



Rapid morphological divergence of cultured cod of the northwest Atlantic from their source population

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ABSTRACT: The performance of aquaculture escapees in the wild depends in part on how their morphology differs from that of wild fish. We compared farmed Atlantic cod *Gadus morhua* morphology to that of wild cod from the same ancestral population. Traditional and geometric morphometrics showed that farmed cod had relatively smaller fins, heads, eyes, and jaws than wild cod for a given size. Conversely, drumming muscle size and metrics of body and liver condition were greater in farmed fish. As the observed differences are likely due to phenotypic plasticity, their fitness consequences for escaped farmed fish may be transient.

KEY WORDS: Wild–farmed interactions · Geometric morphometrics · Farmed phenotype · Aquaculture escapes

INTRODUCTION

Fish exposed to culture develop phenotypes that differ from those of their wild counterparts (Fleming & Gross 1994, Araki et al. 2008, Bailey et al. 2010, Chittenden et al. 2010). The phenotypes they develop may be beneficial under culture but may reduce the fitness of an individual when exposed to another environment (e.g. the wild environment following escape). These cultured phenotypes can be the product of a plastic response whereby different phenotypes can be expressed by a single genotype in response to different environmental conditions (Imre et al. 2002, Skjæraasen et al. 2008, Mayer et al. 2011, Vehanen & Huusko 2011), or these phenotypes may be the result of genetic changes brought about through both intentional and unintentional selection (Fleming et al. 1994, Einum & Fleming 2001, Fleming & Petersson 2001, Hutchings & Fraser 2008, Solberg et al. 2013). The degree of phenotypic

change, and its permanence, are both a function of the time an individual has spent in captive conditions (Pakkasmaa et al. 1998, von Cramon-Taubadel et al. 2005), as well as the degree of genetic change from the ancestral lineage due to captivity (Fleming et al. 1994, Blanchet et al. 2008, reviewed by Hutchings & Fraser 2008, Fraser et al. 2010). Thus, if it is presumed that the phenotypes of wild fish are the product of adaptation to their local environment, then the degree to which the phenotype of cultured fish diverges from the wild type is likely a reflection of how maladaptive the cultured phenotype may be if exposed to the wild environment. Furthermore, the 'permanence' of the cultured fish's phenotype, or the degree to which phenotypic plasticity allows it to (re)converge on a wild-type phenotype over time at liberty, may result in a lifetime fitness difference between the 2 groups that is lower than would be predicted based on morphological differences at the time of escape.

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Through programmes that sought to diversify the Canadian aquaculture industry, experimental Atlantic cod *Gadus morhua* broodstocks were created from wild-caught fish, and their offspring were stocked to commercial cage aquaculture farms. These first-generation farmed cod afforded us the unique opportunity to study the morphological effects of exposure to the aquaculture environment on fish that had not experienced the intensive selection regimes common in more established species (e.g. Atlantic salmon *Salmo salar*). We compared the morphology of wild cod to farmed individuals created from wild-caught parents that were genetically similar to our wild fish. We then discuss the differences in morphology in terms of potential fitness effects on escapees in the wild.

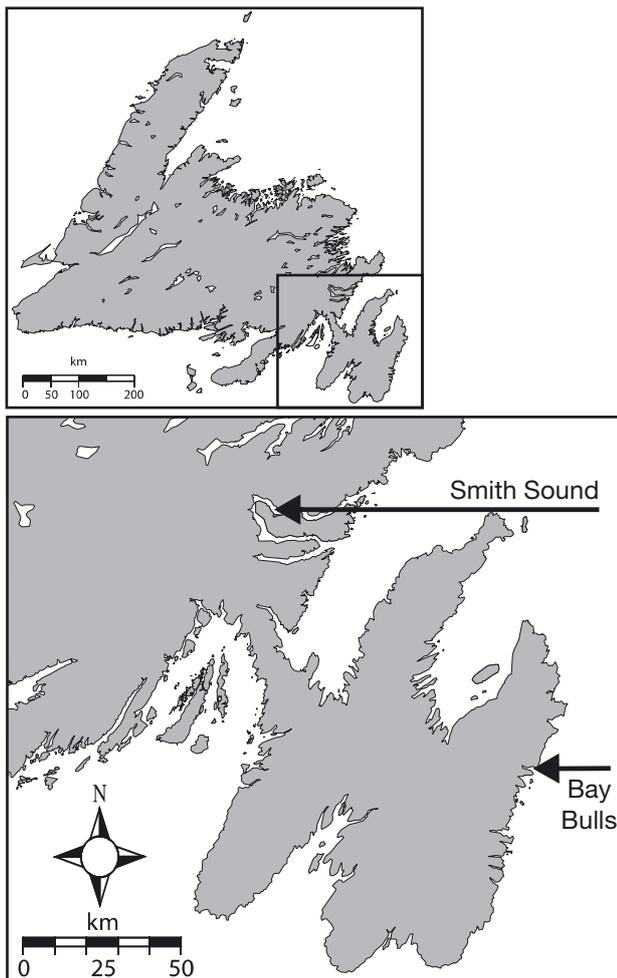


Fig. 1. Island of Newfoundland, showing the locations of sample collection (Bay Bulls: 47° 18' N, 52° 48' W; Smith Sound: 48° 9' N, 53° 44' W)

MATERIALS AND METHODS

Data collection

Farmed cod were the progeny of wild-caught fish from Bay Bulls, Newfoundland, Canada (47° 18' N, 52° 48' W; Northwest Atlantic Fisheries Organization [NAFO] division 3L; Fig. 1), which were spawned between December 2006 and March 2007. The farmed cod were reared in tanks at Memorial University from fertilization until they were transferred en masse to Sapphire Sea Farms' net-pen facility in Bay Bulls on 30 November 2008. Some of them ($n = 112$) were sampled between 4 and 9 November 2009 during the annual harvest.

Wild cod were captured using baited cod pots on 10 and 20 November 2009 ($n = 38$ and $n = 19$, respectively) in Smith Sound, Newfoundland (48° 9' N, 53° 44' W; NAFO division 3L; Fig. 1). Cod of Smith Sound and Bay Bulls are thought to be of the same stock, being genetically similar (Beacham et al. 2002, Bradbury et al. 2010, Rose et al. 2011). The wild fish were held in a tank and measured 2–3 wk after collection. The farmed and wild cod were held without feeding prior to measuring to ensure that gut contents did not bias weight or shape measure, and only fish free of obvious skeletal defect were included in the analysis.

After being killed, fish were kept on ice before being arranged left side up, with their median and caudal fins extended and pinned in place, and photographed with a digital camera (Nikon D300) mounted on a tripod. A ruler was included in each photograph to allow for size calibration.

After photographing, the right and left pelvic fin lengths (distance from the origin of the fin to tip of the longest fin ray) were measured (± 0.01 cm) with digital callipers because they could not be measured from the photographs. Fish were weighed whole (± 0.01 g), sexed when the internal organs were removed, and the liver was weighed separately (± 0.01 g). Following the protocol of Rowe & Hutchings (2004), both the right and left drumming muscles were removed and frozen, before being dried to constant mass and weighed together (± 0.001 g).

Eighteen landmarks were recorded as x - y coordinates from the photographs using ImageJ (Schneider et al. 2012; <http://rsb.info.nih.gov/ij/download.html>; Fig. 2). Standard lengths were measured as the distance between the anterior-most point of the premaxilla and the posterior-most edge of the hypural plate (points 1 and 8 respectively on Fig. 2). The dorsal and anal fin lengths and widths were measured as

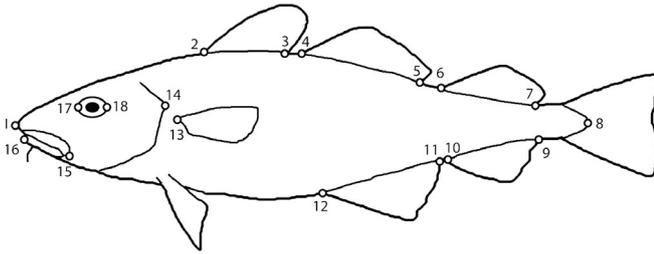


Fig. 2. Landmark points recorded on Atlantic cod *Gadus morhua*; 1: Anterior-most point of premaxilla; 2: origin of first dorsal fin (DF1); 3: insertion of DF1; 4: origin of second dorsal fin (DF2); 5: insertion of DF2; 6: origin of third dorsal fin (DF3); 7: insertion of DF3; 8: posterior-most point of hypural plate; 9: insertion of second anal fin (AF2); 10: origin of AF2; 11: insertion of first anal fin (AF1); 12: origin of AF1; 13: origin of pectoral fin; 14: posterior-most point of operculum; 15: posterior-most point of maxilla; 16: anterior-most point of dentary; 17: anterior-most point of the eye; 18: posterior-most point of the eye directly across from point 17

the distance from the fin origin to the tip of the second fin ray, which was the longest, and as the distance along the fin base from its origin to its distal insertion, respectively (Fig. 2). Unforeseen variation in fin attitude and extension prevented measurement of the size of the left pectoral fin from the digital photographs. A small number of farmed fish (10 of 112) showed malformed fins, and measurements of these fins were excluded from the analysis.

Size standardization and calculation of condition indices

Size standardization was employed so that only relative differences in trait size between the 2 origins (i.e. wild or farmed) were considered. The lengths and widths of the dorsal and anal fins, the lengths of the pelvic fins, and the weight of the drumming muscles were \log_{10} -transformed and then standardized using the method of Reist (1986). Each of these traits was standardized for each fish using the formula $M_{\text{std}} = M_{\text{obs}}(S_{z_{\text{mean}}}/S_{z_{\text{obs}}})^b$, where: M is the trait measure, S_z is the size measure to which samples are standardized, superscript b is the trait-specific common within-groups slope, and the subscripts mean, obs, and std refer to the mean, observed (raw), and the size-standardized measurements, respectively. The weight of the drumming muscles was standardized to a common body weight, while the length and width measurements were standardized to a common centroid size. The centroid size, the square root of the sum of the squared distances of each peripheral landmark (i.e. excluding points 13, 14, 17, and 18 in Fig. 2) to the centroid, was calculated in R (R Devel-

opment Core Team 2011) using the function `gpgen` (`geomorph` package; Adams & Otárola-Castillo 2013).

Condition indices (CI) were calculated for each fish by taking the standardized residuals of the regression of \log_{10} -transformed standard length on the \log_{10} -transformed total weight. The liver indices (LI) were calculated similarly from the regression of the \log_{10} -transformed weight of the liver on the \log_{10} -transformed total weight. The standardized residuals convey the condition status of each fish. Positive residuals indicate that the fish is heavier, or possesses a heavier liver for their size than the average, while negative residuals indicate the opposite.

Traditional morphometric, geometric morphometric, and statistical analyses

All statistical and geometric morphometric analyses were conducted in R (R Development Core Team 2011). The traditional morphometric analyses consisted of testing for differences in size-standardized drumming muscle mass, dorsal and anal fin lengths and widths, pelvic fin lengths, as well as CI, and LI individually between fish origins (i.e. wild or farmed) using a linear model with permutation (`lmp` function, `lmPerm` package; Wheeler 2010) and type III sums-of-squares (`Anova` function, `car` package; Fox & Weisberg 2011) with sex and origin as fixed effects. Using permutation removes the necessity that the data satisfy the assumptions of traditional parametric tests, and allows for the calculation of exact significance levels. The issue of multiple hypothesis testing was addressed by the use of adjusted p-values, with the false discovery rate set to $\alpha = 0.05$ (Benjamini & Hochberg 1995).

Principal component analysis (PCA), with varimax rotation (`prcomp` function, `stats` package; R Development Core Team 2011), was also conducted as part of the traditional morphometric analysis to reduce the number of parameters, using all morphometric measures listed in Table 1, with the exception of standard length, total weight, and drumming muscle mass. Standard length and total weight were excluded because they represent differences in fish size rather than shape (size-standardized). Drumming muscle mass was also excluded because it had missing values, which caused the sample size to drop appreciably. All principal components (PCs) with eigenvalues greater than the mean eigenvalue were considered significant (Jackson 1993).

Geometric morphometric analyses were conducted using the R packages `shapes` (Dryden 2013) and geo-

morph (Adams & Otárola-Castillo 2013). The x - y coordinates collected from the photographs of the fish were first converted to shape coordinates using generalized Procrustes analysis (GPA; Adams et al. 2004). GPA removes the non-shape aspects of size, (scaling), orientation, and location from the raw x - y coordinates, and also standardizes each individual to a common unit centroid size (Rohlf 1999, Adams et al. 2004).

The amount of shape variation attributable to the different origins of the fish (controlling for sex) was quantified using Procrustes ANOVA with permutation, which compares the observed sum-of-squared Procrustes distances to an expected distribution which is calculated through permutation (Goodall 1991). PCA was also conducted on the configuration of the specimens into principal warp space to detect the major features of the shape variation. Differences in PC scores between origins were tested using linear models with sex and origin as fixed effects.

RESULTS

Traditional morphometrics

No interactions were detected between sex and origin. Within origin, the size-adjusted dried mass of the drumming muscles was greater in males than in females (Table 1). However, females were bigger

and their LIs were greater than those of the males (Table 1). All size-adjusted morphometric measures, with the exception of the width of the first dorsal fin, differed significantly between wild and farmed cod (Table 1).

The first 4 PCs all had eigenvalues greater than the mean eigenvalue, and cumulatively explained 74.3% of the variation in traditional morphometric variables (Table 2). The loadings of wild and farmed fish on PCs 1 and 2 differed significantly (t -test, $p < 0.001$), while there was no significant difference on PCs 3 and 4 (t -test, both $p > 0.05$; Fig. 3; PC4 not shown).

The first PC, which explains 44.3% of the variation, was characterized by negative loading of the fin measures, particularly fin lengths (Table 2). PC2 explained 12.6% of the variation, and for the most part is described by positive loadings from CI, LI, and fin widths. Interestingly, on PC2, the fin widths showed moderate to strong positive loadings, while their lengths showed near-zero to moderately negative loadings (Table 2).

Geometric morphometrics

ANOVA with permutation on the Procrustes-aligned coordinates of the wild and farmed cod revealed that there was a significant interaction between sex and origin ($F_{1,140} = 6.112$, $p < 0.001$).

Table 1. Mean (\pm SD) morphometric measures and analyses by sex and farmed or wild origin Atlantic cod *Gadus morhua*. Standard length and weight measures are unstandardized; the calculation of condition index (CI) and liver index (LI) includes an inherent standardization. Drumming muscle weight has been standardized to a common weight, while all other measures have been standardized to a common centroid size. DM: combined dried mass of right and left drumming muscles; DF1, DF2, DF3: first through third dorsal fins; AF1 and AF2: first and second anal fins; PF: pelvic fins. There were no significant interactions between sex and origin for any of the measures. Adjusted p -values are shown, and those significant are in **bold** ($\alpha = 0.05$)

Measure	Farmed male (n = 45)	Wild male (n = 44)	Farmed female (n = 28)	Wild female (n = 19)	Sex		Origin	
					F	p	F	p
Standard length (mm)	419 \pm 40	484 \pm 60	435 \pm 33	516 \pm 51	10.69	0.016	113.63	<0.001
Weight (g)	1056 \pm 279	1377 \pm 409	1158 \pm 321	1583 \pm 367	8.16	0.027	55.01	<0.001
CI	0.10 \pm 1.13	-0.20 \pm 0.84	0.16 \pm 1.11	-0.21 \pm 0.66	0.04	0.837	4.85	0.033
LI	0.48 \pm 0.60	-1.15 \pm 0.72	0.75 \pm 0.54	-0.80 \pm 0.74	9.99	0.016	267.45	<0.001
DM weight (g)	0.23 \pm 0.07	0.18 \pm 0.06	0.20 \pm 0.09	0.12 \pm 0.05	8.33	0.020	3.39	<0.001
DF1 length (mm)	49.61 \pm 3.67	65.71 \pm 4.83	49.26 \pm 3.85	65.63 \pm 7.09	0.14	0.751	333.48	<0.001
DF1 width (mm)	67.70 \pm 4.39	66.20 \pm 5.41	66.89 \pm 4.38	67.44 \pm 4.74	0.19	0.751	0.11	0.737
DF2 length (mm)	45.35 \pm 3.09	53.82 \pm 3.38	44.13 \pm 3.83	54.3 \pm 2.51	2.15	0.411	205.52	<0.001
DF2 width (mm)	100.91 \pm 7.35	104.65 \pm 6.38	99.83 \pm 7.21	106.14 \pm 3.69	0.17	0.751	15.05	<0.001
DF3 length (mm)	42.80 \pm 3.69	53.60 \pm 4.40	42.18 \pm 3.66	52.76 \pm 3.31	1.14	0.699	211.18	<0.001
DF3 width (mm)	66.03 \pm 6.09	73.44 \pm 5.91	65.31 \pm 5.12	72.31 \pm 3.88	0.78	0.713	43.81	<0.001
AF1 length (mm)	44.23 \pm 7.87	52.98 \pm 6.32	42.61 \pm 3.52	55.15 \pm 7.40	0.47	0.751	73.60	<0.001
AF1 width (mm)	91.47 \pm 8.47	95.70 \pm 6.20	91.07 \pm 5.62	99.41 \pm 7.12	0.21	0.751	21.64	<0.001
AF2 length (mm)	38.09 \pm 3.01	50.04 \pm 4.83	37.53 \pm 2.44	50.84 \pm 3.26	0.22	0.751	437.14	<0.001
AF2 width (mm)	61.52 \pm 5.84	67.39 \pm 3.31	60.89 \pm 4.59	67.36 \pm 2.92	0.35	0.751	41.40	<0.001
Right PF length (mm)	47.47 \pm 4.41	62.10 \pm 6.05	45.99 \pm 5.09	58.61 \pm 6.16	5.10	0.087	177.45	<0.001
Left PF length (mm)	50.32 \pm 4.32	61.61 \pm 5.42	48.45 \pm 3.89	59.62 \pm 5.33	6.38	0.054	162.10	<0.001

Table 2. Percentage of explained variance, eigenvalues, and the loadings of the measurements included in the principal component analysis (PCA) (with varimax rotation) on the first 4 principal components (PCs), for farmed and wild *Gadus morhua*. DF, AF, and PF refer to the dorsal, anal, and pelvic fins respectively, and their corresponding numbering begins with the most anterior fin. Condition index (CI) and liver index (LI) are the standardized residuals of the regression of standard length, and liver weight on total weight respectively. Fin sizes were standardized to a common centroid size, while the calculation of CI and LI includes an inherent standardization

Measure	PC1	PC2	PC3	PC4
CI	-0.01	0.54	-0.15	0.51
LI	0.25	0.43	-0.13	0.21
DF1 length (mm)	-0.36	0.04	-0.06	0.09
DF1 width (mm)	0.00	0.43	-0.33	-0.10
DF2 length (mm)	-0.33	0.00	0.14	0.19
DF2 width (mm)	-0.17	0.33	0.61	-0.13
DF3 length (mm)	-0.35	0.01	0.04	0.00
DF3 width (mm)	-0.22	0.12	-0.37	-0.47
AF1 length (mm)	-0.25	-0.17	-0.06	0.04
AF1 width (mm)	-0.22	0.34	0.40	-0.25
AF2 length (mm)	-0.37	0.00	0.06	0.17
AF2 width (mm)	-0.25	0.19	-0.32	-0.42
Right PF length (mm)	-0.31	-0.10	-0.13	0.31
Left PF length (mm)	-0.32	-0.14	-0.20	0.22
Percentage of variance	44.34	12.56	8.84	8.53
Eigenvalue	6.21	1.76	1.24	1.19

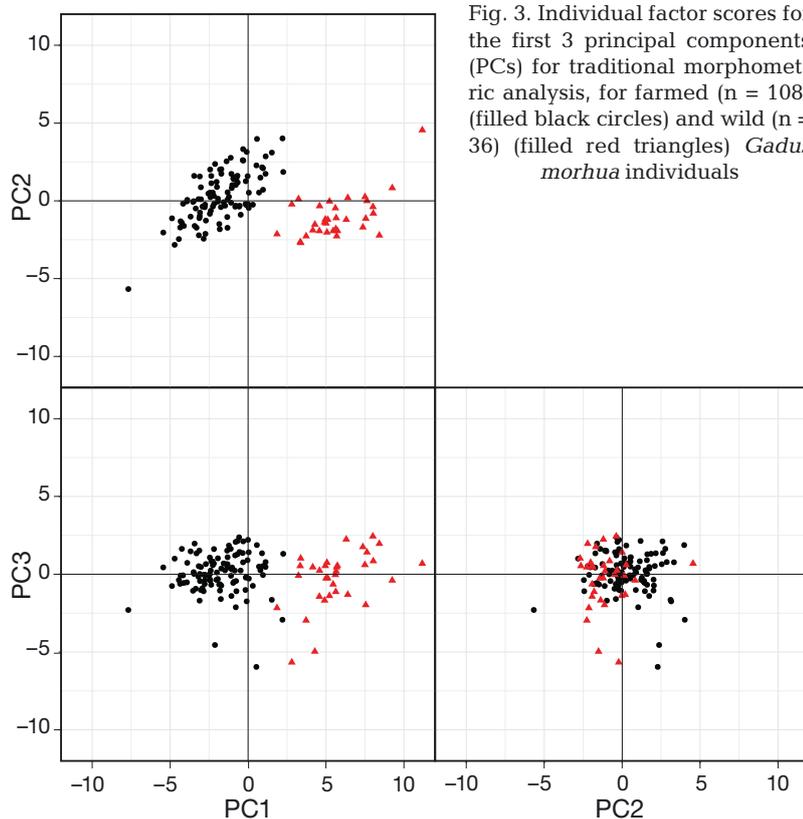


Fig. 3. Individual factor scores for the first 3 principal components (PCs) for traditional morphometric analysis, for farmed (n = 108) (filled black circles) and wild (n = 36) (filled red triangles) *Gadus morhua* individuals

Within-origin analysis showed that the shape of the wild males differed from that of the wild females, and the same was true for farmed males and females (both $p < 0.05$). Testing within sexes, the shape of both farmed females and males was different from that of their wild counterparts (both $p < 0.001$).

PCA of the configuration of the wild and farmed specimens into the principal warp space revealed 7 PCs with eigenvalues greater than the mean eigenvalue, and cumulatively explained 81.90% of the variance. Like the ANOVA above, the scores on PC1 and PC2 showed a significant interaction between sex and origin (both $p < 0.05$; Fig. 4). That said, Fig. 4 shows a clear separation between wild and farmed fish along PC2. PC1 explained 30.17% of the variance, and PC2 18.52%. PC1 was, however, significantly correlated with centroid size (Spearman's rho: -0.259 , $p < 0.01$), indicating that the shape differences described by the first PC were mainly related to size. There were no significant differences in shape between origins, sexes, or any interaction between the two for PCs 3–7 (all $p > 0.05$).

Fig. 5 depicts the difference in shape between farmed females relative to farmed males (Fig. 5a), wild females relative to wild males (Fig. 5b), farmed females relative to wild females (Fig. 5c), and farmed

males relative to wild males (Fig. 5d), and is illustrative of the significant sex \times origin interaction. Despite detecting significant statistical difference in shape between the farmed males and females, their consensus shapes appear to be quite congruent even when differences are magnified 3 \times (Fig. 5a). Wild females appear to be shallower in the abdominal region than the wild males, as indicated by the magnitude of the ventral displacements of points 2, 3, and 4 relative to point 12 (refer to Fig. 2 for description of points and Fig. 5b for relative displacement of points). This difference in body depth seems to be confined to the abdominal region because the displacement of the points on the dorsal surface is offset by the displacement of the points opposite them on the ventral surface in the head (points 1, 13, 15, 16, and 18), and in the caudal regions (points 5, 6, 7, 9, 10, and 11; Fig. 5b).

Farmed males and females both show a reduction in head size and

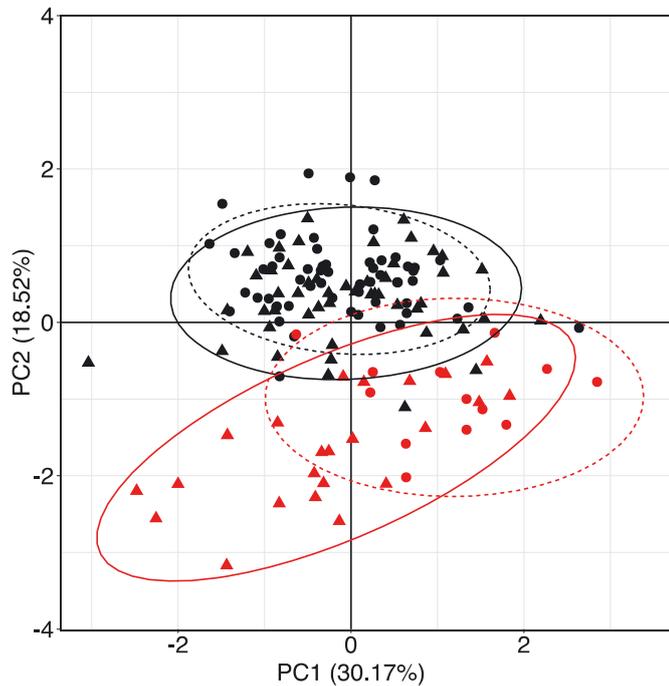


Fig. 4. Ordination plot for configurations of specimens into principal warp space for geometric morphometric analysis of *Gadus morhua*. Individuals are plotted by origin and sex using colour and shape respectively (farmed = black, wild = red, males = triangles, females = circles; farmed males: $n = 58$, farmed females: $n = 50$, wild males: $n = 13$, wild females: $n = 23$). Ellipses represent 95% CI for the groups. The same colour scheme is used to denote origins, but sexes are distinguished by line type (solid = males, dashed = females)

caudal peduncle length relative to their wild counterparts (females: Fig. 5c; males: Fig. 5d). The smaller head size is evidenced by the posterior displacement of points 1, 16, 17, and 18, the anterior displacement of points 13 and 14, and the anteriodorsal displacement of point 15 (Fig. 5c,d). However, farmed males show a greater reduction in jaw length relative to wild males than farmed females do to wild females, (point 15; Fig. 5c,d). The posterior displacement of points 6, 7 (females), 9, and 10, while the midlateral portion of the hypural plate (point 8) remains rela-

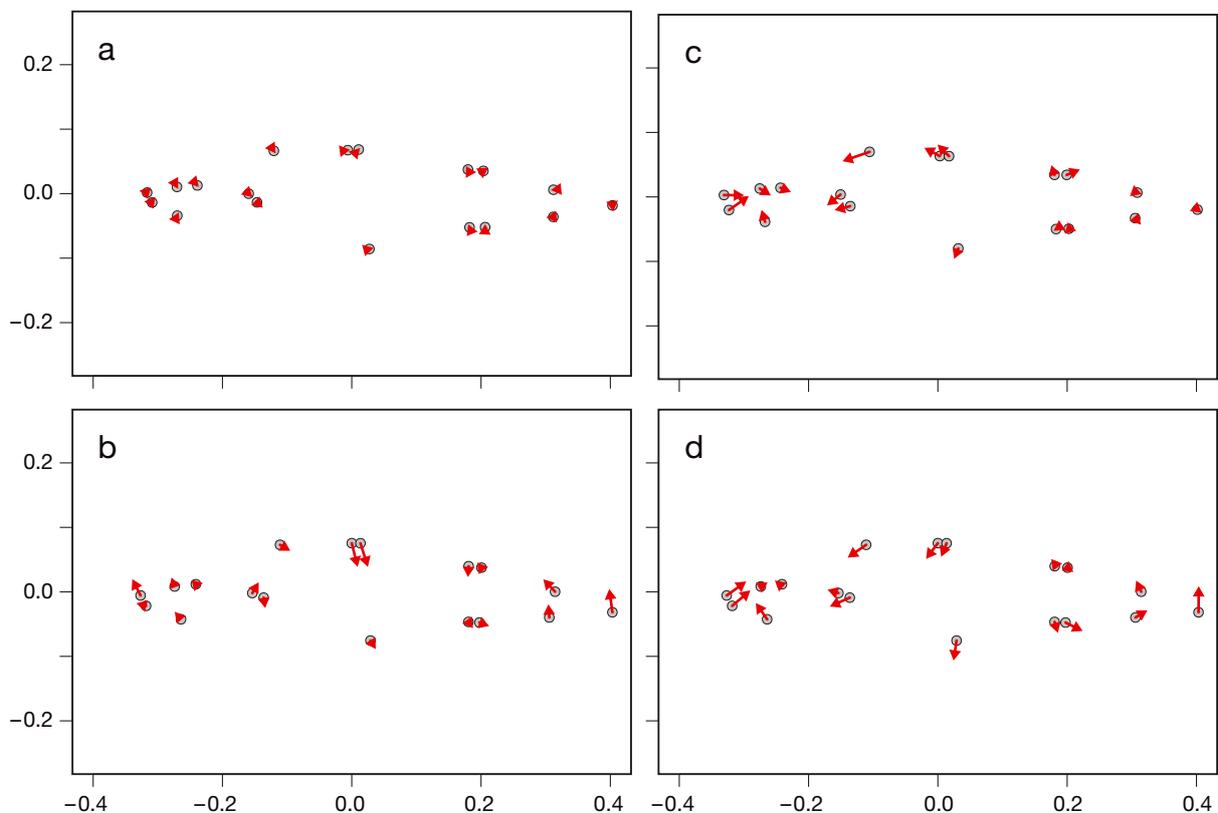


Fig. 5. Magnitude and displacement of the consensus shapes of *Gadus morhua*: (a) farmed females ($n = 50$) relative to farmed males ($n = 58$); (b) wild females ($n = 23$) relative to wild males ($n = 13$); (c) farmed females relative to wild females; (d) farmed males relative to wild males. The direction and degree of displacement (red arrow) of the landmarks of the consensus shape of the first group is shown relative to the location of landmarks on the consensus shape of the second group (\odot). The landmark numbering in (b–d) is the same as that in (a), and landmark numbers and descriptions are given in Fig. 2. Displacements have been magnified 3 \times for easier visualization. Units for both the x- and y-axes are the Procrustes coordinates

tively unchanged along the anteroposterior axis, is indicative of a truncation of the caudal peduncle. Of particular note, the difference in abdominal region body depth between the farmed and wild females appears to be greater than the difference between the farmed and wild males (points 3, 4, and 12; Fig. 5c,d). It is worth noting that the dorsal rotation of point 8 in Fig. 5b,d appears most likely to be the result of subtle differences in the overall rotation, or curvature of the wild male specimens, and likely should be taken as spurious.

DISCUSSION

Differences between wild and farmed fish

Farmed Atlantic cod experience an environment markedly different from that of wild cod. Differences include diet, water temperature and current, fish density, visual and structural complexity, and structure, all of which have been shown to plastically affect the growth, development, and morphology of fishes (Currens et al. 1989, Adams & Huntingford 2002, Marcil et al. 2006, Ambrosio et al. 2008). Not surprisingly, the vast majority of morphological characters we measured differed significantly between wild and farmed individuals, as did their overall shape as evidenced by geometric morphometric analysis. Both traditional and geometric morphometrics indicated that farmed cod had relatively smaller head, jaw, and fin measures, while their body depth, CI, and LI measures were larger than those of the wild cod.

The presence in cultured cod of greater CI and LI than wild cod has been widely documented (e.g. Lie et al. 1986, Svåsand et al. 1996, Grant et al. 1998, Purchase & Brown 2001) and is corroborated by our results. Given that the main site of lipid sequestration in cod is the liver, and liver size and lipid content are directly influenced by the lipid content of the feed, the observed differences in LI are likely reflective of the different diet and physical environment experienced by the wild and farmed cod (Lie et al. 1986, Lambert & Dutil 1997, Morais et al. 2001). Similarly, the greater CI and the greater body depth of the farmed relative to the wild fish in this study are both related to the farmed cod having a higher LI (liver, and as a consequence, visceral mass).

As seen for body depth and LI, the different head morphology in the farmed and wild cod was also likely the result of differences in diet, and perhaps to a lesser extent, physical environment. The jaw and head morphology of fishes have been shown to

be highly phenotypically plastic, and this plastic response is related to and influenced by the fish's diet. While studies on the phenotypic effects of different diets are lacking for cod, studies of other species have indicated that smaller heads and jaws are seen in fish which are fed non-elusive, prepared diets (Meyer 1987, Wintzer & Motta 2005), as well as in fish fed a greater ration (Currens et al. 1989). These features are characteristic of the pellet diet and feeding regime of farmed cod, and relatively smaller heads and jaws have been previously observed in cultured cod (Uglem et al. 2011).

Among the head features that were found to be relatively smaller in the farmed than the wild fish was eye size. Apart from simply being proportional to the head size, Devlin et al. (2012) have suggested that the eye development of rapidly growing fish becomes decoupled from their somatic growth, resulting in a negative allometry.

The most consistently observed differences between multiple species of wild and cultured fish are that cultured fish tend to develop relatively smaller fins of all types (e.g. Lund et al. 1989, Swain et al. 1991, Rogdakis et al. 2011, Patiyal et al. 2014). In some cases, this difference in size is the result of the fins of the cultured fish being either damaged or malformed (Bosakowski & Wagner 1994, Latremouille 2003, Hatlen et al. 2006, Blanchet et al. 2008, Chittenden et al. 2010). However, it is unlikely that contemporary fin damage or malformation affected the results of the present study. The fins of both the wild and farmed fish were checked for signs of damage (e.g. clubbing, or abrasion of fin margin, etc.) or deformity, and measurements from any deformed fins were excluded from the analysis. Whether past damage or abrasion may have resulted in stunting of the size of the farmed cod's fins is also unclear, given the behaviour of cod (decreased wounding with fish size; Hatlen et al. 2006), as well as the great capacity for organ and tissue regeneration present in fish (Azevedo et al. 2011, Shao et al. 2011). It is possible that the smaller fins of the cultured cod resulted in part from a plastic response to water current. Studies in salmonids have shown that lower current velocity and variability experienced in culture can lead to relatively smaller fins (Pakkasmaa & Piironen 2000, Wessel et al. 2006, Keeley et al. 2007). Similarly, when compared to wild fish, farmed cod likely experience similar reductions in water velocity, and hence similar plastic effects on fin size could be expected in our study.

Considering all the observed differences between the farmed and wild cod in our study, the congruence

between our results and those of Uglem et al. (2011), the only other study of differences in adult morphology between wild and farmed cod in which sufficient information is reported to allow comparison, is impressive. This is especially true given that the populations examined are thought to have been isolated for at least 100 000 yr (Bigg et al. 2008). This suggests that the observed differences may represent a stereotypical plastic response of Atlantic cod to culture.

Differences between sexes

Cod drumming-muscle weight (Engen & Folstad 1999, Rowe & Hutchings 2004, Skjæraasen et al. 2006, 2008) and the length of the pelvic fins (Skjæraasen et al. 2006, 2008, 2012) have been shown to be sexually dimorphic in other studies, and our results found this to be true of drumming muscle weight, but marginally not so for pelvic fin length. Both traits are suspected to play important roles in mate choice (Skjæraasen et al. 2006, 2012, Rowe & Hutchings 2008), and in the case of the pelvic fins, in maintaining ventral alignment during gamete release (Skjæraasen et al. 2008).

Sampling time and differences in the maturation schedule of male and female cod likely account for the observed differences in body depth, body mass, and LI, and perhaps to some extent drumming muscle mass. Seasonal gonad ripening in cod from this population generally begins at about the same time these fish were sampled (Rideout & Burton 2000). Male Atlantic cod (cultured and wild) generally begin to mature and have functionally mature gonads earlier in the season than females. During maturation, males cease feeding and exhibit a concomitant decrease in body mass and marked hypertrophy of the testes and drumming muscles, while maintaining an LI lower than that of females throughout their reproductive cycle (Fordham & Trippel 1999, Rideout & Burton 2000, Rowe & Hutchings 2004, Solberg & Willumsen 2008).

Implications

When cultured cod escape from net-pens, they interact with wild cod, and are subjected to the selective pressures of the natural environment (Moe et al. 2007, Damsgård et al. 2012, Zimmermann et al. 2012). It is likely that the morphology developed by the cod in culture will be to some degree maladaptive in the wild, and thus any escapees will experi-

ence lower fitness than their wild counterparts, as has been seen in other species (Fleming et al. 2000, McGinnity et al. 2003, Meager et al. 2010, Skaala et al. 2012).

The differences in fin size and body condition we documented may result in different swimming performance. However, the relationship between them in cod and other species is not always clear (Rose et al. 1995, Reidy et al. 2000). Fitness effects of the fins may also extend to reproduction, with the relatively smaller fins of the farmed cod imparting a competitive disadvantage during both male–male agonistic interaction and courtship display. Extension of the median fins is a component of male Atlantic cod's 'flaunting display' (shown to both males and females; Brawn 1961), and pelvic fins are used both for display (Skjæraasen et al. 2010) and to grasp the female and maintain alignment of their urogenital openings during ventral mount (Brawn 1961, Rowe et al. 2008). Moreover, some evidence suggests pelvic fin size may be related to spawning success (Rowe et al. 2008). Such effects may, however, be mitigated to some extent by transience in the differences in fin sizes resulting from convergence through plasticity towards the wild phenotype following escape, as noted in gilthead sea bream *Sparus aurata* (Arechavala-Lopez et al. 2013), and the same is likely true of condition (CI and LI; Nordeide et al. 1994, Jacobsen & Hansen 2001).

It is worth reiterating that the fish in this study are first-generation offspring of wild-caught parents, and while a single generation in captivity has been shown to affect the fitness of cultured fish (Fleming et al. 1997, Milot et al. 2013), increased generations under selection in a cultured environment can lead to genetic changes (reviewed by Hutchings & Fraser 2008, Nguyen 2015). Such genetic changes could result in permanent phenotypic changes relative to the wild fish, even if they are exposed to the same environment (i.e. after escape; Araki et al. 2008, Christie et al. 2012, Milot et al. 2013). Therefore, any differences in fitness caused by the morphological differentiation between wild and cod observed in this study would likely be inflated by genotypic and consequent phenotypic changes that accumulate over time through both deliberate and inadvertent selection.

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LITERATURE CITED

- Adams CE, Huntingford FA (2002) The functional significance of inherited differences in feeding morphology in a sympatric polymorphic population of Arctic charr. *Evol Ecol* 16:15–25
- Adams DC, Otárola-Castillo E (2013) geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol Evol* 4:393–399
- Adams DC, Rohlf FJ, Slice DE (2004) Geometric morphometrics: ten years of progress following the ‘revolution’. *Ital J Zool* 71:5–16
- Ambrosio PP, Costa C, Sánchez P, Flos R (2008) Stocking density and its influence on shape of Senegalese sole adults. *Aquacult Int* 16:333–343
- Araki H, Berejikian BA, Ford MJ, Blouin MS (2008) Fitness of hatchery-reared salmonids in the wild. *Evol Appl* 1: 342–355
- Arechavala-Lopez P, Sanchez-Jerez P, Izquierdo-Gomez D, Toledo-Guedes K, Bayle-Sempere JT (2013) Does fin damage allow discrimination among wild, escaped and farmed *Sparus aurata* (L.) and *Dicentrarchus labrax* (L.)? *J Appl Ichthyol* 29:352–357
- Azevedo AS, Grotek B, Jacinto A, Weidinger G, Saude L (2011) The regenerative capacity of the zebrafish caudal fin is not affected by repeated amputations. *PLoS ONE* 6: e22820
- Bailey MM, Lachapelle KA, Kinnison MT (2010) Ontogenetic selection on hatchery salmon in the wild: natural selection on artificial phenotypes. *Evol Appl* 3:340–351
- Beacham TD, Bratley J, Müller KM, Le KD, Withler RE (2002) Multiple stock structure of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador determined from genetic variation. *ICES J Mar Sci* 59:650–665
- Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 57:289–300
- Bigg GR, Cunningham CW, Ottersen G, Pogson GH, Wadley MR, Williamson P (2008) Ice-age survival of Atlantic cod: agreement between palaeoecology models and genetics. *Proc R Soc B* 275:163–173
- Blanchet S, Páez DJ, Bernatchez L, Dodson JJ (2008) An integrated comparison of captive-bred and wild Atlantic salmon (*Salmo salar*): implications for supportive breeding programs. *Biol Conserv* 141:1989–1999
- Bosakowski T, Wagner EJ (1994) Assessment of fin erosion by comparison of relative fin length in hatchery and wild trout in Utah. *Can J Fish Aquat Sci* 51:636–641
- Bradbury IR, Hubert S, Higgins B, Borza T and others (2010) Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature. *Proc R Soc B* 277:3725–3734
- Brawn VM (1961) Reproductive behaviour of the cod (*Gadus callarias* L.). *Behaviour* 18:177–198
- Chittenden CM, Biagi CA, Davidsen JG, Davidsen AG and others (2010) Genetic versus rearing-environment effects on phenotype: hatchery and natural rearing effects on hatchery- and wild-born coho salmon. *PLoS ONE* 5:e12261
- Christie MR, Marine ML, French RA, Blouin MS (2012) Genetic adaptation to captivity can occur in a single generation. *Proc Natl Acad Sci USA* 109:238–242
- Currans KP, Sharpe CS, Hjort R, Schreck CB, Li HW (1989) Effects of different feeding regimes on the morphometrics of Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*). *Copeia* 689–695
- Damsgård B, Høy E, Uglem I, Hedger RD, Izquierdo-Gomez D, Bjørn PA (2012) Net-biting and escape behaviour in farmed Atlantic cod *Gadus morhua*: effects of feed stimulants and net traits. *Aquacult Environ Interact* 3:1–9
- Devlin RH, Vandersteen WE, Uh M, Stevens ED (2012) Genetically modified growth affects allometry of eye and brain in salmonids. *Can J Zool* 90:193–202
- Dryden IL (2013) shapes: statistical shape analysis. R package. <http://CRAN.R-project.org/package=shapes>
- Einum S, Fleming IA (2001) Implications of stocking: ecological interactions between wild and released salmonids. *Nord J Freshw Res* 75:56–70
- Engen F, Folstad I (1999) Cod courtship song: a song at the expense of dance? *Can J Zool* 77:542–550
- Fleming IA, Gross MR (1994) Breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. *Evolution* 48:637–657
- Fleming IA, Petersson E (2001) The ability of released, hatchery salmonids to breed and contribute to the natural productivity of wild populations. *Nord J Freshw Res* 75:71–98
- Fleming IA, Jonsson B, Gross MR (1994) Phenotypic divergence of sea-ranched, farmed, and wild salmon. *Can J Fish Aquat Sci* 51:2808–2824
- Fleming IA, Lamberg A, Jonsson B (1997) Effects of early experience on the reproductive performance of Atlantic salmon. *Behav Ecol* 8:470–480
- Fleming IA, Hindar K, Mjølnerød IB, Jonsson B, Balstad T, Lamberg A (2000) Lifetime success and interactions of farm salmon invading a native population. *Proc R Soc B* 267:1517–1523
- Fordham SE, Trippel EA (1999) Feeding behaviour of cod (*Gadus morhua*) in relation to spawning. *J Appl Ichthyol* 15:1–9
- Fox J, Weisberg S (2011) An R companion to applied regression. Sage, Thousand Oaks, CA
- Fraser DJ, Houde ALS, Debes PV, O’Reilly P, Eddington JD, Hutchings JA (2010) Consequences of farmed-wild hybridization across divergent wild populations and multiple traits in salmon. *Ecol Appl* 20:935–953
- Goodall C (1991) Procrustes methods in the statistical analysis of shape. *J R Stat Soc B* 53:285–339
- Grant SM, Brown JA, Boyce DL (1998) Enlarged fatty livers of small juvenile cod: a comparison of laboratory-cultured and wild juveniles. *J Fish Biol* 52:1105–1114
- Hatlen B, Grisdale-Helland B, Helland SJ (2006) Growth variation and fin damage in Atlantic cod (*Gadus morhua* L.) fed at graded levels of feed restriction. *Aquaculture* 261:1212–1221
- Hutchings JA, Fraser DJ (2008) The nature of fisheries- and farming-induced evolution. *Mol Ecol* 17:294–313
- Imre I, McLaughlin RL, Noakes DLG (2002) Phenotypic

- plasticity in brook charr: changes in caudal fin induced by water flow. *J Fish Biol* 61:1171–1181
- Jackson DA (1993) Stopping rules in principal components analysis: a comparison of heuristic and statistical approaches. *Ecology* 74:2204–2214
- Jacobsen JA, Hansen LP (2001) Feeding habits of wild and escaped farmed Atlantic salmon, *Salmo salar* L., in the Northeast Atlantic. *ICES J Mar Sci* 58:916–933
- Keeley ER, Parkinson EA, Taylor EB (2007) The origins of ecotypic variation of rainbow trout: a test of environmental vs. genetically based differences in morphology. *J Evol Biol* 20:725–736
- Lambert Y, Dutil JD (1997) Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of cod (*Gadus morhua*)? *Can J Fish Aquat Sci* 54:104–112
- Latremouille DN (2003) Fin erosion in aquaculture and natural environments. *Rev Fish Sci* 11:315–335
- Lie Ø, Lied E, Lambertsen G (1986) Liver retention of fat and of fatty acids in cod (*Gadus morhua*) fed different oils. *Aquaculture* 59:187–196
- Lund RA, Hansen LP, Järvi T (1989) Identifisering av oppdrettslaks og vill-laks ved ytre morfologi, finnestørrelse og skjellkarakterer. In: NINA Forskningsrapport, Book 1. Norsk Institutt for Naturforskning, Trondheim
- Marcil J, Swain DP, Hutchings JA (2006) Genetic and environmental components of phenotypic variation in body shape among populations of Atlantic cod (*Gadus morhua* L.). *Biol J Linn Soc* 88:351–365
- Mayer I, Meager J, Skjæraasen JE, Rodewald P, Sverdrup G, Fernö A (2011) Domestication causes rapid changes in heart and brain morphology in Atlantic cod (*Gadus morhua*). *Environ Biol Fishes* 92:181–186
- McGinnity P, Prodohl P, Ferguson K, Hynes R and others (2003) Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. *Proc R Soc B* 270: 2443–2450
- Meager JJ, Skjæraasen JE, Fernö A, Løkkeborg S (2010) Reproductive interactions between fugitive farmed and wild Atlantic cod (*Gadus morhua*) in the field. *Can J Fish Aquat Sci* 67:1221–1231
- Meyer A (1987) Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution* 41: 1357–1369
- Milot E, Perrier C, Papillon L, Dodson JJ, Bernatchez L (2013) Reduced fitness of Atlantic salmon released in the wild after one generation of captive breeding. *Evol Appl* 6:472–485
- Moe H, Dempster T, Sunde LM, Winther U, Fredheim A (2007) Technological solutions and operational measures to prevent escapes of Atlantic cod (*Gadus morhua*) from sea cages. *Aquacult Res* 38:91–99
- Morais S, Bell JG, Robertson DA, Roy WJ, Morris PC (2001) Protein/lipid ratios in extruded diets for Atlantic cod (*Gadus morhua* L.): effects on growth, feed utilisation, muscle composition and liver histology. *Aquaculture* 203: 101–119
- Nguyen NH (2015) Genetic improvement for important farmed aquaculture species with a reference to carp, tilapia and prawns in Asia: achievements, lessons and challenges. *Fish Fish* (in press), doi:10.1111/faf.12122
- Nordeide JT, Fosså JH, Salvanes AGV, Smedstad OM (1994) Testing if year-class strength of coastal cod, *Gadus morhua* L., can be determined at the juvenile stage. *Aquacult Fish Manage* 25, Suppl 1:101–116
- Pakkasmaa S, Piironen J (2000) Water velocity shapes juvenile salmonids. *Evol Ecol* 14:721–730
- Pakkasmaa S, Ranta E, Piironen J (1998) A morphometric study on four land-locked salmonid species. *Ann Zool Fenn* 35:131–140
- Patiyal RS, Mir JJ, Sharma RC, Chandra S, Mahanta PC (2014) Pattern of meristic and morphometric variations between wild and captive stocks of endangered *Tor putitora* (Hamilton 1822) using multivariate statistical analysis methods. *Proc Natl Acad Sci India B* 84:123–129
- Purchase CF, Brown JA (2001) Stock-specific changes in growth rates, food conversion efficiencies, and energy allocation in response to temperature change in juvenile Atlantic cod. *J Fish Biol* 58:36–52
- R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Reidy SP, Kerr SR, Nelson JA (2000) Aerobic and anaerobic swimming performance of individual Atlantic cod. *J Exp Biol* 203:347–357
- Reist JD (1986) An empirical evaluation of coefficients used in residual and allometric adjustment of size covariation. *Can J Zool* 64:1363–1368
- Rideout RM, Burton MPM (2000) The reproductive cycle of male Atlantic cod (*Gadus morhua* L.) from Placentia Bay, Newfoundland. *Can J Zool* 78:1017–1025
- Rogdakis YG, Koukou KK, Ramfos A, Dimitriou E, Katselis GN (2011) Comparative morphology of wild, farmed and hatchery released gilthead sea bream (*Sparus aurata*) in western Greece. *Int J Fish Aquacult* 3:1–9
- Rohlf FJ (1999) Shape statistics: Procrustes superimpositions and tangent spaces. *J Classif* 16:197–223
- Rose GA, deYoung B, Colbourne EB (1995) Cod (*Gadus morhua* L.) migration speeds and transport relative to currents on the Northeast Newfoundland Shelf. *ICES J Mar Sci* 52:903–913
- Rose GA, Nelson RJ, Mello LGS (2011) Isolation or meta-population: whence and whither the Smith Sound cod? *Can J Fish Aquat Sci* 68:152–169
- Rowe S, Hutchings JA (2004) The function of sound production by Atlantic cod as inferred from patterns of variation in drumming muscle mass. *Can J Zool* 82:1391–1398
- Rowe S, Hutchings JA (2008) A link between sound producing musculature and mating success in Atlantic cod. *J Fish Biol* 72:500–511
- Rowe S, Hutchings JA, Skjæraasen JE, Bezanson L (2008) Morphological and behavioural correlates of reproductive success in Atlantic cod *Gadus morhua*. *Mar Ecol Prog Ser* 354:257–265
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9: 671–675
- Shao JP, Chen DY, Ye QJ, Cui JL, Li YH, Li L (2011) Tissue regeneration after injury in adult zebrafish: the regenerative potential of the caudal fin. *Dev Dyn* 240:1271–1277
- Skaala O, Glover KA, Barlaup BT, Svåsand T, Besnier F, Hansen MM, Borgstrøm R (2012) Performance of farmed, hybrid, and wild Atlantic salmon (*Salmo salar*) families in a natural river environment. *Can J Fish Aquat Sci* 69: 1994–2006
- Skjæraasen JE, Rowe S, Hutchings JA (2006) Sexual dimorphism in pelvic fin length of Atlantic cod. *Can J Zool* 84: 865–870

- Skjæraasen JE, Meager JJ, Karlsen Ø (2008) The expression of secondary sexual characteristics in recruit- and repeat-spawning farmed and wild Atlantic cod (*Gadus morhua*). ICES J Mar Sci 65:1710–1716
- Skjæraasen JE, Meager JJ, Karlsen Ø, Mayer I, Dahle G, Rudolfsen G, Fernö A (2010) Mating competition between farmed and wild cod *Gadus morhua*. Mar Ecol Prog Ser 412:247–258
- Skjæraasen JE, Meager JJ, Heino M (2012) Secondary sexual characteristics in codfishes (Gadidae) in relation to sound production, habitat use and social behaviour. Mar Biol Res 8:201–209
- Solberg C, Willumsen L (2008) Differences in growth and chemical composition between male and female farmed cod (*Gadus morhua*) throughout a maturation cycle. Aquacult Res 39:619–626
- Solberg MF, Skaala O, Nilsen F, Glover KA (2013) Does domestication cause changes in growth reaction norms? A study of farmed, wild and hybrid Atlantic salmon families exposed to environmental stress. PLoS ONE 8:e54469
- Svåsand T, Jørstad KE, Otterå H, Kjesbu OS (1996) Differences in growth performance between Arcto-Norwegian and Norwegian coastal cod reared under identical conditions. J Fish Biol 49:108–119
- Swain DP, Riddell BE, Murray CB (1991) Morphological differences between hatchery and wild populations of coho salmon (*Oncorhynchus kisutch*): environmental versus genetic origin. Can J Fish Aquat Sci 48:1783–1791
- Uglem I, Berg M, Varne R, Nilsen R, Mork J, Bjørn PA (2011) Discrimination of wild and farmed Atlantic cod (*Gadus morhua*) based on morphology and scale-circuli pattern. ICES J Mar Sci 68:1928–1936
- Vehanen T, Huusko A (2011) Brown trout *Salmo trutta* express different morphometrics due to divergence in the rearing environment. J Fish Biol 79:1167–1181
- von Cramon-Taubadel N, Ling EN, Cotter D, Wilkins NP (2005) Determination of body shape variation in Irish hatchery-reared and wild Atlantic salmon. J Fish Biol 66:1471–1482
- Wessel ML, Smoker WW, Joyce J (2006) Variation of morphology among juvenile Chinook salmon of hatchery, hybrid, and wild origin. Trans Am Fish Soc 135:333–340
- Wheeler B (2010) lmPerm: permutation tests for linear models. R package version 1.1-2. <https://cran.r-project.org/src/contrib/Archive/lmPerm/>
- Wintzer AP, Motta PJ (2005) Diet-induced phenotypic plasticity in the skull morphology of hatchery-reared Florida largemouth bass, *Micropterus salmoides floridanus*. Ecol Freshw Fish 14:311–318
- Zimmermann EW, Purchase CF, Fleming IA (2012) Reducing the incidence of net cage biting and the expression of escape-related behaviors in Atlantic cod (*Gadus morhua*) with feeding and cage enrichment. Appl Anim Behav Sci 141:71–78

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