Rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) develop a more robust body shape under organic rearing

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Abstract

Prolonged exposure to captive conditions has led to the development of a rainbow trout 'farmed' phenotype, which is different from that of wild trout. Selection for desirable productive traits in hatcheries has resulted in the development of some morphological traits that are maladaptive in nature. The recent development of organic aquaculture, guided by the well-being of the fish, could potentially produce a new farmed phenotype that would be more adaptive in nature. In this study, rainbow trout reared in intensive and organic farms were compared by means of shape analysis, to detect patterns of shape variation associated with rearing environment. The results of this study highlight a significant effect of the rearing method on rainbow trout shape: organically reared trout showed a higher body profile, in particular in the head and trunk regions, shorter median fins and a deeper caudal peduncle. A combined effect of density and habitat complexity could have contributed to the observed shape differences: in organic rearing systems, lower densities and steady water could increase territoriality and aggressive interactions, promoting body designs more functional in rapid attacks and escapes.

Keywords: domestication, organic aquaculture, rainbow trout, shape analysis

Introduction

Rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) are one of the most commonly cultured species in Europe and North America due to their adaptability to artificial rearing, and have been selected for aquaculture purposes for more than one century (MacCrimmon 1971). They were introduced to Europe at the end of the 19th century from their native range (Western United States, Alaska and Kamchatka) (MacCrimmon 1971). The prolonged artificial selection for desired traits (i.e. enhanced growth rate and late sexual maturity – Thorpe 2004) carried out throughout the last century has led to the development of a 'farmed type', i.e. domesticated strains sharing phenotypic (e.g. morphological, physiological and behavioural) traits different from the pristine wild ones (Jonsson & Jonsson 2006). Similar phenotypic differences between wild and cultured fish have been observed also in several other salmonids. The environments experienced by cultured and wild animals are, indeed, rather different (Price 1999; Waples 1999) and could generate different phenotypes. Furthermore, reproduction of cultured fish is controlled by man and, thus, competition for mates is avoided. Due to these hatchery environments, farmed fish face different selective pressures than their wild counterparts (Waples 1999; Fleming & Gross 1993): natural selection is generally weaker in captivity than in the wild, whereas artificial selection can be very strong on certain life-history traits (Wedekind, Rudolfsen, Jacob, Urbach & Müller 2007). Disturbances by human treatments and elevated rearing densities lead to high numbers of social encounters and increased stress and aggression levels in farmed trout (Hungtinford 2004). Phenotypic

differences among wild and hatchery fish are, at least in part, genetically inherited (Swain, Riddell & Murray 1991; Einum & Fleming 1997; Fleming & Einum 1997; Hard, Winans & Richardson 1999; Garant, Dodoson & Bernatchez 2003; Metcalfe, Valdimarsson & Morgan 2003; Wessel, Smoker & Joyce 2006), and fitness of hatchery-reared fish can decline rapidly in salmonids (Araki, Cooper & Bluoin 2007; Araki, Berejikian, Ford & Bluoin 2008; Blanchet, Páez, Bernatchez & Dodson 2008). Hatchery practices can cause maladaptive evolutionary changes in morphological, behavioural and physiological traits (Fleming, Jonsson & Gross 1994; McDonald, Milligan, McFarlane, Croke, Currie, Hooke, Angus, Tufts & Davidson 1998 Jonsson & Jonsson 2006; Brockmark & Johnsson 2010; Saikkonen, Kekäläinen & Piironen 2011). For example, traits associated with rapid growth in captivity, such as large body size and increased standard metabolic rate, are likely beneficial in hatcheries, where food is delivered in predictable locations, but might be selected against in natural food-limited habitats (Niva & Jokela 2000; Harwood, Armstrong, Metcalfe & Griffiths 2003; Vøllestad & Quinn 2003; Alvarez & Nicieza 2005). Furthermore, large body size correlates negatively with swimming performance (Reinbold, Thorgaard & Carter 2009) and ability to avoid predators (Berejikian 1995), and positively with aggressiveness (Lahti, Laurila, Enberg & Piironen 2001) and risk-taking behaviour (Johnsson & Abrahams 1991).

The recent rapid rise of salmonid farming has led to several criticisms, largely related to potential impacts of intensive net-pen operations on local ecosystems (Einum & Fleming 1997; Fleming, Hindar, Mjølnerød, Jonsson, Balstad & Lamberg 2000; Krkošek, Lewis, Volpe & Morton 2006) and on living marine resources, which account for high proportions of fish meals and oils in the feed (Tacon 2005). Increasing demands for wholesome and environment-friendly products by consumers have increased interest in 'organic aquaculture', which seeks to combine best environmental practices, preservation of natural resources and high animal welfare standards (EC 834/2007). Husbandry practices, including feeding, system design, stocking densities and quality should ensure that the developmental, physiological and behavioural needs of animals are met (i.e. animals should have access to adequate space, fresh air, outdoors, daylight and shade, and should be free to express their natural behaviour – EC 710/2009). The exposure to a new environment, with different spatial and hydrodynamic characteristics, and lower densities, could result in a new, more natural 'farmed phenotype'.

The aim of this study was to investigate shape differences among intensively and organically reared rainbow trout using an integrated approach for shape analysis combining Geometric Morphometrics (Bookstein 1991) and Euclidean Distance Matrix Analysis (Lele & Richtsmeier 1995, 2001).

Materials and methods

Sampling sites

A total of 645 rainbow trout females were collected in five fish farms: two intensive (named as INT1 and INT2), and three organic (named as ORG1, ORG2 and ORG3), varying in several features (type of ponds, surface and volume of the

Table 1 Characteristics of the farms where fish were collected (in grey organic ones)

	Facility	Certification body	Pond	Surface*	Volume[†]	Water flow[‡]	Temperature[§]	Density[‖]
INT1	Italy (Abruzzo)		Rectangular raceways	800 × 0.7	560	50–100	10–10.5	50–55
INT2	Italy (Piemonte)		Rectangular raceways	1000/1300 × 0.5	500–650	100	12.5	35–40
ORG1	Italy (Lazio)	Naturland	Squared earth ponds	800/1300 × 0.8	650–1150	50–100	10.5–11	12–15
ORG2	Italy (Piemonte)	Ecocert	Rectangular earth ponds	600/1000 × 0.6	300	300	10–10.5	12
ORG3	Switzerland	Biosuisse	Rectangular, vegetated earth ponds	720 × 0.6	430	100	8	12

** Surface = m2 × m; † Volume = m3; ‡ Water flow = l×s^{-1}; § Temperature = °C; || Density = kg×m^{-3}.

rearing tanks, water flow, temperature and fish density; Table 1). The three organic farms followed the standards for organic production developed by the Naturland, Ecocert and Biosuisse certification bodies respectively. As juveniles from organic broodstock are unavailable, intensive hatcheries provide organic farms with non-organically produced juveniles. Thus, individuals shared the same rearing conditions until they attained the weight of about 10 g (water = ~10°C; dissolved oxygen (DO) = 12 ppm; density: ~13 kg · m^{-3}). The ORG1 fish originated from INT1 and ORG2 from INT2. The ORG3 has its own hatchery, where the same standard rearing conditions were applied for all fish. The grow-out phase was then completed in the five above-mentioned farms.

Different strains were sampled in each farm (Table 2): Italian fish were collected from all the farms except ORG3, French fish were collected from INT1, Spanish fish from INT2, American fish from ORG2 and Swiss ones from ORG3.

All these strains originally derived from native American rainbow trout, extensively introduced from the USA to various European nations during the early days of introductions (1880–1900). *O. mykiss* was first introduced in Italy in 1895 (Gandolfi, Zerunian & Torricelli 1991; Bianco & Ketmaier 2001). The strains used in this study have been maintained in isolation by farmers since the 1980s (M. Grispan & A. Fuselli pers. comm.). Successful introductions occurred in France in 1829 (Welcomme 1988; Lever 1996; Keith & Allardi 1998). However, the strain analysed in this study was not a pure French strain, as it was originated from a recent crossing between Brittany (France) and American strains (Washington, USA) (M. Grispan, pers. comm.). The Spanish strain originated from the first introductions of rainbow trout from North America (1880–1900; Welcomme 1988; Elvira 1998), whereas the Swiss strain came from several early

intra-European transfers of rainbow trout from German hatcheries (Welcomme 1988; Koli 1990; Holcík 1991; Frier 1994; Lever 1996; Flajshans, Linhart & Slechtová 1999; Blanchet, Loot, Grenouillet & Brosse 2007).

Finally, American trout were purchased as eyed eggs from the Troutlodge facility (Soap Lake, Washington, USA), developed from strains originating in the Pacific Northwest USA.

After capture, fish were immediately anaesthetized and killed with a lethal dose of 2-phenoxyethanol, and total length (TL) was recorded (Table 2).

Shape analysis

On each specimen, 13 landmarks (Fig. 1) were digitized using TpsDig2.10 software (Rohlf 2006). To ensure overall shape coverage (Zelditch, Swiderki, Sheets & Fink 2004), three outline curves were recorded on each specimen. The beginning and end of each outline corresponded: 1) to the anterior tip of premaxilla and the origin of dorsal fin; 2) to the insertion of the dorsal fin and the origin of adipose fin; 3) to the insertion of the anal fin and the insertion of the operculum on the ventral profile. A series of two, one and three equally spaced semi-landmarks (Zelditch *et al.* 2004; Perez, Bernal & Gonzalez 2006), respectively, were automatically chosen along each curve using TpsDig.

Semi-landmarks were iteratively allowed to slide along the outline curve to minimize the bending energy of the thin-plate spline interpolation function computed between each specimen and the sample Procrustes average. This procedure was carried out using the algorithm of Bookstein (Gunz, Mitteroecker & Bookstein 2005; Gunz & Harvati 2007) that allows points to slide along tangents to the outline in the so-called Spline-relaxation

Table 2 Genetic origin, number (*n*) and total length (mean ± standard deviation) of rainbow trout collected for this study. Data about year of introduction of *Oncorhynchus mykiss* for rearing and source populations are from MacCrimmon (1971) & Crawford and Muir (2008)

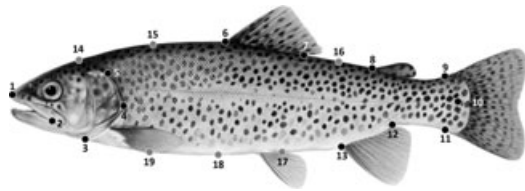| Farm	Origin	Year of introduction	Source	*n*	TL ± SD
INT1	Italy	1895	USA	46	28.7 ± 3.2
	France	1829	USA	120	30.1 ± 4.4
INT2	Italy	1895	USA	45	33.5 ± 4.7
	Spain	1890	France	78	12.6 ± 1.1
ORG1	Italy	1895	USA	111	31.7 ± 3.4
ORG2	Italy	1895	USA	30	24.7 ± 4.4
	USA			138	25.5 ± 3.1
ORG3	Switzerland	1888	Germany	77	21.6 ± 2.7

Figure 1 Landmarks (black circles) and semi-landmarks (grey circles) collected on rainbow trout specimens. (1) Anterior tip of premaxilla; (2) most posterior point of maxilla; (3) insertion of the operculum on the ventral profile; (4) most posterior point of the operculum; (5) uppermost point of the operculum; (6) origin and (7) insertion of the dorsal fin; (8) origin of adipose fin; (9) anterior attachment of dorsal membrane from caudal fin; (10) base of middle caudal fin rays; (11) anterior attachment of ventral membrane from caudal fin; (12) origin and (13) insertion of anal fin; (14–15) semi-landmarks collected along the anterior dorsal profile; (16) semi-landmark collected along the posterior dorsal profile; (17–19) semi-landmarks collected along the ventral profile.

procedure. These tangents were approximated for each semi-landmark by converting the vector between the two neighbouring points to unit length. Spline-relaxation removes the effects of 'digitizing error' in the tangent direction that results from the practical necessity of placing the semi-landmarks somewhere along the curve. After relaxation, these semi-landmarks can be treated in multivariate analyses as if they had been homologous points in the first place (Gunz *et al.* 2005; Gunz & Harvati 2007). The landmarks and sliding-landmarks were converted to shape coordinates using Procrustes superimposition (GPA, Rohlf & Slice 1990). This procedure removes information about location and orientation from the raw coordinates and standardizes each specimen to unit centroid size (CS), a size-measure computed as the square root of the summed squared Euclidean distances from each landmark to the specimen's centroid. Residuals from the registration were analysed using the thin-plate spline (TPS) interpolating function (Bookstein 1991), producing principal warps.

The configurations of the specimens into the principal warps space (the so-called partial warps) were analysed using a principal component analysis (PCA), to display the major features of shape variation. To highlight shape variation related to differences among lots, a PCA was computed for the farms (INT1, INT2 and ORG 2)

where more than one genetic strain was sampled. The normality of distribution of PCA scores was tested (Shapiro-Wilk's test), and, subsequently, a Student's t-test was performed to test for the significance of difference between the scores of each lot along PC1 and PC2.

A comprehensive PCA was also performed for all the lots sampled, pooled in intensively and organically reared lots, to investigate possible shape differences related to rearing approach.

To better visualize shape variation related to rearing condition, a Discriminant Analysis (DA), computed on the weight matrix (the matrix of partial warp scores, together with the uniform component, for a sample of shapes – Bookstein 1991) was performed. Discriminant Analysis is a supervisionate analysis that starts with an initially defined grouping of objects and tries to determine to which extent a set of quantitative descriptors can efficiently explain this grouping (Legendre & Legendre 1998). In this case, individuals were grouped relative to the rearing method (intensive or organic). In addition, the procedure implemented in MorphoJ carries out a leave-one-out cross-validation, based on resampling (10000 permutations), to assess the statistical reliability of classifications (Weiss & Kulikowski 1991). Splines relative to the extreme positions of the canonical axes were computed (tps Regr; Rohlf 2000), so that shape differences could be visualized and discussed. All these analytical steps were computed using MorphoJ software (Klingenberg 2007).

Morphometrics softwares of the Tps series are freely available at: http://life.bio.sunysb.edu/morph/.

MorphoJ is freely available at: http://www.flywings.org.uk/MorphoJ_page.htm.

Euclidean Distance Matrix Analysis (EDMA) is a free-coordinate method independent of the position of the specimens in the space. This analysis was performed to test and localize morphological differences between intensively and organically reared fish. Each form was represented by a matrix showing all the distances between each pair of landmarks. Forms were compared by producing a Form Difference Matrix (FDM) in which each interlandmark value was represented by the ratio between the interlandmark distance of the first form and the interlandmark distance of the second (Richtsmeier, Cheverud & Lele 1992). Similar ratios throughout the matrix suggest isometry, whereas different ratios reflect changes in form.

Interlandmark distances exceeding 2 standard deviations from the mean value of the whole Form Difference Matrix (FDM) were considered as determinant interlandmark distances. Landmarks with a median distance value exceeding 5% of the average median value were considered influential landmarks. The mean or median value of all the interlandmark differences represents the average difference in size between the two forms. The significance of the shape differences was tested with the T statistic (Lele & Richtsmeier 1991). As no tabled critical values exist for this statistic, a bootstrapping procedure (Efron & Tibshirani 1993; Davison & Hinkley 1997) was used to assess the significance of the T value. The analysis was performed using the software WinEDMA (Cole 2002; freely available at: http://oshima.anthro.psu.edu).

Although Geometric Morphometrics techniques provide clear graphic outputs, such as deformation grids and splines, the output of EDMA is a list of interlandmark distances, which is complicated to visualize. One recently developed visualization approach is to map the average interlandmark distances on two dimensions using functions aimed at describing the changes from one point to another, interpolating the variations along the surface. The differences between the Euclidean distance matrixes from the configurations of the different fish lines (pairwise comparisons) were mapped using a precise function (inverse to the power of two). Precise functions are forced to exactly pass through the value at each point. Maps were computed using Surfer 7.0 (Golden Software, Inc).

Results

Shape analysis

Shape differences within facilities

Principal component analysis was performed for INT1, INT2 and ORG2, farms where more than one rainbow trout strain was available. Except for INT2, no shape differences related to origin were detected along the first axis (PC1) (Fig. 2). This was confirmed by the Student's t-test results: $t_{INT1} = 1.05$, $p_{INT1} = 0.29$; $t_{ORG2} = 1.42$, $p_{ORG2} = 0.16$. A significant difference between means of French and Italian strains (INT1) was detected along PC2: $t_{INT1} = 6.49$, $p_{INT1} < 0.001$. A deep morphological differentiation was evident between the Italian and Spanish strains (INT2):

$t_{INT2} = 18.33$, $p_{INT2} < 0.001$. However, a highly significant correlation between PC1 and size (expressed as centroid size, CS) was detected (Spearman's $R = -0.78$; $P < 0.0001$). Therefore, these shape differences were mainly due to size variation among the two lots, i.e. the Spanish lot was significantly smaller than the Italian one ($U = 8.17$; $P < 0.001$). The comprehensive analysis was thus performed on a reduced sample of 567 specimens, excluding the fish from the Spanish lot (INT2).

Shape differences between intensively and organically reared fish

Even if the effect of genetic differences between lots was excluded within each facility, a potential interaction between genetic background and rearing method could not be completely excluded, as different strains were used in the two different treatment groups (intensive and organic). Thus, a preliminary Principal Component Analysis (PCA) was performed taking into account only the Italian strains, available in both rearing conditions. Individuals collected in organic and intensive farms were significantly ($t = -2.63$; $P < 0.01$) differentiated along PC1 (25.8%). As visualized by the splines relative to the extreme configurations observed along PC1, a more streamlined body shape and narrower caudal peduncle were characteristic of intensive rearing conditions. On the other hand, organically reared specimens were characterized by a higher body profile, in particular in the head and trunk regions, shorter median fins and a deeper caudal peduncle (Fig. 3).

The same analysis was performed on the entire sample, pooling lots depending on the rearing method, to investigate if the overall pattern of shape variation was consistent with that observed for Italian lots. The first two PCA axes accounted for 42% of the overall variance. Along PC1 (24.7%) specimens, even if widely overlapped, tended to separate using rearing method: the positive extreme of the axis was almost exclusively populated by intensively reared fish, and the negative extreme by organically reared ones (Fig. 4a). The Student's t-test revealed highly significant differences between the means of both the PC1 ($t = 11.18$; $P < 0.0001$) and PC2 ($t = -3.52$; $P < 0.001$) scores of the two groups.

As visualized by the splines in figure 4, shape variation between intensive and organic lots was consistent with that for Italian samples. Along the
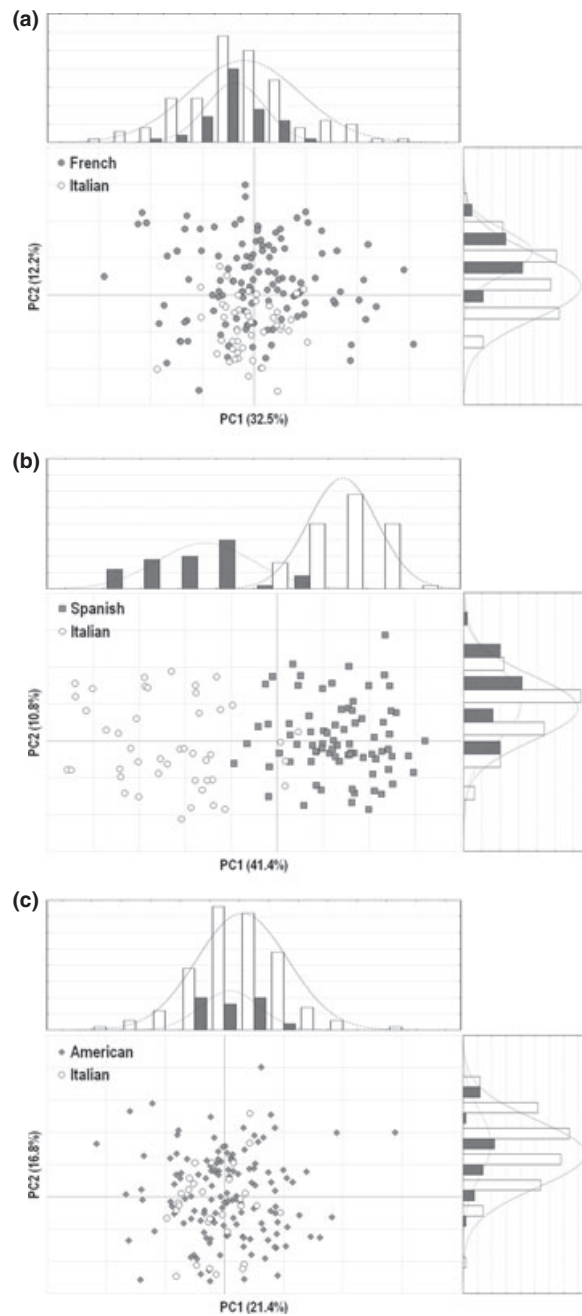
Figure 2 Principal component analysis ordination plot for (a) INT1, (b) INT2 and (c) ORG2. Histograms show scores distribution along PC1 and PC2 for each lot of different origin.

discriminant axis, the two clusters were significantly separated ($T^2 = 630.340$; $P < 0.0001$) (Fig.4b). The percentages of correct classification after leave-one-out cross-validation (no. of permutations = 10,000) (Table. 3) were always greater than 80%, indicating a good separation of the groups in relation to shape descriptors. The overall grand mean (± St. Dev.) for the organic sample was 1.05 ± 0.02, indicating that the organically reared configuration was on average 5% larger than the intensively reared one. Points showing a larger median ratio in organically reared speci-
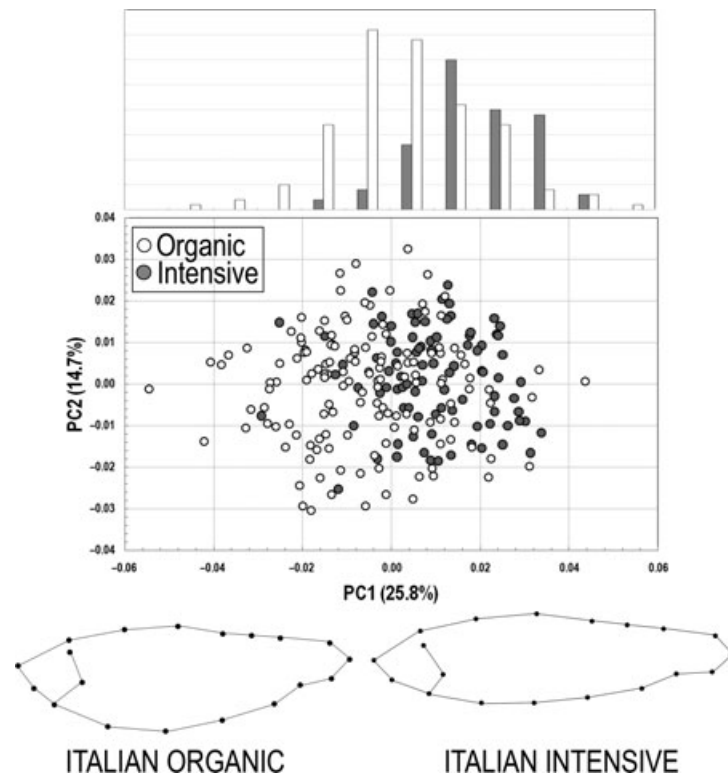
Figure 3 Principal component analysis ordination plot for Italian strains collected in organic (ORG1 and ORG2) and intensive (INT1 and INT2) farms. Histogram shows scores distribution along PC1 for specimens reared under the two rearing methods. Splines relative to shape configurations at the extremes of PC1 are reported.

mens (that is, points included in interlandmark distances generally longer in the organically reared configuration) were those landmarks located on the head, in particular the insertion of the operculum on the ventral profile (3), the most posterior point (4) and the uppermost point (5) of the operculum, and landmarks in the posterior dorsal profile (9, 10). Conversely, landmarks proximal to the dorsal (7), adipose (16, 8) and anal fins (12, 13) showed the smallest values, thus they were closer to the rest of the profile when compared with the intensively reared configuration. The mean values at each landmark can be mapped using a precise function, as described above. These functions take into account the mean values at each point using a chromatic scale to mark areas of average longer/shorter interlandmark distances, then using these values to interpolate the differences along the surface of the geometrical model. This approach can be used to visualize the information of the Form Difference Matrix (Fig. 5).

Discussion

We found evidence for morphological differences between rainbow trout reared in a traditional intensive aquaculture system and with a novel organic approach. A relationship between shape and genetic origin of lots cannot be completely ruled out in this study, as different genetic strains were available for the comparison for the two rearing methods. However, the analysis carried out on only the Italian strains, available in both intensive and organic farms, showed results consistent with those obtained for the overall sample, allowing us to attribute the shape variation to rearing methodology. Such shape variation is apparently consistent with the observations of several other studies comparing wild and hatchery salmonid species (Fleming & Gross 1989; Fleming *et al.* 1994; Hard, Berejikian, Tezak, Schroder, Knudsen & Parkerd 2000; Wessel *et al.* 2006). A more streamlined body with a narrower caudal peduncle appears to be characteristic of fish cul-
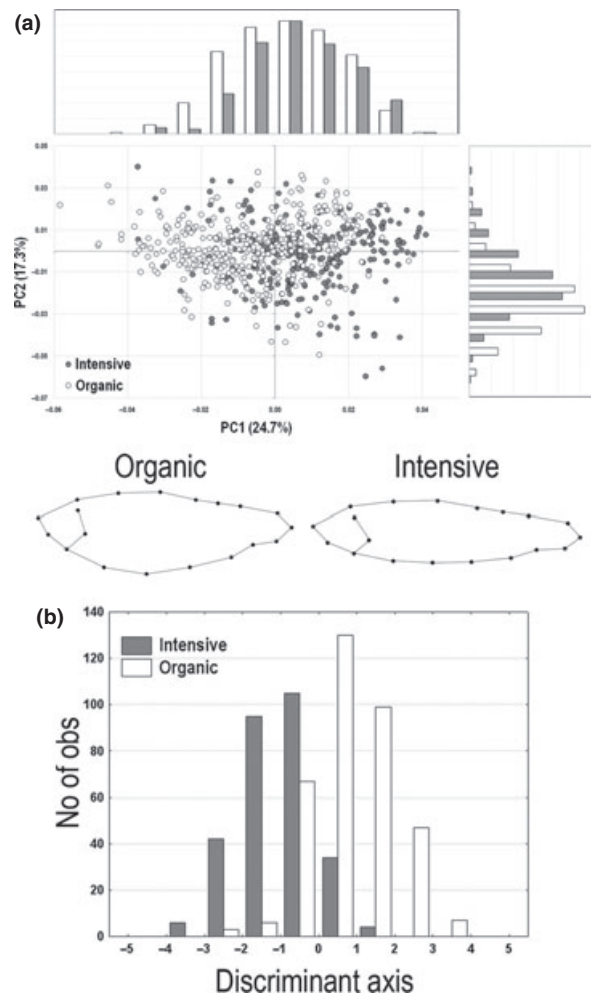
Figure 4 (a) Principal component analysis ordination plot for intensively vs. organically reared specimens. Histograms of scores distributions along PC1 and PC2 are displayed. Splines relative to shape configurations at the extremes of PC1 are reported. (b) Histogram of the scores along the discriminant axis for the grouping variable 'rearing approach'.

tured in raceways, where water flow is one-way and constant, whereas a higher body profile with a deeper peduncle characterizes wild fish. However, morphological adaptive response of salmonids to rearing environment is still not clear: several studies have attempted to identify morphological divergence between hatchery and wild salmonids, without finding any consistent differences (Taylor 1986; Sundell, Dellefors & Björnsson 1998; Swain *et al.* 1991; Fleming *et al.* 1994; Fleming & Einum 1997; Fleming & Gross 1989; Hard *et al.* 2000; Poole, Nolan, Wevers, Dillane, Cotter & Tully 2003; Fiske, Lund & Hansen 2005; von Cramon-Taubadel, Ling, Cotter & Wilkins 2005; Wessel *et al.* 2006; Nichols, Edo, Wheeler &

Thorgaard 2008). Although the above-mentioned findings vary greatly by species, developmental stage and rearing conditions, a functional explanation of our findings can be proposed. The rainbow trout is a body and caudal fin (BCF) generalist (Webb 1977, 1988), and its body shape is the result of a trade-off between the need to sustain steady swimming during migrations while also giving a fast-start performance to escape from predators and catch prey. As the locomotor systems for these two types of swimming in rainbow trout are the same (coupled locomotor systems – see Blake 2004), increasing the performance in one causes a trade-off in the other. Both fast-start ability and steady swimming are dependent on

Table 3 Discriminant analysis classification/misclassification table after cross-validation

True	Allocated to			
	Intensive	Organic	Tot	%*
Intensive	196	15	211	92.9
Organic	64	292	356	82.0

*Percentages of correct classification.

body form. The optimal functional design for steady swimming maximizes thrust and minimizes drag, and is characterized by a lunate caudal fin, joining the body via a narrow caudal peduncle, and a relatively rigid streamlined body shape (Wu 1971; Lighthill 1975; Webb 1975, 1984; Blake 1983). Fast-start performance, however, is optimized by large body depth and area, in particular in the caudal region, by a flexible body and by a large muscle mass relative to body mass (Webb 1984).

Wessel *et al.* (2006) found hatchery Chinook salmon to be more dorso-ventrally compressed, with narrower head and caudal peduncle than wild fish. They hypothesized these differences may be adaptive, as a deeper and more robust body, such that of wild fish, may be optimal for rapid bursts and, thus, advantageous for predator avoidance and food capture in nature. However, relaxed selection in hatcheries, together with invariant water flows, could shape morphology towards a design optimal for prolonged swimming against counter-current movements in raceways. Differences in predator pressure or prey capture cannot bias our results, as both the intensive and organic environments are captive. However, organic ponds had variable water flows, with some areas having high water flow while other having nearly steady water. Such a hydrodynamic pattern results in a differential food distribution, which could select for phenotypes better adapted to rapid manoeuvres and acceleration. This could result in a shift from the generalist cruiser design towards one better adapted to fast-start propulsion.

The strong territoriality of salmonids is also a key factor related to morphology. Salmonids actively compete to monopolize and defend a favourable territory (Grant & Kramer 1990). Such intra-specific interactions are density-dependent and affected by habitat complexity (Deverill, Adams & Bean 1999; Jenkins, Diehl, Kratz & Cooper 1999; Bohlin, Sundström, Johnsson, Höjesjö & Pettersson 2002; Sundström, Bohlin & Johnsson 2004). The inhibitory effect of high densities experienced in intensive rearing systems on intra-specific competition has been demonstrated for Artic charr (*Salvelinus alpinus* – Brown, Brown & Srivastava 1992), which initiated significantly fewer agonistic interactions and spent significantly more time shoaling at high densities. Habitat complexity reduced aggression levels, promoted visual isolation and reduced territory size in Atlantic salmon (*Salmo salar*) (Blanchet, Dodson & Brosse 2006). Aggressive interactions have been
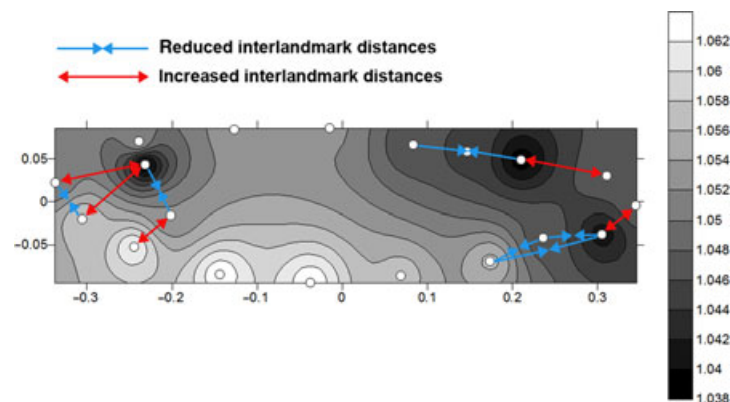

Figure 5 Euclidean Distance Matrix Analysis comparison between rainbow trout reared in intensive and organic farms. Form Difference Map shows the interpolated values (half configuration) using the average ratio for each landmark (dark: average lengthening in the organically reared specimens; light: average shortening. Interlandmark distances exceeding 2 standard deviations from the mean value of the whole FDM are reported in red, those lowering 2 standard deviations are reported in azure.

observed to increase in pools relative to riffles, were aggression is reduced due to environmental complexity structured by water velocity. Therefore, a combined effect of density and habitat complexity could have contributed to the observed shape differences: in organic rearing systems, lower densities and steady water could increase territoriality and, therefore, aggressive interactions, promoting body designs more functional in rapid attacks and escapes.

Some authors found the pattern of morphometric variation in salmonids to associate with genetic differences among the stocks (Swain *et al.* 1991; Hard *et al.* 1999; Wessel *et al.* 2006; Keeley, Parkinson & Taylor 2007), whereas others attributed morphological differences between wild and hatchery populations primarily to environmental effects (Taylor 1986; Fleming *et al.* 1994; Hedenskog, Petersson, Jarvi & Khamis 1997; Hard *et al.* 2000; von von Cramon-Taubadel *et al.* 2005). In this study, we cannot exclude the interaction between genetics and rearing methods as a factor influencing shape, because different strains were available for the two rearing conditions. However, two observations allow us to conclude that rearing method significantly affected rainbow trout shape: (1) different strains collected in the same farms showed similar morphologies (Fig. 2), and (2) the same Italian strain reared in intensive and organic farms showed shape differences consistent with those detected for the combined lots (Fig. 5). Furthermore, the 'organic' morphology was achieved within a single generation, as the organic facilities do not have a broodstock and are continuously supplied from intensive hatcheries. Therefore, the observed morphological modifications appear to be solely environmentally induced, without any genetic basis.

However, morphology is just one of the phenotypic modifications induced by domestication, and the effect of organic aquaculture on the suppression of the anti-predator behaviour (Berejikian 1995; Reisenbichler & Rubin 1999), reduction in the reproductive success (Berejikian & Ford 2004), impoverishment of sensorial skills (Marchetti & Nevitt 2003), and other causes of the lower fitness of hatchery fish in nature (Lynch & O'Hely 2001; Ford 2002; Frankham, Briscoe & Ballou 2002) must be investigated. More studies are needed to investigate the effect of semi-intensive/organic aquaculture on other rainbow trout phenotypic traits, i.e. aggressive, agonistic, and anti-predator behaviour, growth rate, swimming performance, and to experimentally test the relative fitness of organically and intensively reared individuals in the wild.

Acknowledgments

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