ICES Journal of Marine Science (2011), 68(9), 1928–1936. doi:10.1093/icesjms/fsr120

Discrimination of wild and farmed Atlantic cod (*Gadus morhua*) based on morphology and scale-circuli pattern

Ingebrigt Uglem[1]*, Marius Berg[1], Rebecca Varne[2], Rune Nilsen[3], Jarle Mork[2], and Pål Arne Bjørn[3]

[1]*Norwegian Institute of Nature Research, Tungasletta 2, N-7485 Trondheim, Norway*
[2]*Trondhjem Biological Station, Department of Biology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway*
[3]*Nofima Marin, Muninbakken 9-13, PO Box 6122, N-9291 Tromsø, Norway*

**Corresponding Author: tel: +47 73 801400; fax: +47 73 801401; e-mail: ingebrigt.uglem@nina.no.*

Uglem, I., Berg, M., Varne, R., Nilsen, R., Mork, J., and Bjørn, P. A. 2011. Discrimination of wild and farmed Atlantic cod (*Gadus morhua*) based on morphology and scale-circuli pattern. – ICES Journal of Marine Science, 68: 1928 – 1936.

Received 14 January 2011; accepted 22 June 2011; advance access publication 25 July 2011.

To evaluate the spatio–temporal distribution and ecological impacts of escaped farmed Atlantic cod (*Gadus morhua*), it is necessary that escapees can be traced in the wild. To do this, simple, reliable, and fast methods for determining the origin of cod are required. The aim of this proof-of-concept study was to evaluate whether simple analyses of scales and body morphology can distinguish between wild and farmed cod. Digital images of fish and scales from adult cod from two farms, and wild cod caught near these farms, were analysed by computer-based image analyses. By combining mean breadth of circuli and length-adjusted scale radius in a discriminant analysis, 86 and 80% of wild and farmed fish, respectively, were correctly classified. Moreover, using three simple morphometric measures representing dorsal fin size, neck curvature, and length of lower jaw, 100 and 95% of wild and farmed cod, respectively, were classified correctly. To validate these discrimination methods further, an expanded analysis of additional farmed and wild cod populations is required. The results pave the way for the development of a reliable and standardized methodology for classification of the origin of cod caught in the wild.

Keywords: aquaculture, Atlantic cod, fish escape, *Gadus morhua*, morphological variation scale analyses.

Introduction

In 2009, almost 20 000 t of farmed Atlantic cod (*Gadus morhua*) were produced in Norway (Norwegian Directorate of Fisheries, 2010). Knowledge of the ecological and genetic impacts of cod farming is still sparse, but the potential for negative ecological consequences is significant (Bekkevold *et al.*, 2006). Escaped farmed cod are present in the spawning areas of wild cod during the spawning season, and wild and escaped cod are likely to interbreed (Uglem *et al.*, 2008; Meager *et al.*, 2009). Within their sea cages, farmed cod can also produce viable larvae that subsequently mix with larvae from wild cod in the areas around the cod farms (Jørstad *et al.*, 2008). Hence, cod farming may result in unfavourable genetic changes in wild populations of cod similar to that found for Atlantic salmon (*Salmo salar*; Hindar *et al.*, 2006). Further, escaped farmed cod may transmit pathogens to wild populations (Øines *et al.*, 2006) and also increase the predation pressure on wild salmon smolt (Brooking *et al.*, 2006) and other fish species.

It has been suggested that farmed cod are more prone to escape from marine net-pen farms than, for instance, Atlantic salmon (Moe *et al.*, 2007). Estimates of escaped farmed cod were not recorded systematically until 2004, but Moe *et al.* (2007) estimated that up to 6% of the annual farmed stock may have escaped during the years 2000–2005. Between 2004 and 2009, a total of 1.13 million farmed cod escaped in Norway (Norwegian Directorate of Fisheries, 2010). On average, this corresponds to 1.1% of the farm stock at the end of each year (Norwegian Directorate of Fisheries, 2010). The proportion of escaped fish in cod farming has so far been higher than in salmon farming, where, on average over the years 2004–2009, 0.2% of the farmed stock at the end of each year was reported to have escaped (Norwegian Directorate of Fisheries, 2010).

To map the spatio–temporal distribution and possible ecological impacts of escaped farmed cod, it is necessary to be able to trace escapees in the wild. To do this, simple, reliable, and fast methods for determining the origin of cod are required. The importance of simple determination of the origin of cod caught in the wild is illustrated by frequent reports in the Norwegian media during recent years regarding catches of abnormal and assumed escaped farmed cod. In many of these cases, it has hitherto been difficult to verify that such fish were of farm origin, because genetic samples were not taken. Analyses of scales and body morphology can distinguish farmed and wild salmonids with a relatively high degree of certainty (Lund and Hansen, 1991; Fleming *et al.*, 1994; Fiske *et al.*, 2006). The primary intent of this proof-of-concept study, therefore, was to evaluate for Atlantic cod whether simple analyses of scales and body morphology have the potential for determining origin. This was done by analysing digital images of fish and scales of farmed cod from two farms and wild cod near the same farms, using computer-based image analyses.

Material and methods

Farmed Atlantic cod were sampled randomly from two fish farms, one located outside the island of Frøya, close to Mausund

Table 1. Length, weights, *K*-factors, and sex ratios for the Atlantic cod used in the (top panel) scale and (bottom panel) morphological analyses.

Location	Type	Date	*n*	Mean length (mm) ± s.d.	Mean weight (g) ± s.d.	Mean *K*-factor ± s.d.	Sex ratio (%) (male:female)
Frøya	Wild	3 November 2009	30	456 ± 55	947 ± 359	0.96 ± 0.06	53.3:47.7
	Farmed	5 November 2009	30	370 ± 55	619 ± 286	1.12 ± 0.18	56.7:43.3
Ytterøya	Wild	9 April 2010	49	580 ± 41	2 100 ± 434	1.06 ± 0.12	61.2:38.8
	Farmed	9 March 2010	50	541 ± 40	1 953 ± 480	1.23 ± 0.24	40.0:60.0
Frøya	Wild	3 November 2009	49	444.3 ± 63.0	902.8 ± 378.2	0.98 ± 0.12	55.1:44.9
	Farmed	5 November 2009	100	380.4 ± 58.1	675.8 ± 307.2	1.12 ± 0.17	53.0:47.0
Ytterøya	Wild	9 April 2010	50	580.8 ± 40.2	2 097.0 ± 429.9	1.06 ± 0.12	60.0:40.0
	Farmed	9 March 2010	50	545.0 ± 40.7	1 953.2 ± 480.2	1.19 ± 0.17	40.0:60.0

(63°52′10″N 08°38′52″E), and one at the island of Ytterøya in the inner part of the Trondheimsfjord (63°40′01″N 11°02′93″E; Table 1). The farmed cod from Frøya and Ytterøya were some 1.3 and 2.5 years old (±3 months) when they were sampled. The background of the farmed cod is unknown because they originated from a mixed broodstock consisting of both coastal cod and Northeast Arctic cod. Wild cod of approximately the same size as the farmed fish were sampled near the farms (<15 km) using fykenets and cod pots (Table 1). Cod captured in the wild were evaluated visually as being wild if they lacked obvious culture-related traits, i.e. neck or mouth deformities, fin damage, or other morphological features typical for escaped cod. The probability that escaped farmed cod were determined to be wild fish was judged to be low, but it is not possible to rule out completely the possibility that some of the wild-caught fish were, in fact, escaped farmed cod. No escape incidents were reported from the farm at Frøya, but two larger escape incidents were reported for the Ytterøya farm before the sampling of wild cod.

Length and weight were measured to the nearest millimetre and gramme for each fish. Otoliths of all wild fish were removed and stored dry in marked paper envelopes for subsequent estimation of age. Before age determination, the otoliths were broken through the nucleus, and age zones were classified as translucent or opaque according to the method outlined by Williams and Bedford (1974). Most wild fish from Frøya were between 2 and 4 years old, and most of the wild fish from Ytterøya were between 3 and 5 years old (Table 2).

Table 2. Estimated ages from otoliths for wild cod from Frøya and Ytterøya, with age categories representing the number of fish up to 1 year older than a given age.

	Number of fish at age (years)				
Location	1+	2+	3+	4+	5+
Frøya ($n = 49$)	2	36	10	0	1
Ytterøya ($n = 50$)	0	1	28	15	6

Scale analyses

Scales were sampled from the same position on all fish, above the lateral line under the third dorsal fin (Figure 1), by first using the blunt side of a knife to remove mucus and then the sharp side to remove between 30 and 60 scales. The scales were dried and stored in marked paper envelopes to await analysis. Before measurement of circuli pattern, the scales were rolled onto a translucent film. The scale-circuli patterns were analysed by capturing digital images of the scales using a Leica Z6-APO macroscope. The images were then examined by computer-based image analysis (ImagePro plus, Media Cybernetics, Inc.). Scale radius was measured from the centre to the edge of the scale (Figure 1). The distances between individual circuli were measured along the same axis as that used to measure the scale radius (Figure 1).

Morphological analyses

After capture/collection, the fish were killed and immediately stored on ice (<5 h) until they were photographed, before onset of rigor mortis, using a digital camera (Canon G10) mounted on a tripod. The fish were placed on a uniform, light-grey background, with the true left side of the fish up, and illuminated from four sides to avoid shadows. All fins were extended to their natural shape and held in place with needles. An object of known dimension (25 × 100 mm) was placed close to each fish to ensure correct calibration in the subsequent image analyses. Altogether, 19 morphological measures (Figure 1, Table 3) were recorded as x–y coordinates using the image analysis software ImageTool (V. 3.0, UTHSCSAN, http://ddsdx.uthscsa.edu/dig/). Repeatability was determined by measuring ten wild and ten farmed fish from Frøya five times. The average coefficient of variation (*CV*) of all measurements was 2.7%.

The morphological measures were selected to represent relatively easily measured parameters for basic differentiation between farmed and wild fish, and not primarily for describing body shape in detail. As farmed cod often have damaged fins (Hatlen *et al.*, 2006), the areas of the three dorsal fins, the caudal fin, and the two ventral fins, as well as pectoral fin length, were measured (Figure 1, Table 3). Further, the angle between the anterior fin root of the posterior dorsal fin and the tip of the second fin ray of this fin and the posterior fin root was measured. This angle is easy to measure in a field situation, without digital image analysis. Distances between fins may vary among different cod stocks (B. J. McAdam, pers. comm.), possibly as a result of variation in environmental conditions during ontogeny. Hence, the distances between dorsal, ventral, and caudal fin roots were also measured (Figure 1, Table 3). A large proportion of farmed cod have deformities in the most cranial vertebrae, which may result in both abnormal upward and downward curvature in the cranial region (Grotmol *et al.*, 2005; Fjelldal *et al.*, 2009). To be able to evaluate morphological variation in the head region, five distances were measured (Figure 1, Table 3). In addition, the angle from the lowest point on the dorsal side of the head to the highest point posterior and anterior of the lowest point was measured (Figure 1, Table 3). The different morphological

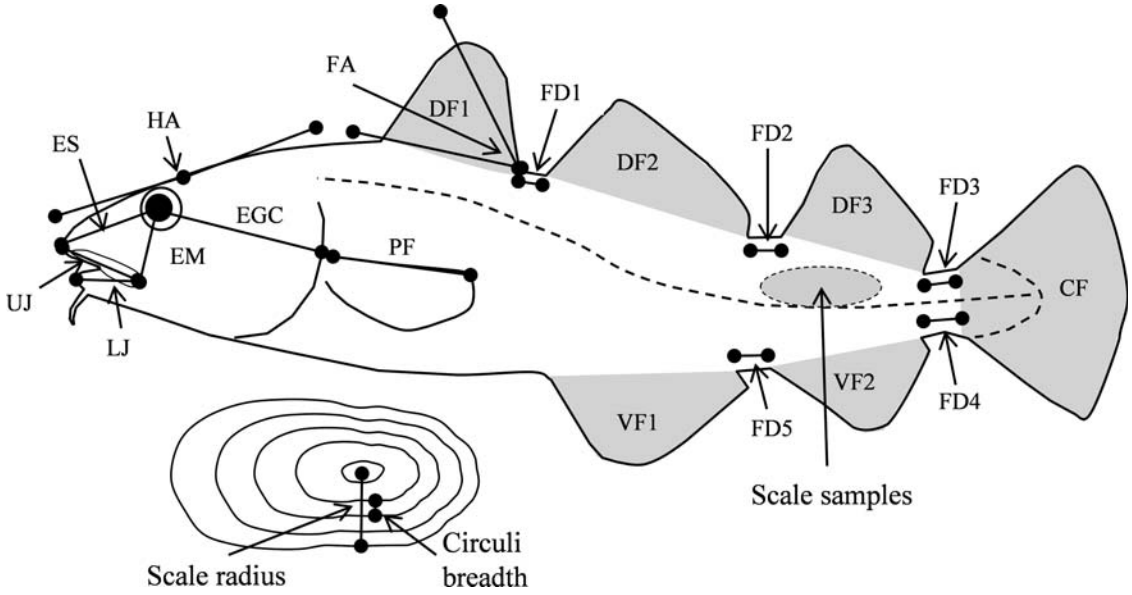

Figure 1. Morphological and scale measures (abbreviations listed in Table 3). The area from where the scale samples were taken is indicated under the last dorsal fin.

Table 3. Abbreviations and description of morphological measures, component loadings, percentage of variance, and eigenvalues for the PCs (with varimax rotation).

Code	Description	PC1	PC2	PC3
SE	Distance from the snout to the centre of the eye	0.935		
EGC	Distance from the centre of the eye to the end of the gill cover	0.848		
PF	Length of the pectoral fin	0.836		
EM	Distance from the centre of the eye to the corner of the mouth	0.922		
LJ	Length of the lower jaw	0.862		
UJ	Length of the upper jaw	0.875		
DF1	Area of dorsal fin 1	0.821		
DF2	Area of dorsal fin 2	0.893		
DF3	Area of dorsal fin 3	0.928		
CF	Area of caudal fin	0.948		
VF1	Area of ventral fin 1	0.910		
VF2	Area of ventral fin 2	0.917		
FD1	Distance from dorsal fin 1 to dorsal fin 2		0.454	
FD2	Distance from dorsal fin 2 to dorsal fin 3		0.589	
FD3	Distance from dorsal fin 3 to the caudal fin		0.561	
FD4	Distance from ventral fin 2 to the caudal fin		0.693	
FD5	Distance from ventral fin 1 to ventral fin 2		0.734	
HA	Angle to the lowest point on neck, to indicate neck deformity			0.829
Percentage of variance		53.8	11.0	8.3
Eigenvalue		9.69	1.97	1.5

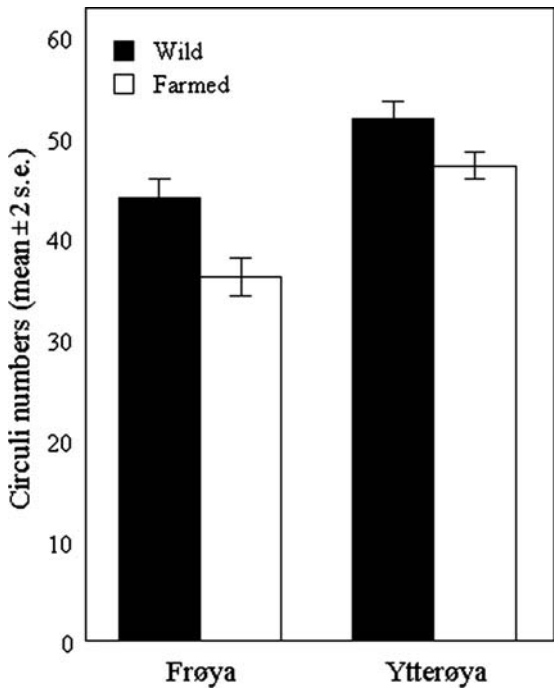

Figure 2. Mean number of circuli per scale for farmed and wild Atlantic cod from two locations, Frøya and Ytterøya.

measurements are hereafter referred to according to the codes described in Figure 1 and Table 3.

Data analysis

In a practical situation, use of scale-circuli patterns to determine the origin of a fish could take place without knowing the exact age of the fish, because age determination based on scale-circuli

patterns is usually impossible because of the presence of unclear seasonal zones for most of the farmed fish. Hence, further discrimination between wild and farmed cod using scale measures is not based on age or data on seasonal zone spacing. The circuli numbers varied both among groups and individuals, and cumulative circuli breadths were only calculated for the first 40 circuli, because almost 90% of the fish had at least this number of circuli. Univariate GLM type III sums of squares analyses with gender, fish type (farmed or wild), and location (Frøya or Ytterøya) as fixed factors, and fish length as covariates, were used for testing whether the scale parameters varied in relation to gender and fish size. Gender was not significantly associated with any of the three scale measures ($F < 1.23$, $p > 0.27$). Scale radius ($F = 41.4$, $p < 0.001$) and circuli number ($F = 42.4$, $p < 0.001$) were significantly related to fish length, so were length-adjusted before further analysis. Mean sclerite distance was not associated with fish length ($F = 3.73$, $p = 0.06$). Discriminant analysis based on selected scale parameters was used to classify fish as either farmed or wild.

All morphological measures were length-adjusted according to the method outlined by Reist (1986), i.e. by transforming all measures in the allometric equation

$$\tilde{Y}_i = \ln Y_i - b(\ln X_i - \ln X_{mean}), \quad (1)$$

where $\tilde{Y}_i$ is the natural logarithm of the correlated trait for fish i, Y_i the original unadjusted measurement, X_i the measured length of the individual, X_{mean} the mean length for all fish, and b the allometric coefficient (the slope of the relationship between ln Y and ln X). These transformations were made separately for each location and for farmed or wild fish. The length-adjusted measures from Equation (1) were further standardized to a mean of zero and s.d. of 1 (Z-standardization). Whether length-adjusted morphological measures were associated with age of the wild fish was tested using one-way ANOVA for ages 2+ and 3+ from Frøya, and ages 3+ and 4+ from Ytterøya (Table 2). The sample sizes of other ages were judged to be too low to be included in such analyses (Table 1). Apart from the relative caudal fin area for wild fish from Ytterøya ($F = 5.74$, $p = 0.02$), none of the morphological measures were associated with age for either Frøya ($F < 3.2$, $p > 0.08$) or Ytterøya ($F < 2.34$, $p > 0.13$). Univariate GLM type III sums-of-squares tests with fish type (farmed or wild) and location (Frøya or Ytterøya) as fixed factors were used to test for differences between morphological measures for farmed and wild fish. To minimize the number of parameters, all morphological measures apart from the angle between the anterior fin root of the posterior dorsal fin and the tip of the second fin ray of this fin and the posterior fin root (i.e. FA; Figure 1) were analysed using principal component analysis (PCA) with varimax rotation. The angle FA was not included in this analysis because, in principle, it is the same measure as DF1, i.e. both measures represent the area of the first dorsal fin. All PCAs with eigenvalues >1.00 were considered to be significant (Chatfield and Collins, 1980). Discriminant analysis, based on individual scores for different PCs and also on selected morphological parameters, was then used to classify fish into either farmed or wild categories. The data were analysed using PASW Statistics (SPSS, v. 18.0.2), with the significance level established at $p < 0.05$.

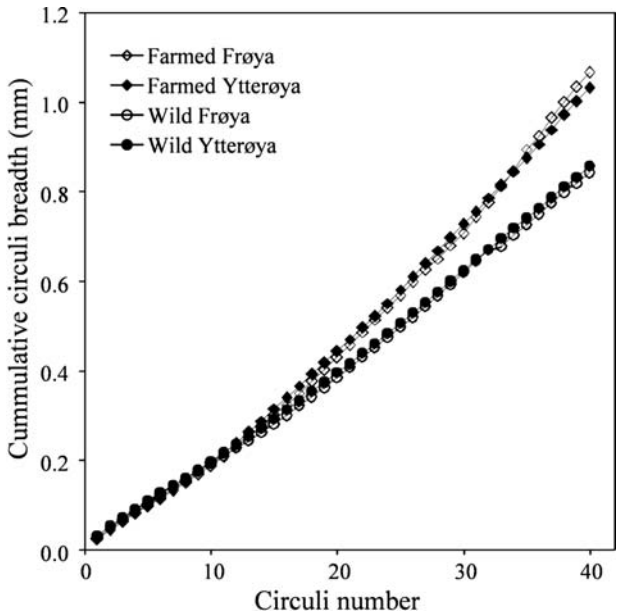

Figure 3. Cumulative circuli breadth (first 40 circuli) for farmed and wild Atlantic cod from Frøya and Ytterøya.

Results

Scales

The number of circuli per scale was lower for fish from Frøya than from Ytterøya ($F = 115.6$, $p < 0.001$), and the examined farmed fish also had fewer circuli than wild fish ($F = 49.9$, $p < 0.001$; Figure 2). There was no significant interaction between fish type and location ($F = 3.3$, $p = 0.073$) with respect to numbers of circuli per scale.

The cumulative circuli breadth did not differ between farmed and wild fish until circuli 14 (Figure 3; $F = 5.0$, $p = 0.026$). From circuli 14 to circuli 40, the cumulative circuli breadth was significantly larger for farmed fish than for wild fish (Figure 3; circuli 40, $F = 36.5$, $p < 0.001$). Location was significantly associated with variation in cumulative circuli breadth for circuli 40 ($F = 16.3$, $p < 0.01$). Further, there was a significant interaction

Table 4. Data from discriminant analysis for scale and morphological parameters, including the proportion of wild and farmed Atlantic cod being classified correctly.

							Correct classification (%)		
Model	***F***	**Eigenvalue**	**Canonical correlation**	**Wilk's λ**	**χ^2**	**d.f.**	***p*-value**	**Wild**	**Farmed**
Mean circuli breadth and length-adjusted scale radius	1	0.51	0.58	0.66	64.7	2	<0.001	86.1	80.0
PC1, PC2, and PC3	1	3.16	0.87	0.24	346.4	2	<0.001	97	96
LJ, HA, and FA	1	4.03	0.9	0.2	395	3	<0.001	100	95

Original classification and cross-validation is identical. The codes for the morphological parameters are described in detail in Figure 1 and Table 3.

Table 5. Means and s.d. for the size-correlated morphometric parameters, with results from univariate GLM analyses.

	Morphometric data (size-correlated according to Reist, 1986)								Univariate GLM statistics					
									Location		Type		Location × Type	
Measure	Wild Frøya (mean ± s.d.)		Farmed Frøya (mean ± s.d.)		Wild Ytterøya (mean ± s.d.)		Farmed Ytterøya (mean ± s.d.)		*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
FD1	2.38	0.17	2.38	0.31	2.47	0.29	2.71	0.34	28.4	<0.001	9.6	0.002	9.8	0.002
FD2	2.54	0.20	2.55	0.23	2.65	0.33	2.80	0.25	30.1	<0.001	5.2	0.023	5.1	0.025
FD3	2.76	0.21	2.89	0.17	2.85	0.30	2.92	0.32	9.2	0.003	3.9	0.049	0.9	0.356
FD4	2.81	0.22	2.89	0.15	3.07	0.33	2.95	0.21	29.6	<0.001	0.6	0.446	12.7	<0.001
FD5	2.77	0.22	2.82	0.20	3.00	0.36	2.77	0.28	7.1	0.008	6.7	0.010	16.2	<0.001
SE	3.78	0.06	3.46	0.07	4.14	0.08	3.83	0.06	1 662.7	<0.001	1 236.0	<0.001	0.6	0.442
EGC	4.27	0.07	3.92	0.23	4.50	0.07	4.35	0.06	253.1	<0.001	149.5	<0.001	22.6	<0.001
PF	4.19	0.08	3.76	0.26	4.35	0.16	4.30	0.06	200.1	<0.001	92.3	<0.001	55.6	<0.001
EM	3.51	0.10	3.22	0.07	3.82	0.09	3.59	0.07	993.4	<0.001	561.6	<0.001	9.5	0.002
LJ	3.36	0.09	2.94	0.13	3.63	0.18	3.26	0.15	244.0	<0.001	456.4	<0.001	2.3	0.127
UJ	3.64	0.11	3.26	0.14	3.86	0.14	3.59	0.10	256.4	<0.001	362.4	<0.001	9.7	0.002
DF1	7.54	0.14	6.36	0.42	7.80	0.14	6.95	0.33	99.9	<0.001	572.5	<0.001	16.0	<0.001
DF2	7.87	0.14	7.15	0.33	8.03	0.13	7.89	0.15	209.9	<0.001	190.0	<0.001	90.4	<0.001
DF3	7.46	0.15	6.99	0.14	7.67	0.14	7.68	0.13	570.6	<0.001	156.6	<0.001	166.2	<0.001
C	8.48	0.10	8.06	0.10	8.86	0.08	8.82	0.09	2 057.9	<0.001	340.1	<0.001	230.7	<0.001
VF1	7.23	0.14	6.81	0.12	7.36	0.18	7.43	0.12	414.9	<0.001	95.1	<0.001	183.4	<0.001
VF2	7.78	0.14	7.23	0.16	7.95	0.18	7.72	0.16	244.9	<0.001	337.3	<0.001	55.1	<0.001
HA	5.18	0.02	5.23	0.05	5.18	0.04	5.26	0.02	9.2	0.003	180.1	<0.001	15.9	<0.001
FA	3.94	0.14	3.24	0.39	3.97	0.16	3.17	0.38	0.2	0.678	328.6	<0.001	1.4	0.230

Abbreviations are described in detail in Figure 1 and Table 3.

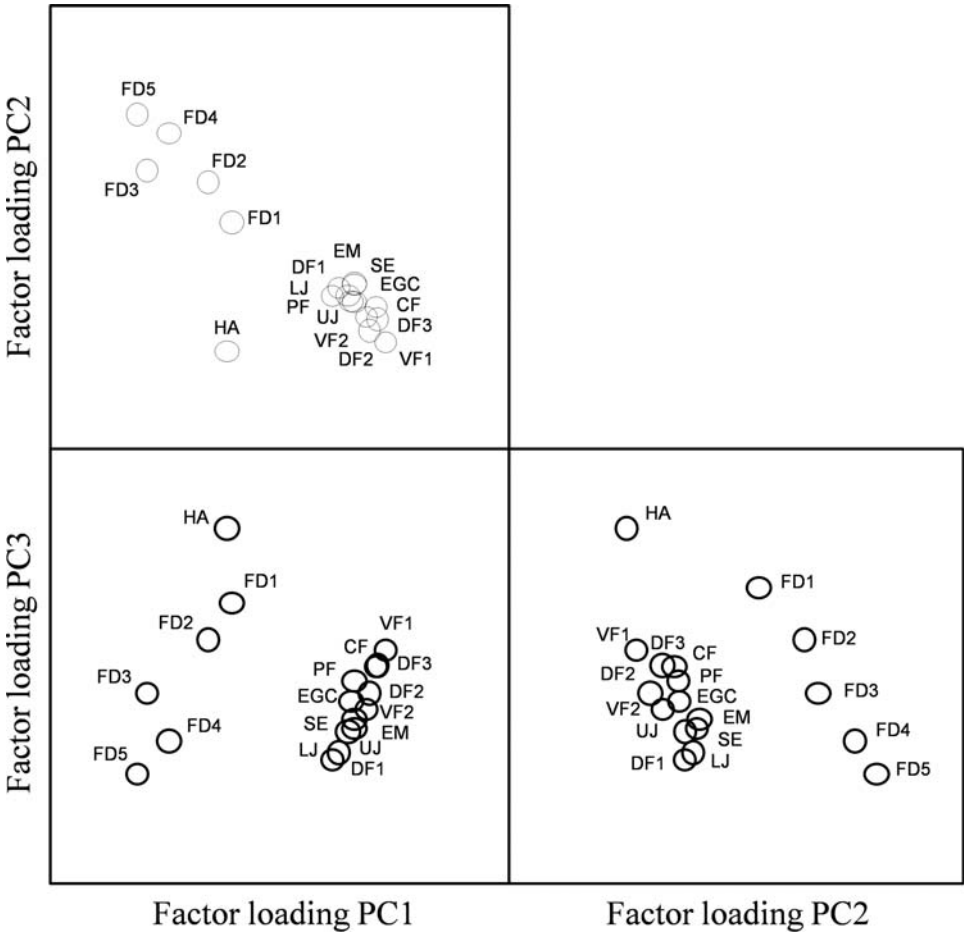

Figure 4. Factor loadings for the three significant PCs in relation to the morphological measures (abbreviations listed in Table 3).

for location and type for circuli 40 ($F = 11.0$, $p = 0.001$), indicating that the cumulative circuli breadth was slightly higher for farmed cod from Frøya than from Ytterøya, whereas the cumulative circuli breadth tended to be lower for wild fish from Frøya than from Ytterøya (Figure 3).

Mean circuli breadth and length-adjusted scale radius differed between wild and farmed fish (Univariate GLM type III sums-of-squares analysis, fixed factors: location and fish type; mean circuli breadth: $F = 67.7$, $p < 0.001$; scale radius: $F = 43.9$, $p < 0.001$). However, variation in length-adjusted circuli numbers per scale was not associated with fish type (Univariate GLM type III sums-of-squares analysis, fixed factors: location and fish type, $F < 0.1$, $p = 0.87$). Mean circuli breadth and length-adjusted scale radius were, therefore, selected for evaluation of the possibility of using scale parameters to discriminate between farmed and wild cod. A discriminant analysis with these parameters showed that 86.1 and 80% of wild and farmed fish, respectively, were correctly classified (Table 4).

Morphology

Apart from FD4 and FA, all morphological measures differed significantly among locations and farmed and wild fish (Table 5). FA did not differ between the two locations, whereas FD4 did not differ between farmed and wild fish (Table 5). Apart from FD3, SE, LJ, and FA, there were significant interaction effects for location and fish type for the other 15 morphological parameters (Table 5).

A combination of three principal components (PCs) explained 73.2% of the variation in size-adjusted body morphology variables (Table 3). The first PC comprised parameters describing the head region of the fish and fin areas, and PC2 represented the distances between fins (Figure 4, Table 3). PC3 represented variation in HA (Figure 4, Table 3). The relationship among the factor scores indicates morphological variation both between and within the different wild and farmed fish groups (Figure 5). In particular, the farmed fish from Frøya appeared to differ from the other groups with respect to variation in PC1, i.e. different measures in the head region and fin areas (Figure 5). A discriminant analysis of the individual scores of these three PCs showed that 97 and 96% of wild and farmed fish, respectively, were classified correctly (Table 4).

Three parameters were selected for considering the possibility of using a few simple measurements to discriminate between farmed and wild cod. The primary selection criterion was that these parameters would be easy to measure in the field. FA was selected because (i) it was highly correlated with the area of the posterior dorsal fin, (ii) there was no difference between locations, and (iii) there were no significant interaction effects between location and type. Likewise, LJ was selected because there was no interaction effect among location and type. HA

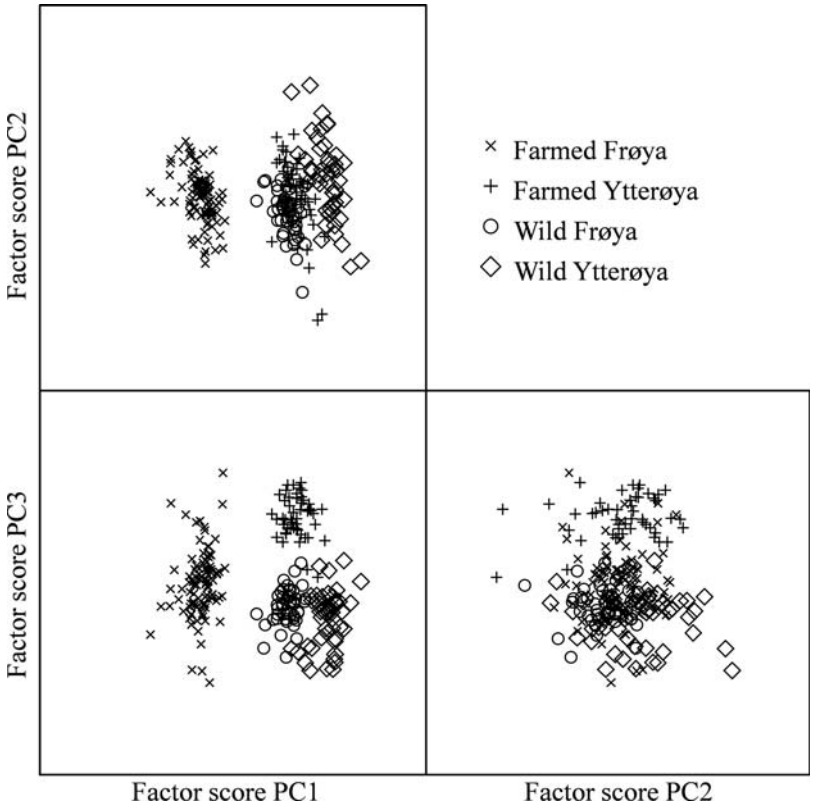

Figure 5. Individual factor scores for the three significant PCs in relation to location and fish type.

was selected because a large proportion of farmed cod have abnormal curvatures in the neck region. A discriminant analysis with FA, LJ, and HA showed that 100% of the wild fish were classified correctly and that 95% of the farmed fish were classified correctly (Table 4).

Discussion

This proof-of-concept study shows that variation in scale-circuli pattern and body morphology has the potential to distinguish between wild and farmed Atlantic cod, concurring with the results of earlier studies on farmed and wild Atlantic salmon (e.g. Lund and Hansen, 1991; Fleming *et al.*, 1994). Both morphological and scale analyses are commonly used for determination of proportions of escaped Atlantic salmon in Norwegian commercial and recreational fisheries and have therefore proven to be valuable management tools (Fiske *et al.*, 2006).

The variation in scale-circuli pattern and morphological traits between farmed and wild cod could be caused by a range of factors. In the same way as for Atlantic salmon (Fiske *et al.*, 2006), the differences in scale-circuli pattern between wild and farmed cod is most likely associated with varying growth patterns caused by variation in external and/or internal factors at different life stages. Initially, the growth patterns of the two groups are relatively similar. Later, farmed cod appear to grow faster than wild fish, as indicated by an increasingly larger cumulative circuli breadth and relatively larger-scale radius for farmed than for wild cod. It is reasonable to assume that the faster growth of farmed cod is a result of greater food availability through an abundance of artificial fish food, an energetically less costly life style because of a life in captivity, and optimal physical conditions during early life stages in intensive culture compared with their wild counterparts. However, genetic factors cannot be ruled out, although attempts to reveal genotypic differences in the growth of wild cod have provided inconsistent results (Mork *et al.*, 1984; Jørstad and Nævdal, 1994; Gjerde *et al.*, 2004; Jørstad *et al.*, 2006).

The morphological differences between wild and farmed cod caused by the culture process might be of both a relatively permanent nature and a direct cause of the duration of the cultivation period. For instance, deformations in neck curvature are probably determined in early life (Grotmol *et al.*, 2005; Fjelldal *et al.*, 2009) and will be persistent throughout the entire lifespan of the fish. On the other hand, the degree of fin damage would most likely increase throughout the culture period through, for example, social interactions or handling (e.g. Kindschi *et al.*, 2001; Person-Le Ruyet and Le Bayon, 2009). Morphological variation among different cod populations may also be related to phenotypic plasticity caused by environmental or genetic variation (Marcil *et al.*, 2006). Hence, it is important to bear in mind the fact that both scale and morphological parameters of farmed and wild cod may vary as a result of both environmental and genetic factors. Indeed, the results from this proof-of-concept study indicate that both the morphology and scale-circuli patterns vary both between and within farmed and wild fish populations.

Our results suggest that there is a need to examine more farmed and wild fish populations, as well as several year classes and ages, before a functional and reliable methodology for discrimination between wild and farmed cod can be developed. Another factor that must be taken into account during development of such

methodologies is that the occurrence of production-related deformities and damage to farmed cod may decrease over time because of the ongoing breeding of farmed cod and optimization of production methods. As many of the morphological traits examined in the current study are production-related deformities and damages, perhaps the opportunity to make reliable distinctions between wild and farmed fish based on morphology will be reduced in future. Moreover, calibration of methods for distinguishing between wild and farmed fish based on scale and morphological traits needs to be accompanied by genetic analyses to ensure that cod caught in the wild are truly wild-origin fish. As large numbers of farmed cod escape each year, either as fish or as fertilized eggs, genetic analysis is a prerequisite for verifying the origin of wild fish (Glover *et al.*, 2011). Finally, it will also be necessary to verify the precision of methods for distinguishing between escapees and wild fish through blind tests, i.e. testing datasets not originally used to develop the statistical models. However, the results from the current proof-of-concept study show that scale and morphological analyses have the potential to distinguish between wild and farmed cod.

Discrimination between escaped farmed cod and wild cod may also be achieved by other means than scale and morphological parameters. For instance, recent developments within genetics have led to increasingly more efficient and less costly ways not only for distinguishing between farmed and wild fish, but also for determining the actual farm from which an escapee originated (Glover *et al.*, 2008, 2010; Glover, 2010; Karlsson *et al.*, 2011). However, Glover *et al.* (2011) observed that some morphologically characterized wild-caught cod closely resembled escapees when screening wild and farmed cod for ten microsatellite loci and the *Pan I* locus. Therefore, it may be difficult to distinguish wild and farmed fish based on neutral or nearly neutral genetic markers in cases where cod are farmed in the same region as their broodstock or where escapees originate from several sources (Glover *et al.*, 2011). Further, trace-element composition in scales and otoliths has proven to be effective in distinguishing between wild and farmed salmon (Veinott and Porter, 2005; Adey *et al.*, 2009). Such analysis may also find application in cod. In addition, variation in fatty acid composition in body tissues could be used as a tool for determining origin because the commercial fish feed used in aquaculture would affect the fatty acid composition of farmed fish contra wild fish, which feed on natural organisms (Fernandez-Jover *et al.*, 2007).

Although the alternative methods for recognizing fish origin may have greater precision than scale and morphological analyses, they also require advanced technological equipment and a level of professional expertise not always readily available. Therefore, often, the selection of a method for distinguishing between wild and farmed fish will be a trade-off between reliability, processing speed, costs, and practical applicability. Sometimes a field-based determination of the origin of a fish may be an advantage. Also, methods that can be used by non-professionals will be useful. For instance, the results from this proof-of-concept study indicate that a large proportion of Atlantic cod may be correctly classified as either farmed or wild based on three simple morphological measures that could be collected either from images or from live fish after anaesthesia. If it is possible to develop a standardized methodology for using scales and/or morphological traits for distinguishing escaped farmed cod from wild fish, this would represent a practical approach for evaluating the origin of cod that also may supplement more advanced methods.

Acknowledgements

We thank the workers at the cod farms at Mausund, Frøya, and Ytterøya, Ronny Jakobsen from the Norwegian Institute of Food, Fisheries and Aquaculture Research, and Mamiko Mitamura and Iver Tanem for their support and help during the course of the study and John G. Taylor for language correction. The research was funded by the Norwegian Research Council projects EcoMA and iCoast (Project number: 192429) and the EU-project PreventEscape (project number: 226885).

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