



Diet induces phenotypic plasticity of *Percichthys trucha* (Valenciennes, 1833) (Perciformes, Percichthyidae) in Patagonia

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ABSTRACT

The South American fish genus *Percichthys*, due to its great morphological variation, has included several nominal species throughout a long history. However, current genetic analyses signal the existence of only two extant species, *Percichthys melanops* Girard in Chile (west of the Andes) and *P. trucha* (Valenciennes) in Chile and Argentina (west and east of the Andes). Here the morphological variation of free embryos, larvae, and juveniles of *P. trucha* was analyzed using linear measurements and geometric morphometrics. Early morphological variation and compensatory growth were examined using sibling free embryos and larva. Morphological consequences of experimentally controlled food treatments were explored in juveniles. Our results showed individual variation in the size of the yolk-sac of free embryos and in the duration of the mixed feeding period. Phenotypic convergence of the upper jaw length from larva to juveniles and adults, and the causal relationship between diet and head shape was found to change as a consequence of controlled feeding. Embryonic, larval, and juvenile morphological variation and phenotypic plasticity observed in *P. trucha* pinpoint a possible cause for the shape variation observed in the wild. Phenotypic plasticity allows *P. trucha* to exploit different trophic resources and to occupy different habitats in low biodiversity lakes of Patagonia, being a major factor taking part in the past and present success of *P. trucha*.

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1. Introduction

The family *Percichthyidae* (Jordan and Eigenman, 1890) includes one extant freshwater genus in South America; *Percichthys* (Girard, 1845) (Mac Donagh, 1953; Nelson, 1994). All species within the genus are endemic to southern South America (Ringuelet et al., 1967; Arratia, 1982) and are distributed from Tunuyán and Maipo rivers (33° S) to the southern tip of continental Patagonia (Santa Cruz river, 50° S, Ruzzante et al., 2011). Although Ringuelet et al. (1967) and Arratia et al. (1983) reported *Percichthys* species in Tierra del Fuego, no survey has detected it on the island thereafter.

The ranges of several diagnostic characters of the species overlap within the genus. Mac Donagh and Thormählen (1945) pointed out the wide variation within *Percichthys trucha* (Valenciennes, 1833) whereas Ringuelet et al. (1967) differentiated *Percichthys* species based on the ratio between several morphometric traits (e.g., dorsal fin height, upper jaw length, mouth size, interorbital distance, and head and body size). Arratia (1982) described *P. trucha* and *P. melanops* (Girard, 1855) in Chile, paying particular attention

to discrete characters of the external and internal anatomy as well as meristic characters, such as the number of fin rays and the number of vertebrae, concluding in the existence of three species, *P. altispinis* (Regan, 1905), *P. trucha* and *P. melanops*. Arratia et al. (1983) also included *Percichthys colhuapiensis* (MacDonagh, 1955) and *Percichthys vinciguerrae* (Perugia, 1891) in the genus. However, López-Arbarello (1993) stated that *P. vinciguerrae*, *P. colhuapiensis* and *P. trucha* are very similar and very difficult to distinguish morphologically. Consequently López-Arbarello (2004) collapsed three of the species (*P. trucha*, *P. vinciguerrae*, and *P. altispinnis*) into a single one (*P. trucha*) and resurrected a new one (*Percichthys laevis* (Jenyns, 1840)), resulting in the consideration of five valid species: *P. colhuapiensis*, *P. trucha*, *P. laevis*, *Percichthys chilensis* (Girard, 1855) and *P. melanops*. All these species differ mainly in mouth size, dorsal fin height, and number of gill rakers (Fig. 1). However, López-Arbarello (2004) highlighted that the efficiency of her key was quite poor and that the identification of individuals was often impossible due to the frequent presence of intermediate forms. For example, the first set of characters of the key of López-Arbarello (2004) separated *P. chilensis* based on the number of gill rakers of the first branchial arch. The considered specimens ($n = 2$) were paralectotypes of *P. trucha*, their distribution was pre-Andean streams of central and southern Chile (Arratia, 2003), and López-Arbarello (2004) mentioned that the species was probably present in the

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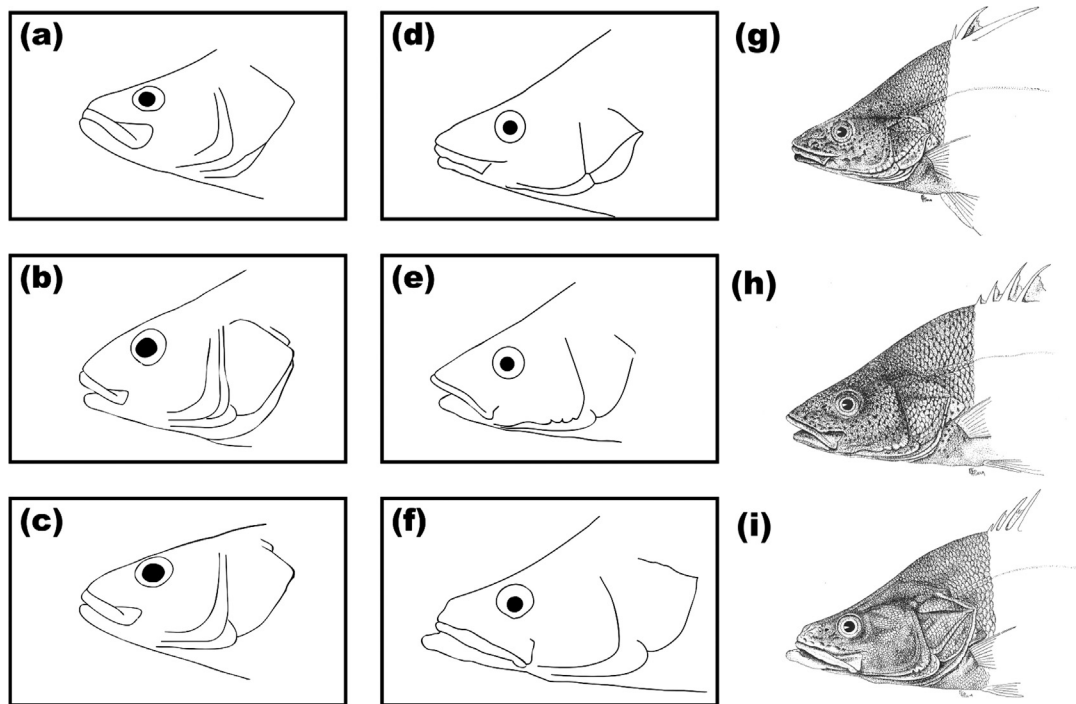


Fig. 1. Schematic lateral view of the head of different nominal forms of *Percichthys trucha*. (a) *Percichthys colhuapiensis*, (b) *Percichthys trucha*, (c) *Percichthys laevis*, (d and g) *Percichthys* sp. of Limay River, (e and h) *Percichthys* sp. of Lake Nahuel Huapi, and (f and i) *Percichthys* sp. of Lake Pellegrini. Schemes (a)–(c) were re-drawn after López-Arbarello (2004) and (d)–(f) after Fuster de Plaza and Plaza (1955). (g)–(i) The original ink drawings made by M.L. Fuster and kindly facilitated by E.E. Boschi to VEC.

Tunuyán river, Mendoza, Argentina. Recently, individuals captured in the Tunuyán river basin have been identified as *P. trucha* by the molecular analyses (Ruzzante et al., 2011), and no other reference to the existence of *P. chilensis* was found in the literature (Campos and Gavilán, 1996; Dyer, 2000; Habit et al., 2006).

In agreement with morphological studies of Campos and Gavilán (1996), genetic results (based on mtDNA and nuclear genes) only supported the existence of *P. melanops* and *P. trucha* (Ruzzante et al., 2006). Paterson et al. (2008) isolated and characterized 13 microsatellite loci for *P. trucha*, with which J.P. Barriga and D. Ruzzante (Pers. Comm.) did not observe significant differences between sympatric morphs. The use of synonyms persisted (Pascual et al., 2007; López et al., 2008; Lopez Cazorla and Sidorkewicz, 2008, 2011) and Ruzzante et al. (2011) expanded the number of localities where the molecular analyses were performed, concluding that (a) all *Percichthys* populations in Patagonia east of the Andes belong to the same species, *P. trucha*, and (b) the combination of the differences in predation and resource regimes, both related to lake productivity, are likely to be responsible for the morphological diversity within this species.

The intra-specific polymorphism of *P. trucha* was linked with the use of different trophic resources (Cussac et al., 1998; Ruzzante et al., 1998; Logan et al., 2000). Morphological changes of *P. trucha* were also detectable after short periods, i.e., upstream and downstream of a hydroelectric dam, only a few years after it was built (Cussac et al., 1998), or after stocking in a fishless lake, where *P. trucha* was successively named as different nominal species while the lake's trophic web changed (Ortubay et al., 2006). These records indicate an important phenotypic plasticity within populations.

Phenotypic plasticity is considered as the existence, within a population, of genotypes that exhibit phenotypic variation when allowed to develop in different environments (e.g., Guntrip and Sibly, 1998; Balon, 2004); as the ability of an organism to react to environmental input with changes in form, state, movement, or rate of activity (West-Eberhard, 2003); or also as the ability

of individual genotypes to produce different phenotypes when exposed to different environmental conditions (Pigliucci, 2001; Pigliucci et al., 2006). Evidence of phenotypic plasticity is widely recognized in fishes (Balon, 1990, 2004). Cephalic morphological variation usually includes phenotypic plasticity phenomena. Thus, juvenile *Perca fluviatilis* (Linnaeus, 1758) show variations in body height and head length that can be experimentally related to feeding (Hjelm et al., 2001) and, in the field, to its preferred habitat (Svanbäck and Eklöv, 2002). The causal relationship between diet and cephalic morphology has been pointed out for *Salvelinus alpinus* (Linnaeus, 1758) (Skúlason et al., 1989; Sigursteinsdóttir and Kristjánsson, 2005; Adams et al., 2007) and *Haplochromis greenwoodi* (Seehausen and Bouton, 1998) (Bouton et al., 2002). Similarly, *Gasterosteus aculeatus* (Linnaeus, 1758) may adapt to a novel environment faster than would be predicted by conventional models of biological differentiation (Walter and Bell, 2000; Kristjánsson et al., 2002). In addition, a notable morphological reversion in the head of *Micropterus salmoides* (Lacepède, 1802) had been experimentally related to food quality (Wintzer and Motta, 2005). Diet also induced morphological changes in *Parachromis managuensis* (Günther, 1867) and *Lepomis humilis* (Girard, 1858) (Meyer, 1987; Hegrenes, 2001).

Ruzzante et al. (2011) pointed out a paradox: there exists a high level of morphological variability among and within *Percichthys* populations, sufficiently high, that variants from different drainages have been proposed to be different species; however there is no evidence whatsoever of a deep divergence in the entire haplotype tree for *Percichthys* east of the Andes, just a very shallow phylogeographic structure for the region.

The aim of this work is (a) to study the early ontogenetic morphological variation and phenotypic plasticity of *Percichthys trucha*; working with sibling free embryos and larvae, (b) to explore experimentally the morphological adaptations to different diets during the juvenile period; and (c) to integrate these results into previous taxonomic, genetic, and biological information, in order to improve the knowledge of phenotypic plasticity in fish.

2. Materials and methods

2.1. Morphology of early ontogenetic stages

Adult *P. trucha* were caught in Lake Morenito (41°05' S, 71°30' W, 758 m a.s.l.) in December 2006 and 2007, and in Lake Laguna Blanca (39°02' S, 70°21' W, 1230 m a.s.l.) in November 2007, in the littoral zone of the lakes using gillnets (15, 30 and 40 mm bar mesh). Individuals were considered reproductive according to [Fuster de Plaza and Plaza \(1955\)](#) criteria, i.e. whether they released ova or milt under slight pressure. All individuals corresponded to the “deep benthic” morphotype ([Ruzzante et al., 1998](#)) with a ratio of 0.073 between upper jaw and standard length (SL) (range 0.054–0.090).

Ova and milt were obtained by stripping and eggs were produced by dry fertilization ([Barnabé, 1990](#)). Ontogenetic periods namely embryo, free embryo, larva, and juvenile were defined by fertilization, hatching, first feeding, and metamorphosis respectively, following [Balon \(1990\)](#).

Eggs from Lake Morenito carried alive to the laboratory in 2006 comprised two sets of ova from the same female, each fertilized by a different male. Each fertilized ova set was split into two subsets and each incubated at one of two controlled water temperatures: 11 °C and 18 °C (± 1 °C). Once eggs hatched, water temperature for the four subsets was maintained at room temperature ranging from 10 to 15 °C, with a mean of 12.4 °C. The following year (2007) eggs from Lake Morenito (coming from 1 parental couple) and from Laguna Blanca (coming from 1 parental couple) were carried alive to the laboratory and again each batch was split into two subsets, each one incubated at one of these controlled water temperatures during the entire experiment: 15 °C and 19 °C (± 1 °C) for Lake Morenito subsets, and 10 °C and 19 °C (± 1 °C) for Laguna Blanca subsets. The temperature for normal development of this species ranges from 8 to 20 °C ([Fuster de Plaza and Plaza, 1955](#)) and the preferred temperatures for juvenile *P. trucha* range from 12.9 to 21.2 °C ([Aigo, 2010](#)).

Newly hatched free embryos and larva were anaesthetized, photographed, and measured under a stereomicroscope. Height (HYS) and length of the yolk sac (LYS), anterior–posterior eye diameter (AED), length of the upper (LUJ) and lower jaw (LLJ), and SL were recorded. Newly hatched free embryos (“Morenito 2006”, 11 °C, $n = 100$, “Morenito 2007”, 15 and 19 °C, “Laguna Blanca 2007”, 10 and 19 °C, $n = 30$ for each temperature) were reared in a 100 L aquarium. Live zooplankton, cladocerans and copepods, were offered ad libitum. The number of individuals feeding for the first time (and the time at which this happened) was recorded within a subset of individuals hatching the same day (504 h after activation and 231 cumulative thermal units, Morenito 2006, 11 °C, $n = 100$).

Fifteen and 30 days after hatching (DAH) (Morenito 2006, 11 °C, Morenito 2007, 15 and 19 °C, and Laguna Blanca 2007, 10 and 19 °C), larvae were anaesthetized with 0.8% benzocaine and photographed (Nikon D70). Body shape was quantified by digitizing the Cartesian coordinates of 15 homologous landmarks on the left side of the head and trunk of each individual and examined with Geometric Morphometric Analysis (GMA). These landmarks were: (1) anterior tip of the lower jaw, (2) posterior lower tip of the lower jaw, (3) posterior lower tip of the upper jaw, (4) posterior lower tip of the retroarticular bone, (5) inner angle of the first branchial arch (between epiphyal and ceratobranchial bones), (6) anus, (7) posterior end of the basis of the median fin fold vestige, (8) vertical tie of landmark 7 at the dorsal line, (9) anterior tip of the medulla, (10) anterior tip of the supraoccipital bone, (11) center of the olfactory placode, (12) dorsal tip of the premaxillary bone, (13), (14) anterior and posterior border of the eye, and (15) anterior tip of the epiphyal 1 ([Fig. 2a](#)). Discriminant Analysis (DA) was performed to test for morphological differences among parents and incubation temperatures using the shape matrix provided by GMA. Then,

each group was analyzed separately, relating shape (i.e., Relative Warps RW for each group), with residuals obtained from the regression of base 10 logarithm of linear measures (AED, LUJ, LLJ) with log₁₀ SL. All analyses and graphs were performed using SPSS® and SIGMAPLOT®.

2.2. Morphological variation of juveniles

Juvenile *P. trucha* were caught with seine in Negro river (38°59' S; 67°59' W late summer 2007), Traful river (40°43' S; 71°05' W, 760 m.a.s.l., late summer 2008), and Piedra del Águila reservoir (40°27' S; 70°39' W, late summer 2009), as well as with the use of a backpack electro fishing unit (Smith-Root 12B model, Vancouver, WA, USA) in Caleufu river (40°23' S; 70°44' W, spring 2007) and Pichileufu river (41°05' S; 70°50' W, late summer 2009). Once in the laboratory, individuals were anaesthetized and photographed (lateral view; whole body and head).

2.3. Induction of plasticity in juveniles

At the laboratory (Centro de Salmonicultura Bariloche, CEN-SALBA), a subset of individuals caught in Traful river were anaesthetized with 0.8% benzocaine and photographed in lateral view of whole body and head shape. Afterwards, all individuals were put in six aquaria (200 L each), at room temperature (mean 12.4 °C, range 10–15 °C), with natural photoperiod, and fed the following three different diets (two aquaria by diet): pellets, zooplankton, and *Tubifex* sp. Pellets formulated according to [Hualde et al. \(2011\)](#) were used as hard, immobile food. Zooplankton included mostly *Daphnia* sp. (70%) and copepods (calanoids and cyclopoids), and was used as food with high mobility and small size. *Tubifex* sp. was used as large food with low mobility. Diets were selected in order to have different degrees of similarity to natural diets, already known for juveniles (10.6–77.8 mm SL); chironomid larvae, *Daphnia* sp. and cyclopoid copepods ([Ruzzante et al., 2003](#)). Food was offered ad libitum twice a day. After 70 days of captivity, all individuals were anaesthetized with 0.8% benzocaine and photographed. Individuals fed with *Tubifex* sp. were anaesthetized and photographed again at 110 and 150 days of captivity.

Body and head shape were quantified by digitizing the Cartesian coordinates of 12 homologous landmarks on the left side of the body ([Fig. 3b](#)) and 12 landmarks on the left side of the head of each individual ([Fig. 3c](#) and [d](#)), using GMA.

Landmarks of the body: (1) Anterior tip of upper jaw, (2) Posterior tip of upper jaw, (3) Lower tip of the intersection between operculum and preoperculum, (4) Posterior tip of operculum, (5) Anus, (6) Ventral point of smallest transversal section of the caudal peduncle, (7) Middle point of the caudal fin base, (8) Dorsal tip of smallest transversal section of the caudal peduncle, (9) Posterior tip of dorsal fin base, (10) Dorsal fin origin, (11) Eye center, (12) Body midpoint on the anus. Three homologous landmarks (1, 7, and 12) were used to unbend curved whole body lateral views.

Landmarks of the head: (1) Anterior tip of upper jaw, (2) Anterior tip of lower jaw, (3) Posterior tip of upper jaw, (4) Posterior tip of lower jaw, (5) Lower tip of the intersection between operculum and preoperculum, (6) Posterior tip of operculum, (7) Dorsal tip of preoperculum, (8, 9, 10, and 11) Anterior, posterior, ventral, and dorsal edges of eye, and (12) Center of nostril.

Discriminant analysis (DA) was performed using the uniform and non-uniform component of Partial Warps. Comparisons between food types and between treatment times were performed using SPSS® and SIGMAPLOT®.

The following comparisons were performed using DA: (a) Body and head shape between newly captured individuals from Traful, Pichileufu, Caleufu, and Negro rivers and Piedra del Águila reservoir (whole body shape analysis) and from Traful, Pichileufu rivers

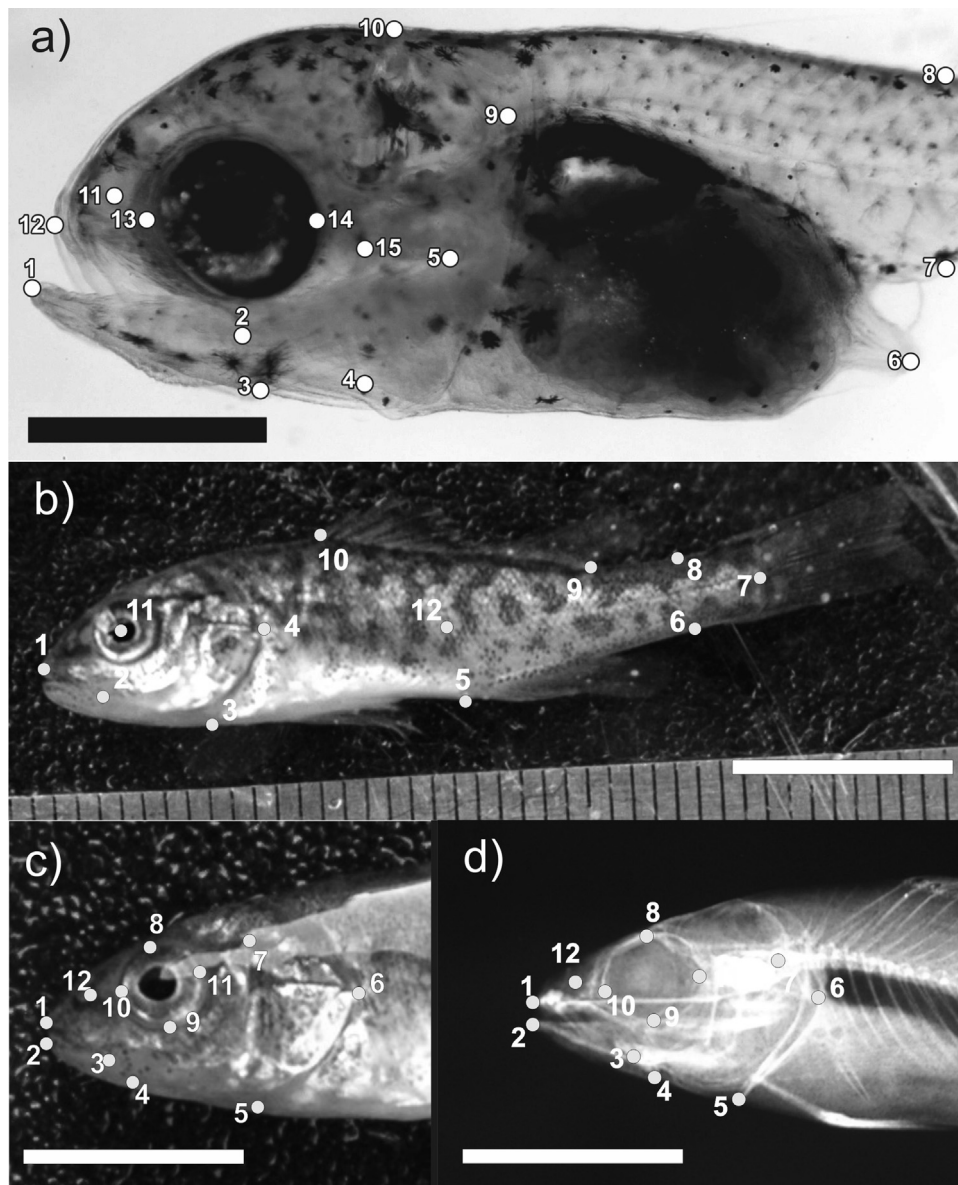


Fig. 2. (a) Position of the landmarks in a 15 DAH larva. (1) anterior tip of the lower jaw, (2) posterior lower tip of the lower jaw, (3) posterior lower tip of upper jaw, (4) posterior lower tip of the retroarticular bone, (5) inner angle of the first branchial arch (between epihyal and ceratobranchial bones), (6) anus, (7) posterior end of the basis of the median fin fold vestige, (8) vertical tie of landmark 7 at the dorsal line, (9) anterior tip of the supraoccipital bone, (10) anterior tip of the olfactory placode, (11) center of the olfactory placode, (12) dorsal tip of the premaxillary bone, (13), (14) anterior and posterior border of the eye, and (15) anterior tip of the epihyal 1. Black bar = 1 mm. (b)–(d) Homologous landmarks in juvenile of *P. trucha* on a) the left side of the body, (b) the head, and (c) on a radiographic image (left side of the head) of individuals from Traful River. Landmarks of the body: (1) anterior tip of upper jaw, (2) posterior tip of upper jaw, (3) lower tip of the intersection between operculum and preoperculum, (4) posterior tip of operculum, (5) anus, (6) ventral point of smallest transversal section of the caudal peduncle, (7) middle point of the caudal fin base, (8) dorsal point of smallest transversal section of the caudal peduncle, (9) posterior tip of dorsal fin base, (10) dorsal fin origin, (11) eye center, (12) body midpoint on the anus. Three homologous landmarks (1, 7, and 12) were used to unbend curved whole body lateral views. Landmarks of the head: (1) anterior tip of upper jaw, (2) anterior tip of lower jaw, (3) posterior tip of upper jaw, (4) posterior tip of lower jaw, (5) lower tip of the intersection between operculum and preoperculum, (6) posterior tip of operculum, (7) dorsal tip of preoperculum, (8–11) anterior, posterior, ventral, and dorsal edges of eye, and (12) center of nostril. With bar = 10 mm.

and Piedra del Águila reservoir (head shape analysis); (b) Body and head shape of three diet groups of individuals from Traful river fed during 70 days, in relation to the shape of newly captured individuals, and c) Body and head shape of newly captured individuals and individuals fed with *Tubifex* sp. for 70, 110 and 150 days, all from Traful river (Table 1).

In all cases, shape was quantified using the GMA approach of thin-plate splines (Bookstein, 1991; Rohlf and Marcus, 1993; Parsons et al., 2003; Adams et al., 2004). Digitized specimens were first scaled 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 (<http://life.bio.sunysb.edu/morph/index.html>).

3. Results

3.1. Morphology of early ontogenetic stages

After hatching (“Morenito 2006”), the yolk sac diameter (mean = 380, ranging from 288 to 462 μm , $n = 15$) diminished. One DAH (“Morenito 2006”, 11 °C; “Morenito 2007”, 15 and 19 °C; “Laguna Blanca”, 10 and 19 °C), the LYS showed 89.2% of variation (100 range mean⁻¹), ranging from 19.0 to 44.3% when considered

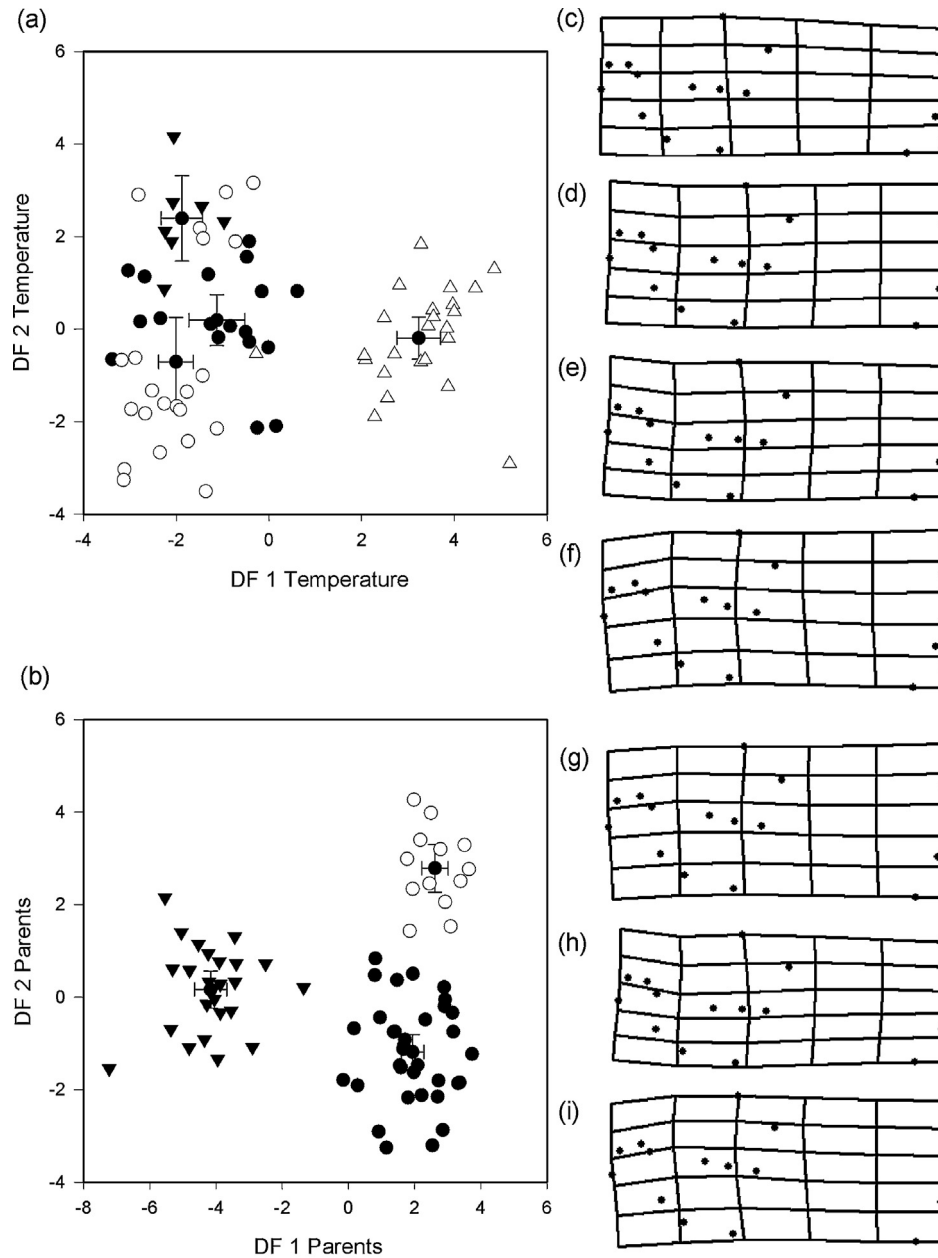


Fig. 3. Shape of 15 DAH larvae. Discriminant Function 2 (DF2) versus Discriminant Function 1 (DF1) for the Discriminant analyses among (a) temperatures of incubation and (b) parents. Group centroids and 95% confidence intervals are indicated. (c)–(f) Deformation grids for each group centroid on analyses of temperatures related to black triangles demonstrate individuals incubated at 15 °C, black circles at 10 °C, white triangles at 11 °C, and white circles at 19 °C, respectively. (g)–(i) Deformation grids for each group centroid on analyses of parents related to white circles demonstrate individuals incubated at 19 °C, black triangles at 15 °C, and black circles at 10 °C, respectively.

Table 1
 Groups compared for body and head shape using geometrics morphometrics data and discriminant analysis. Number of significant discriminant functions, cases correctly classified and variation explained are indicated.

Body shape	Groups	Trafal river Pichileufu river Caleufu river Negro river Piedra del Águila reservoir	Trafal river (just captured) <i>Tubifex</i> (70 days) Pet food (70 days) Zooplankton (70 days)	Trafal river (just captured) <i>Tubifex</i> (70 days) <i>Tubifex</i> (110 days) <i>Tubifex</i> (150 days)
	Significant DFs	4	2	3
	Cases correctly classified	95.6%	89.2%	91.4%
	Variation explained	100%	95.3%	100%
Head shape	Groups	Trafal river Pichileufu river Piedra del Águila reservoir	Just captured <i>Tubifex</i> (70 days) Pet food (70 days)	Just captured <i>Tubifex</i> (70 days) <i>Tubifex</i> (110 days)
	Significant DFs	2	1	3
	Cases correctly classified	95.4%	85.8%	91.7%
	Variation explained	100%	100%	100%

Table 2

Geometric morphometrics and morphological measurements of *P. trucha* larvae. Significant correlations and percentage of variation (100 range mean⁻¹) of the length of the upper jaw (LUJ) are indicated.

	Sampling site	Morenito 2006	Morenito 2007	Laguna Blanca		
	Parental Couple	A	B	C	D	E
	Incubation Temperature (°C)	11	15	19	10	19
15 DHA	n=	24	7	6	18	16
	Variation explained by the first three RWs (%)	66	78	83	57	62
	RW1 vs. SL Pearson Corr./P<	0.455/0.025		-0.857/0.029	-0.515/0.029	
	RW2 vs. res log ₁₀ LUJ Pearson Corr./P<	-0.423/0.039			0.497/0.036	
	RW3 vs. SL Pearson Corr./P<					-0.508/0.045
	RW3 vs. res log ₁₀ LUJ Pearson Corr./P<				-0.540/0.021	
	RW3 vs. res log ₁₀ LLJ Pearson Corr./P<				-0.625/0.006	
RW3 vs. res log ₁₀ AED Pearson Corr./P<	0.453/0.026			-0.491/0.038		-0.537/0.032
	100. LUJ range. LUJ mean ⁻¹	47	49	15	32	49
30 DHA	n=	12	7	6	14	8
	res log ₁₀ LUJ vs. res log ₁₀ AED Pearson Corr./P<	0.761/0.004	0.934/0.002			0.887/0.003
	res log ₁₀ LLJ vs. res log ₁₀ AED Pearson Corr./P<	0.650/0.023	0.980/0.001		0.784/0.001	0.840/0.009
	100. LUJ range. LUJ mean ⁻¹	43	57	38	34	21

by parents and temperatures (Table 2). Four DAH, only the oil globule could be observed, with its consumption occurring during the following two days. Although live food (mostly copepods, 227 µm width) was offered since hatching, only 30% of the larva began feeding at 2 DAH. The remaining 70% of the larva began to feed between 3 and 4 DAH.

At 15 DAH (all parental couples and incubation temperatures), 54% of the morphological variation among larva was explained by the first three RWs. DA among incubation temperatures showed three significant discriminant functions (DFs) with 100% of cases correctly classified and explaining 100% of total variance (DF1, Wilks' lambda=0.040, n=71, P<0.001, DF2, Wilks' lambda=0.272, n=71, P<0.001, and DF3, Wilks' lambda=0.636, n=71, P<0.001, Fig. 3a). DA among parents (females) showed two significant DFs (DF1, Wilks' lambda=0.030, n=71, P<0.001, DF2, Wilks' lambda=0.313, n=71, P<0.001, Fig. 3b) with 100% of cases correctly classified and explaining 100% of total variance. The dependence of shape (RWs obtained for each parental couple and incubation temperature) on SL, and the relationship between RWs and linear measured magnitudes, changed according to parents and incubation temperatures (Table 2).

After 30 DAH, differences among parents and incubation temperatures (MANOVA) were significant for the residual log₁₀ AED (parental couples, F=8.447, n=47, P<0.006, temperature, F=19.570, n=47, P<0.001) and residual log₁₀ LLJ (parental couples, F=6.557, n=47, P<0.014, temperature, F=15.885, n=47, P<0.001). Significant correlations between residual log₁₀ AED and both residual log₁₀ LUJ and residual log₁₀ LLJ (Fig. 4, Table 2) were also observed.

The percentage of variation (100 range mean⁻¹) of the LUJ for each parental couple and temperature, both at 15 and 30 DAH (Table 2), was even higher when considering pooled data, both for 15 DAH (78%) and 30 DAH (85%).

3.2. Morphological variation of juveniles

GMA of body shape of individuals from four rivers and one reservoir showed 51.42% of variation, explained by the first two RWs (RW1=39.45; RW2=11.97; N=203). DA among the 5 water bodies showed four significant DFs that correctly classified 95.6% of cases and explained 100% of the variation (DF1, Wilks' lambda=0.016, N=203, P<0.001; DF2 Wilks' lambda=0.118, N=203, P<0.001; DF3, Wilks' lambda=0.346, N=203, P<0.001; DF4 Wilks' lambda=0.697, N=203, P<0.001, Table 1).

GMA of head shape of individuals from two rivers (Trafal and Pichileufu) and one reservoir showed 65.97% of variation for the first two RWs (RW1=53.84; RW2=12.13; N=151). DA among the

three water bodies showed two significant DFs that correctly classified 95.4% of cases and explained 100% of variation (DF1, Wilks' lambda=0.048, N=151, P<0.001 and DF2 Wilks' lambda=0.361, N=151, P<0.001, Table 1).

3.3. Induction of plasticity in juveniles

3.3.1. Trafal river, wild and treated individuals, body shape obtained with different diets along 70 days

DA among recently captured individuals and those fed with pet food, zooplankton and *Tubifex* sp. showed two significant DFs that correctly classified 89.2% of cases and explained 95.3% of the variation (DF1, Wilks' lambda=0.100, N=130, P<0.001 and DF2 Wilks' lambda=0.397, N=130, P<0.001, Table 1). The DF1 separated recently captured from treated individuals and the DF2 individuals fed (70 days) with *Tubifex* sp. from individuals fed (70 days) with pet food and zooplankton (Fig. 5). Treated individuals showed more robust bodies and longer jaws than wild individuals.

3.3.2. Trafal river, wild and treated individuals, body shape obtained feeding with *Tubifex* sp. during 70, 110, and 150 days

DA among recently captured individuals and individuals fed with *Tubifex* sp. for 70, 110, and 150 days showed three significant DFs. These correctly classified 91.4% of cases and explained 100% of the variation (DF1 Wilks' lambda=0.050, N=162, P<0.001, DF2 Wilks' lambda=0.263, N=162, P<0.001, and DF3 Wilks' lambda=0.557, N=162, P<0.001, Table 1). The deformation grids for group centroids of recently captured individuals and individuals fed with *Tubifex* sp. during 70, 110, and 150 days, allowed us to follow the trajectory of body shape change (Fig. 6). Throughout this trajectory we were able to observe and elongation of the jaw length (70 days) and then a shortening of the jaw length (110 days), reaching a length (150 days) greater than that observed in wild individuals

3.3.3. Trafal river, wild and treated individuals, head shape obtained with different diets along 70 days

DA among recently captured individuals and individuals fed with pet food, zooplankton and *Tubifex* sp., showed one significant DF that classified 85.8% of cases and explained 100% of the variation (DF1, Wilks' lambda=0.139, N=106, P<0.001, Table 1). This DF1 separated recently captured from all treated individuals (Fig. 7). Treated individuals showed larger jaws and bigger eyes than those recently captured.

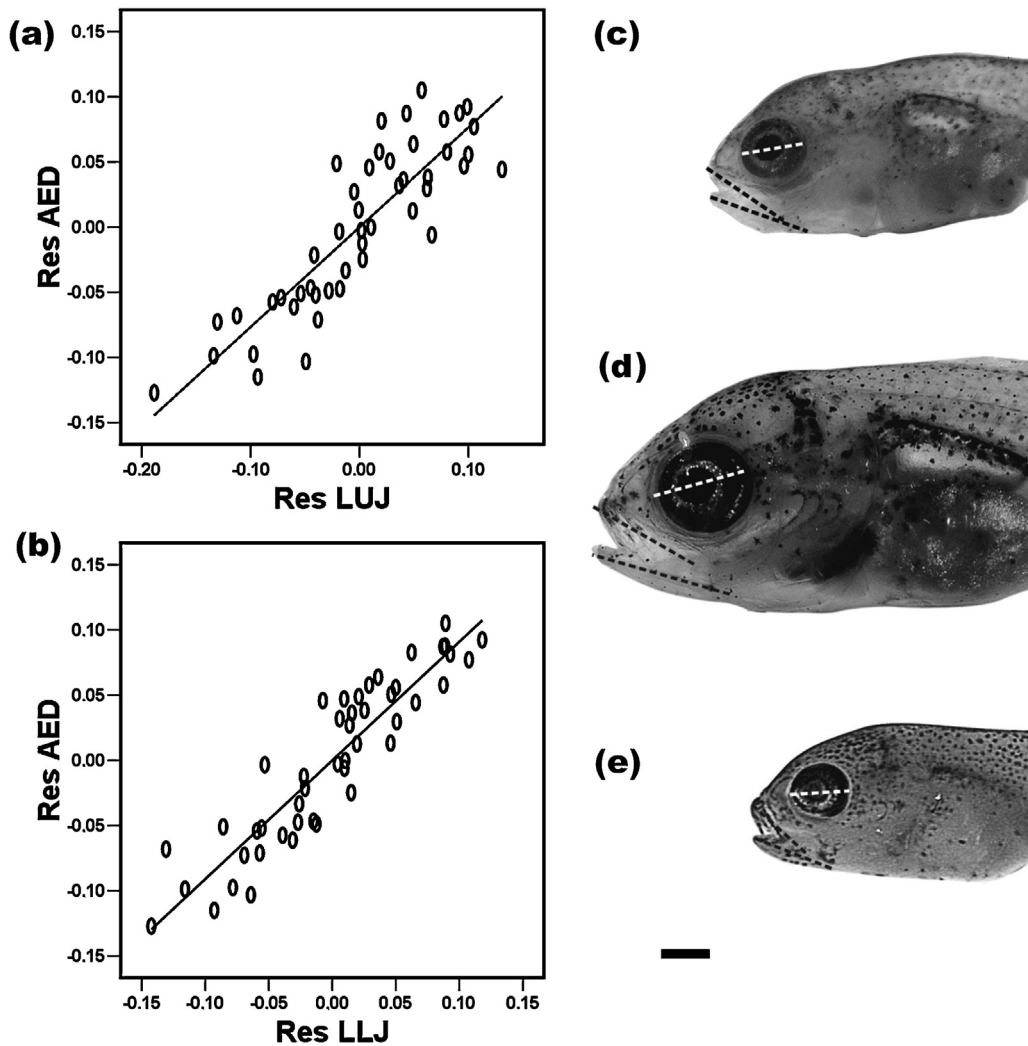


Fig. 4. Correlations among cephalic measurements in 30 DAH larvae between: (a) anterior–posterior eye diameter (Res AED) and length of upper jaw (Res LUJ) and (b) between Res AED and length of lower jaw (Res LLJ). Fitted lines are indicated to show the tendency. All variables are residuals of the double log10 regression versus standard length. Images (c)–(e) correspond to individuals found at the upper tip, middle, and lower tip of the fitted lines, respectively. Dashed drawings on images show AED, LUJ, and LLJ, at the same scale (bar = 1 mm).

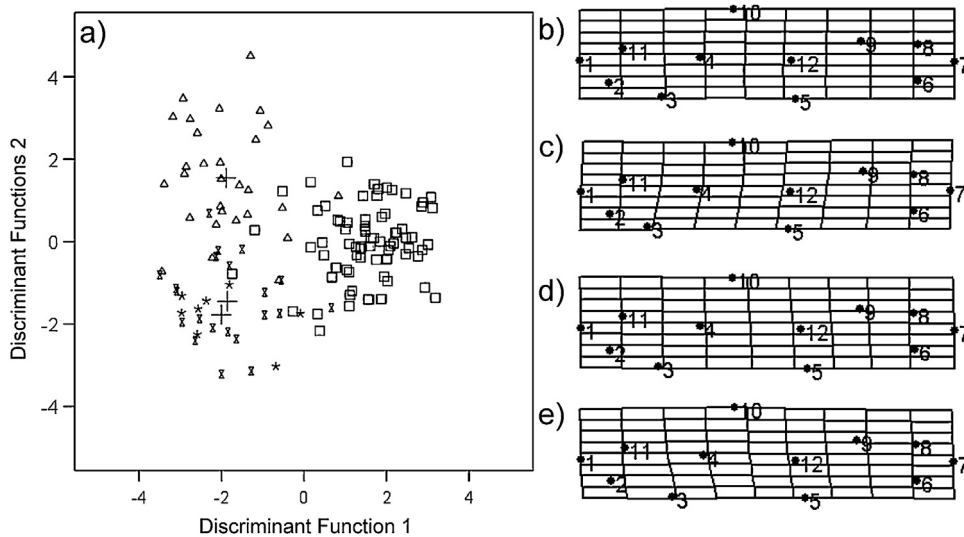


Fig. 5. Whole body shape of juvenile *P. trucha* from Traful River. (a) Discriminant function 2 (DF2) versus Discriminant function 1 (DF1) for individuals fed with four different diets. Deformation grids of individuals, (b) fed with *Tubifex* sp. (triangles), (c) recently captured (square), (d) fed with zooplankton (bows) and (e) fed with pet food (stars).

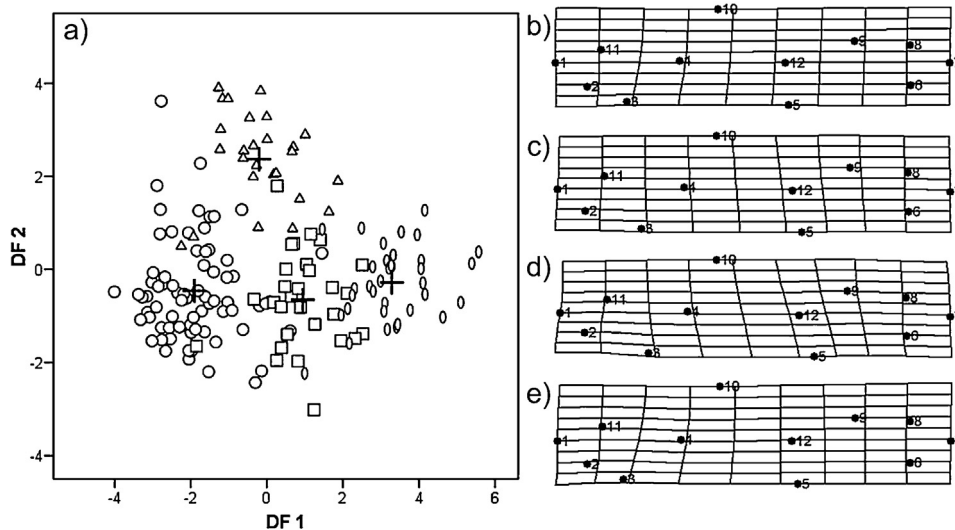


Fig. 6. Whole body shape of juvenile *P. trucha* from Traful River. (a) Discriminant function 2 (DF2) versus Discriminant function 1 (DF1) for recently captured individuals and individuals fed with *Tubifex* sp. during 70, 110, and 150 days. Deformation grids of (b) recently captured individuals (circles), (c) individuals fed with *Tubifex* sp. during 70 days (squares), (d) individuals fed with *Tubifex* sp. during 110 days (ovals), and (e) individuals fed with *Tubifex* sp. during 150 days (triangles).

3.3.4. Traful river, wild and treated individuals, head shape, *Tubifex* sp. during 70, 110, and 150 days

DA among recently captured individuals and individuals fed with *Tubifex* sp. for 70, 110, and 150 days produced three significant DFs that correctly classified 91.7% of cases and explained 100% of the variation (DF1, Wilks' lambda = 0.037, $P < 0.001$, DF2 (Wilks' lambda = 0.161, $P < 0.001$ and DF3, Wilks' lambda = 0.458, $P < 0.001$, Table 1). DF1 separated individuals fed with *Tubifex* sp. for 110 days and DF2 separated individuals fed with *Tubifex* sp. for 150 days (Fig. 8). Throughout this trajectory, the length of the upper jaw decreases (70 days) and later increases (110 days), reaching a length (150 days) greater than the observed in wild individuals.

4. Discussion

In the present work, a significant morphological variation in embryos, larvae and juveniles of *P. trucha* was recorded. Moreover, diet induced changes in head shape, outside the natural range of variation, were experimentally demonstrated. These results are integrated into previous taxonomic, genetic, and biological data.

The embryonic development of *P. trucha* was consistent with previous observations by Fuster de Plaza and Plaza (1955) and is similar to most teleosts (Kunz, 2004). Variation of yolk length among newly hatched sibling free embryos incubated at the same temperature ranged from 19.0 to 44.3% (100 range mean⁻¹), but the percentage scales to 89.2% when considering all the combinations of parents and temperatures (Table 2). This variation could be related to the heterogeneity of vitellogenin intake by each oocyte during oogenesis and in the subsequent embryo development (Kunz, 2004).

Variation of egg sizes in fish populations has been identified as constant across a wide range of species and likely to be significantly modulated by the environment during oogenesis. Similar to many perciform species such as *Morone saxatilis* (Walbaum, 1792) (Eldridge et al., 1982), *Dicentrarchus labrax* (Linnaeus, 1758) (Rønnestad et al., 1998), among others (Kamler, 2008), *P. trucha* consumed the yolk first and the oil globule afterwards. First feeding ranged between 2 and 4 DAH. However, yolk lasted up to 4 DAH and the oil globule up to 6 DAH. In consequence, the mixed period (Balon, 1990) differed between individuals. This mixed

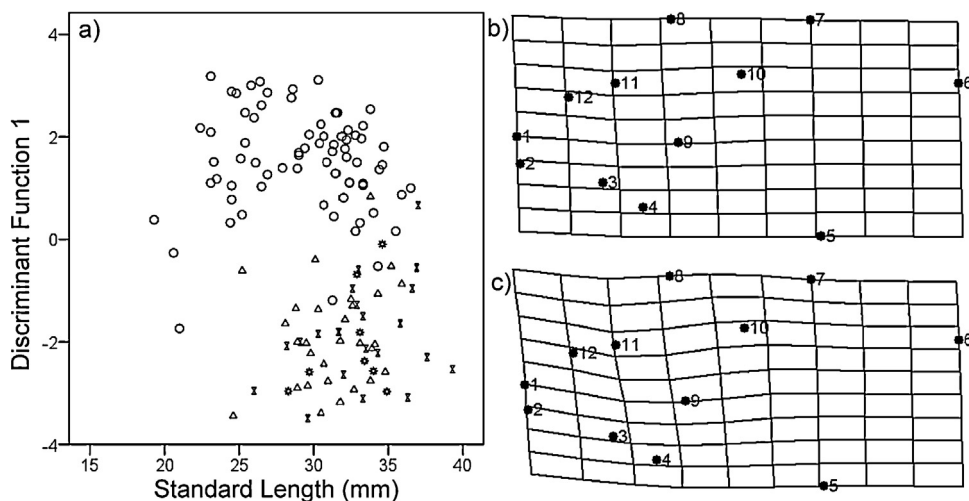


Fig. 7. Head shape of juvenile *P. trucha* from Traful River. (a) Discriminant function 1 (DF1) versus standard length (mm) for individuals fed with four different diets. Deformation grids of individuals (b) recently captured (circles), (c) fed with *Tubifex* sp. (triangles), pet food (stars) and zooplankton (bows).

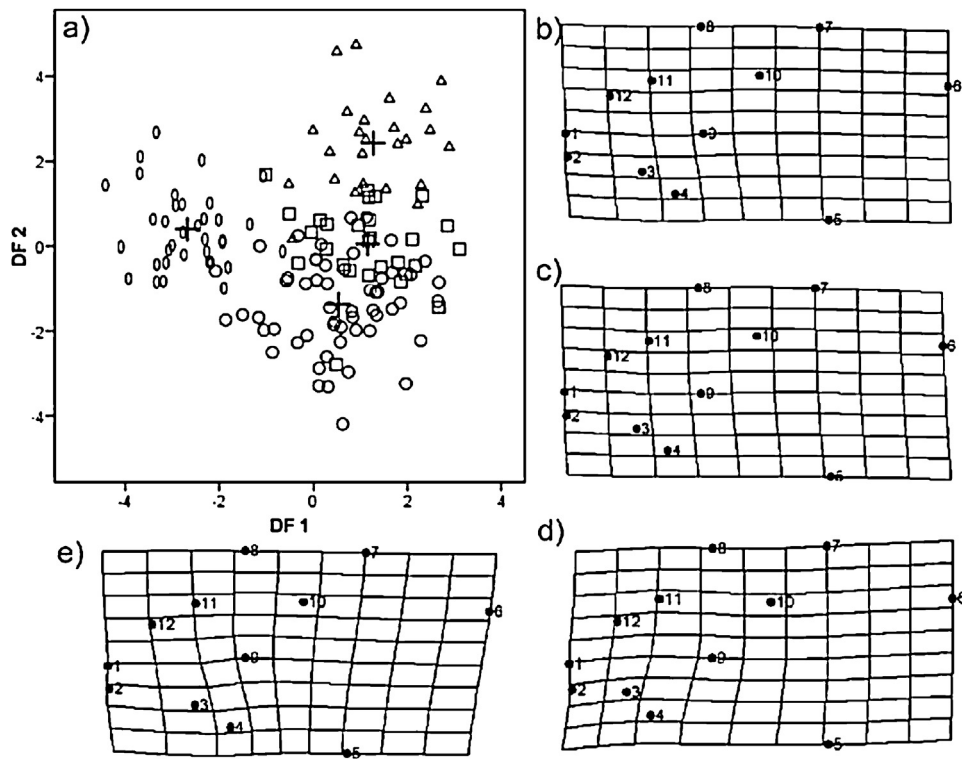


Fig. 8. Head shape of juvenile *P. trucha* from Trafal River. (a) Discriminant function 2 (DF2) versus Discriminant function 1 (DF1) for recently captured individuals and individuals fed with *Tubifex* sp. during 70, 110, and 150 days. Deformation grids of (b) recently captured individuals (circles), (c) individuals fed with *Tubifex* sp. for 70 days (squares), (d) in individuals fed with *Tubifex* sp. for 110 days (ovals), and (e) individuals fed with *Tubifex* sp. for 150 days (triangles).

feeding phenomenon slows the rate of yolk absorption in *Theragra chalcogramma* (Pallas, 1811) and *Salmo trutta* (Linnaeus, 1758) (Hamai et al., 1974; Raciborski, 1987). In other species, an access to external food accelerates yolk absorption rate or has no effect (Hemming and Buddington, 1988; Kamler, 2008). Mixed feeding could be another source of variation, operating at the threshold between embryonic and larval periods (Balon, 1990).

Fifteen DAH, *P. trucha* larvae showed a size-dependent variation of the cephalic shape and a positive relationship between cephalic shape, the size of the upper jaw, and the eye diameter. Thirty DAH, the length of the upper jaw and the eye diameter show a positive relationship. Part of the size variation of larva, juveniles, and adults could be the result of the propagation of initial size differences among individuals throughout growth (Chambers and Leggett, 1996). Commonly reported in fish, birds, and mammals, compensatory growth is an important mechanism of canalization, buffering adult phenotype against genetic or environmental effects, defined as the tendency of growth trajectories to converge on a reduced range of phenotypes (Wilson and Reale, 2006). Phenotypic convergence requires a decrease in variance so that compensatory growth can be inferred from reductions in variance components over ontogeny. In *P. trucha*, the percentage difference 100 range mean⁻¹ for the length of the upper jaw varies from 78% in 15 DAH larvae from two lakes (15–49% within parental couples and temperatures), 85% in 30 DAH larvae from two lakes (21–57% within parents and temperatures), and 24% in juveniles from three lakes (Ruzzante et al., 2003), to 13% in adults from six lakes (Ruzzante et al., 1998). However, such convergence fails to reach a complete buffering of the adult phenotype, allowing the existence of littoral and deep-benthic morphs with significantly different diets (Logan et al., 2000) within the same lake (Ruzzante et al., 1998). Thus, the morphological variation of the head of *P. trucha* could be related to the early amount of yolk and the timing of the first exogenous feeding, among several other factors. The

magnitude of this variation would be subjected to different degrees of compensatory growth and environmental canalization, resulting in the different adult morphologies already observed.

The morphological variation observed in juvenile *P. trucha* is noteworthy (Ruzzante et al., 2003). Both body and head shape of newly captured juveniles showed high percentages of variance explained by the first RWs. Differences between recently captured juveniles and individuals fed with controlled diets were most notorious in head shape. Treated individuals showed a more robust body, longer jaws, and bigger eyes than recently captured wild individuals. This is consistent with the findings of Meyer (1987) in *Parachromis managuensis* and Hegrenes (2001) in *Lepomis humilis*. Both authors detected that individuals fed with large prey items (*Tenebrio* sp. larvae) developed a taller, deep-bodied shape and a blunt snout after 8 months of treatment, whereas individuals fed with nauplii of *Artemia* sp. developed an elongated, fusiform shape with a sharply angled snout (Meyer, 1987; Hegrenes, 2001). Furthermore, individuals that feed primarily on zooplankton tend to develop more streamlined bodies and many long, closely spaced gill rakes that can capture small pelagic prey, whereas individuals feeding on benthic prey tend to develop deeper bodies, and sometimes longer, more robust jaws (Adams et al., 2003; Andersson et al., 2005; Berner et al., 2008). In the same way, Andersson et al. (2005) point that in a structurally complex habitat, rich in macroinvertebrates, fish develop deeper bodies and a downward positioned tip of the snout, compared to a structurally simple habitat, rich in zooplankton. Head shape analyses of *P. trucha* showed that most of the variation was found in the size of the eyes, the upper, and the lower jaw. Similarly, cichlid fishes showed the most prevalent changes in relation to the size of the preorbital region of the skull. In this way, large effects on feeding biomechanics and expansion into a wide array of niches along a benthic–pelagic ecomorphological axis have been observed in cichlids, suggesting that the preorbital region probably constitutes a distinct developmental and evolutionary

module (Cooper et al., 2010). Our results show that controlled diet regimes such as *Tubifex* sp. can induce variation of both body and head shape within a given juvenile body size cohort.

The question about the implicit involvement of growth into differences between just captured and controlled fed individuals could be addressed considering the range of body size. Total length is a more explicative variable than time for physiological rates (Fuiman et al., 1998; Sæle and Pittman, 2010; Chalde et al., 2012). At least in size ranges between the smallest treated *P. trucha* individual and the largest recently captured individual, the feeding treatments can induce shapes different from those observed in the field.

It is tempting to ascribe the morphology of embryos, larvae, or juveniles to a given adult morphology. For example, Fig. 4 shows changes in the length of the upper and lower jaw in relation to the position of the eye, which would correspond to different species of López-Arbarello (2004). For instance, Fig. 4c shows a morphology that would correspond to *Percichthys* sp. in Fig. 1f. In contrast, Fig. 4e shows a morphology that could correspond to *P. trucha* (Fig. 1b) or *P. laevis* (Fig. 1c) or *Percichthys* sp. (Fig. 1d and e). However, it must be considered that the expression of genomic information and the phenotypic plasticity operate all along the ontogeny, from egg to adult, and that one cause can operate in the same or in the opposite direction that a previous one. Thus, the prediction of the final adult phenotype is not a simple matter.

The morphological variation and phenotypic plasticity of *P. trucha* should be visualized in the framework of the environmental and evolutionary history of the species. Up to date, *P. trucha* is distributed along more than 17 latitudinal degrees, in lakes, reservoirs and rivers of the Andean region of Southern South America (Baigún and Ferriz, 2003; Liotta, 2006; Aigo et al., 2008; López et al., 2008; Cussac et al., 2009). The *Percichthyidae* family seems to have originated in freshwater, as a secondary radiation from marine protopercoid ancestors (Jerry et al., 2001). The adaptive potential of estuarine fishes to invade freshwater was emphasized by Beheregaray and Sunnucks (2001) and Beheregaray et al. (2002) for Atherinopsidae. In the same way, Australian percichthyids have developed unique, complex, and flexible migratory, reproductive, and life history strategies to cope with highly variable and unpredictable environmental conditions (Roberts et al., 2008; Walsh et al., 2011, 2012). Recently, Czesny et al. (2012) documented regulatory modifications of gene expression between freshwater and marine morphs of *Alosa pseudoharengus* (Wilson, 1811). Probably the success of South American *Percichthyidae* in freshwater involve similar mechanisms. The *Percichthys* lineage successfully overcame a long history of environmental changes in Patagonia, such as cooling (Cione and Báez, 2007; Cione et al., 2007), the upraise of the Andes, aridization, marine incursions (Hubert and Renno, 2006), glaciations (Ruzzante et al., 2006, 2008, 2011), hydrological variability (Piovano et al., 2009), the recent introduction of exotic salmonids (Pascual et al., 2007), and climate change (Aigo et al., 2008; Cussac et al., 2009).

Our results demonstrate that early morphological variation and phenotypic plasticity observed in *P. trucha* are two possible causes for the shape variation observed in the wild (Cussac et al., 1998; Ruzzante et al., 1998, 2003, 2011). The taxonomical value of morphology lies on its heritability, i.e., the higher the phenotypic plasticity the lower the taxonomical value of morphology will be. Considering both the molecular results (Ruzzante et al., 2006, 2011) and the natural and diet induced morphological variation recorded here, we conclude that the taxonomy of the genus needs an urgent revision.

Phenotypic plasticity allowed *P. trucha* to explore different trophic resources (Logan et al., 2000) and occupy different habitats (Ruzzante et al., 1998) in the species-poor lakes of Patagonia. Thus, phenotypic plasticity is a major factor affecting the past and present evolutionary success of *P. trucha*. The low number of species

within the genus should be considered in adequate management guidelines for species conservation.

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