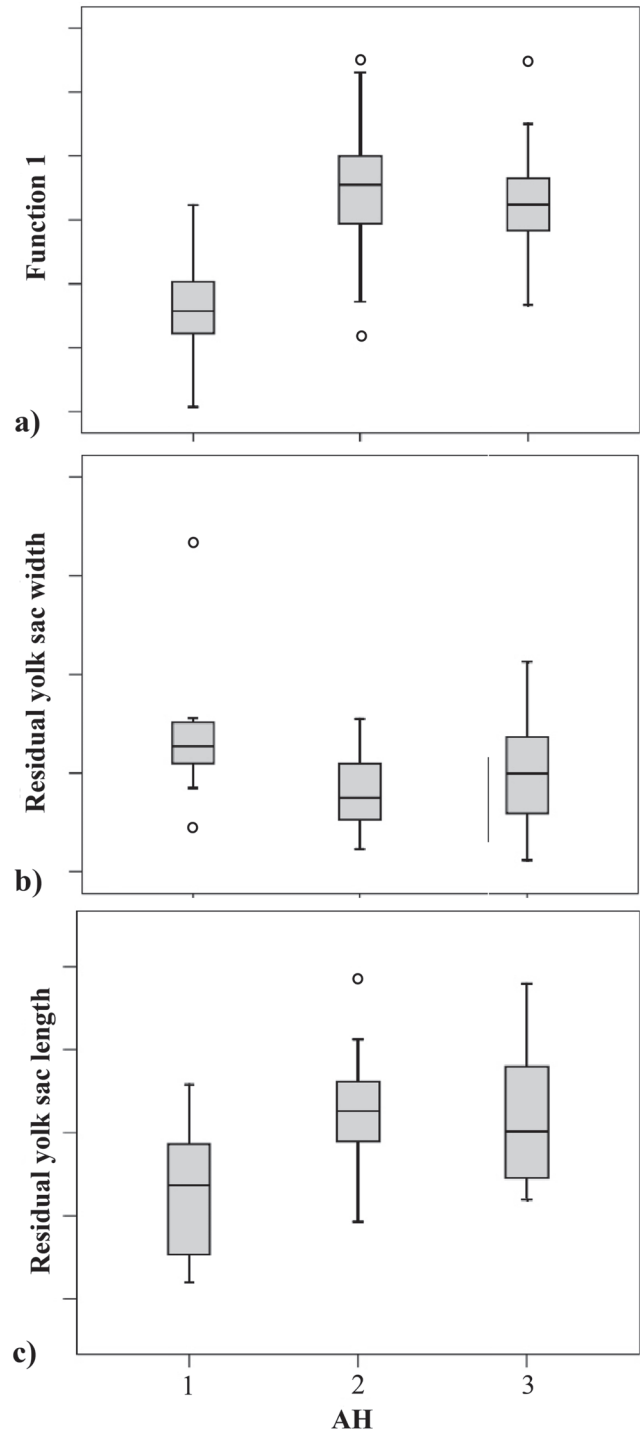
### Discussion

Hatching is not a fixed threshold, but is triggered by environmental cues at different times during the embryonic period, thus even sibling individuals can hatch at very different

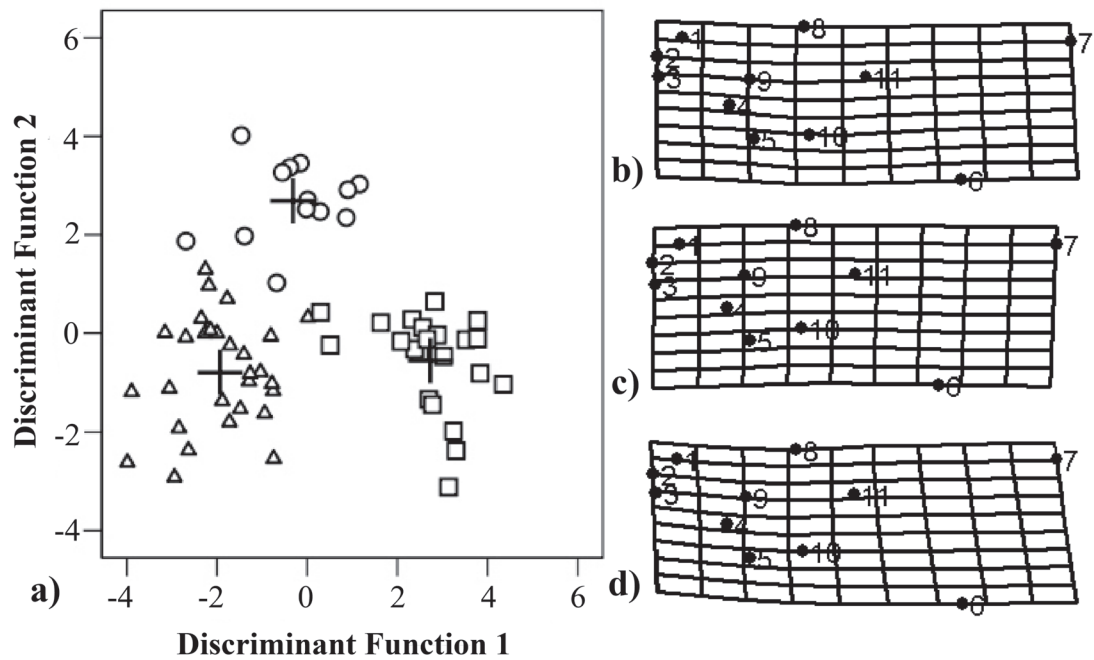


**Fig. 5.** Deformation grids for newly hatched individuals (AH 1), coming from two PC and incubated at **a)** 13°C and **b)** 18°C (see Table 1).

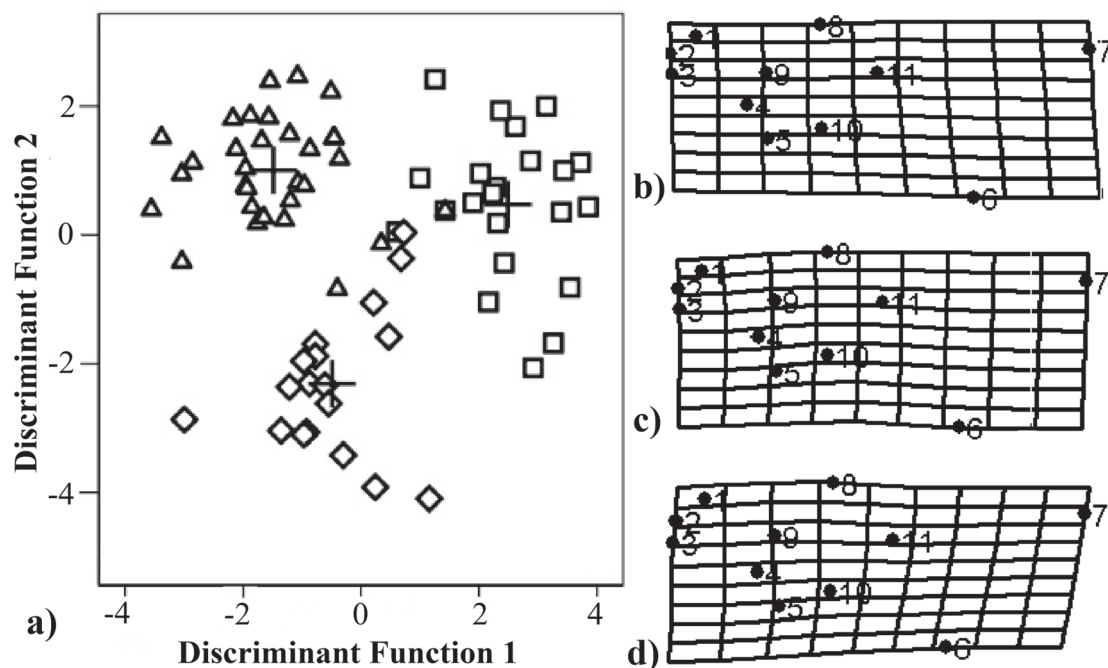
stages of development (Yamagami, 1988; Balon, 1999). Our results showed that morphological variation at hatching in *O. hatcheri* is related to the bending of the embryo over the yolk sac, increasing the head-trunk angle due to the straightening of the embryo along time, as was described by Kimmel *et al.*



**Fig. 6.** Box plots for linear measurements of newly hatched free embryos incubated at 18°C: **a)** first discriminant function (Function 1), **b)** residuals yolk sac width, and **c)** residuals yolk sac length, all *versus* age at hatching (AH).



**Fig. 7.** a) Discriminant function 1 versus discriminant function 2 for head shape of juvenile individuals fed with different food types; zooplankton (circles), *Tubifex* sp. (triangles), and just captured individuals (squares). Crosses indicate group centroids. Deformation grids correspond to (b) individuals fed with zooplankton and (c) *Tubifex* sp., and (d) just captured individuals.



**Fig. 8.** a) Discriminant function 1 versus discriminant function 2 for head shape of just captured individuals (squares), individuals fed with *Tubifex* sp. for 60 days (triangles), and individuals fed with *Tubifex* for 240 days (diamonds). Deformation grids correspond to (b) just captured individuals, (c) individuals fed with *Tubifex* sp. for 60 days, and (d) individuals fed with *Tubifex* sp. for 240 days.

(1995) in zebrafish. In the fish's dorsal view, it became apparent that earlier hatched *O. hatcheri* embryos (AH 1) had bigger yolk sacs than individuals that hatched later.

The effect of parental couples seems to be less conspicuous than AH. Although DA between PC with different AH and the same DAF and temperature (PCAH, Table 1) brought a significant DF, the DA between PC with different DAF and the same temperature and AH (PCDAF, Table 1) failed. Our data (comparison TPCDAF in Table 1) suggest that, at hatching, *O. hatcheri* embryos incubated at higher temperature were more bent. Probably, the size of the yolk sac and the bending of the embryo after hatching will be major factors operating on the beginning of exogenous feeding (Battini *et al.*, 1995). This is consistent with the differential consumption of yolk observed in *Anarhichas minor* (Sund & Falk-Petersen, 2005), where decreased consumption occurred at higher temperature and thus the embryos had more yolk at hatching and were more bent over the yolk sac. Sund & Falk-Petersen (2005) and Peterson *et al.* (2004) also observed in *Anarhichas minor* and *Gadus morhua* that embryos incubated at lower temperature hatched with larger length. Furthermore, water temperature directs the sexual differentiation process in the pejerrey *Odontesthes bonariensis* (Valenciennes) and affects larval condition and growth rate (Strüssmann & Patiño, 1995; Ito *et al.*, 2005; Chalde *et al.*, 2011).

Regarding juvenile feeding, morphological variation increased when, in addition to just captured wild individuals, those subjected to controlled feeding were considered. In all comparisons, the slenderness of the head, size of premaxilla and jaw, and position of the eyes showed appreciable variation in the RW1. Both individuals fed only with zooplankton and those fed only with *Tubifex* sp. had slenderer heads than just captured wild individuals. Furthermore, shapes obtained throughout feeding with *Tubifex* sp. during increasing time lapses included bigger eyes and slenderer heads. In the same way, Grünbaum *et al.* (2007) found different levels of developmental plasticity in critical ontogenetic periods of *Salvelinus alpinus* that are most responsive to environmental constraints. Shape variation has been also induced by controlled diet in other fishes. *Cichlasoma managuense* (= *Parachromis managuensis*) (Meyer, 1987) and *Lepomis humilis* (Hegrenes, 2001) fed with *Artemia* nauplii or *Tenebrio* larvae, showed that individuals fed with large prey (*Tenebrio* larvae) developed an elongated, fusiform shape with a sharply angled snout, after eight months of treatment. In the same way, another native Patagonian fish, *Percichthys trucha* (Valenciennes), showed juveniles fed with zooplankton as having slenderer head and body, and longer jaw than individuals fed with *Tubifex* sp. (large prey) after 70 days of treatment (Crichigno, 2012).

Head shape, size and position of the eyes, and size of premaxilla and jaws are likely to have performance consequences for prey capture and consumption (Carson & Wainwright, 2010). Patagonian lakes show a wide range of photic habitats (Lattuca *et al.*, 2007) and, particularly, the abundance of zooplanktophagous fish like *O. hatcheri* can modify planktonic food webs and water transparency (Reissig *et al.*, 2006). Although an overview of the diet of *O. hatcheri*

indicates an omnivorous diet, a more detailed observation shows that diet changes greatly between lakes, with most of the intralacustrine variation ascribed to the ontogenetic shift (Ferriz, 1987; Grosman & Rudzik, 1990; Cervellini *et al.*, 1993; Macchi *et al.*, 1999). This succession of more or less stenophagous ontogenetic periods could be imposing precise requirements for the oropharyngeal apparatus of the fish.

In conclusion, temperature and hatching timing could act on the head shape during the early life of *O. hatcheri*. Later on, juvenile feeding adds another variable to head shape variation.

Plastic induction of head shape of *O. hatcheri* would provide, by mechanistic, epigenetic processes, additional morphological variation and in consequence a menu of capabilities for prey catching under changing selection selective pressures. In this context, our results could contribute to the comprehension of the plastic component of the morphological variation of the species.

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