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A radiological study on vertebral deformities in cultured and wild Atlantic cod (*Gadus morhua*, L.)

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ABSTRACT

Vertebral deformities are a major challenge in cod culture. To study which vertebral deformities are present in cultured and wild cod, we examined two year-classes (14.4±1.5 cm (mean length±SD) and 15.8±1.6 cm) of intensively cultured cod, two year-classes (13.9±1.9 cm and 16.3±1.6 cm) of extensively cultured cod, and wild cod caught in the autumn of 2005 and 2006 (17.2±3.4 cm and 23.6±7.7 cm), for vertebral deformities (radiology). The prevalence of individuals with one or more deformed vertebrae was significantly higher in the extensively (37.0 and 30.0%) and intensively (44.8 and 45.0%) cultured cod than in the wild cod (5.8 and 6.1%). Both the intensively (12.5 and 17.2%) and extensively (5.0 and 14.8%) cultured groups had a curvature (lordosis) in the region of the vertebral column, located between the second and third dorsal fin. Furthermore, a group of individually tagged intensively (35.1 cm±1.9 cm) and semi-intensively (36.7±2.6 cm) cultured cod was radiographed twice, with one year interval, to investigate how deformities develop with time. In some cases, normal vertebrae became deformed with time; in other cases deformed vertebrae became more severely deformed.

1. Introduction

The Norwegian production of farmed Atlantic cod is expanding, and cod is now rated as the third most important aquaculture species after Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Cultured cod are fed live prey at start feeding, and then switched to formulated feed later on. Both extensive and intensive rearing systems are used. The intensive approach implies the use of indoor tank systems where the cod larvae are fed cultured rotifers and *Artemia*. In contrast, the extensive systems confine enclosed lagoons where the cod larvae feed on natural zooplankton, mainly copepods (Hamre, 2006; Rosenlund and Halldórsson, 2007). One modification of the extensive rearing is the semi-intensive method where the lagoon is solely used as a plankton production unit, equipped with filters to concentrate and fractionate copepods that are fed cod larvae in large floating plastic bags or tanks on land (van der Meeren and Naas, 1997).

One of the major challenges in cod culture is vertebral deformities (Grotmol et al., 2005; Fitzsimmons and Perutz, 2006). Factors that may influence on the development of this pathological condition in gadoids are; marine protein source in weaning diets (cod) (Opstad et al., 2006), and dietary levels of phosphorous (haddock, *Melanogrammus aeglefinus*) (Roy et al., 2002) and vitamin K (haddock) (Roy and Lall, 2007). Also the type of live feed used during intensive start feeding may affect the occurrence of deformities in cod (Imsland et al., 2006). Intensive cultured cod are prone to develop a curvature in the cranial region of the vertebral column (Grotmol et al., 2005), and incubation of fertilized eggs of wild cod in a stagnant system gave larvae with a high prevalence of scoliosis, kyphosis and lordosis at hatching (Fitzsimmons and Perutz, 2006). Several research papers have focused on the development of different vertebral body deformities in cultured salmon (Witten et al., 2005, 2006; Fjelldal et al., 2007a). However, there is a lack of knowledge regarding types and occurrence of vertebral deformities in the cultured and wild Atlantic cod that habituate the Norwegian coastal waters. Thus, the aim of the present study was to elucidate which types of vertebral body deformities are present in cultured and wild cod in Norway, as well as to describe how deformations develop over time. To investigate this, we compared young wild cod with cod juveniles originating from various production methods. This included two different year classes of intensive and extensive cultured cod and wild cod. In addition we X-rayed members of two groups of individually tagged intensively and semi-intensively cultured cod twice with one year interval.

2. Material and methods

2.1. Fish material

Wild cod were caught from shallow water in Masfjorden (60° N, 5° E, Western Norway) by fine-meshed fyke nets designed for eel capture in

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October 2005 and 2006 (Table 1). The age of the wild cod was determined by counting of growth marks in otoliths (Rollefsen, 1933; Kalish et al., 1995). Further, in October 2005 and 2006 extensively cultured cod juveniles were also sampled from the Institute of Marine Research's (IMR) lagoon facility "Parisvatn" in Øygarden, west coast of Norway (Table 1). At this facility, larval cod were stocked in the lagoon in late March and reared on zooplankton as described by Blom et al. (1991). In late April, the larvae were weaned onto commercial formulated feed (2005: Dan-ex 1362, Dana Feed A/S, Horsens, Denmark; 2006: Gemma, Nutreco-Skretting AS, Stavanger, Norway) directly in the lagoon system. At size of approximately 5 cm, the juveniles were harvested by a lift net after attracted by feed, and placed in 125 m^3 net cages in the fjord channel outside the lagoon with Gemma as feed. The extensively cultured cod were all subjected to the natural photoperiod at 60°N. In late March, temperature was 5 and 3 °C in 2005 and 2006, respectively; increasing to 11 °C in mid-May when harvesting and transfer to the cages started. Further increase to 16 °C (2005) and 20 °C (2006) took place during summer, with decrease to between 10 and 11 °C at the time of sampling.

Intensively cultured cod juveniles were sampled from the IMR's, research facilities at Austevoll, west coast of Norway in November 2004, and from a commercial farm in September 2005 (Table 1). At Austevoll, the larval rearing conditions and feed were identical to what are detailed in van der Meeren et al. (2007), with the exception that algal paste made from *Nannocloropsis* sp. was used. This briefly implies larval rearing temperature between 11 and 12 °C and 24 h continuous light, rotifers during the first 30 days, 2 weeks with *Artemia* as food, and weaning on AgloNorse formulated feed (Trofi AS, Tromsø, Norway). The last four months before sampling, the water temperature was reduced to between 8 and 9 °C, and Ewos Marine (Ewos AS, Bergen, Norway) were used as feed. At the commercial farm, cod larvae were reared on rotifers with 24 h continuous light and green water. Through a transition period with *Artemia* as food, the larvae were weaned on formulated feed. The weaned cod fry at the commercial farm were held in indoor tanks until sampled.

Individually tagged intensively and semi-intensively cultured cod were radiographed in June 2005 and reared in a 5×5 m sea cage under natural photoperiod and then radiographed again in June 2006 (Table 1). These cod were originating from one egg group that was split between the two rearing methods in April 2004. The intensive group was sampled from the same batch as described above for the IMR Austevoll facility. The semi-intensive culture took place in 120 m^3 plastic bags at the IMR lagoon facility in Øygarden. The basic outline of the semi-intensive method (van der Meeren and Naas, 1997) implies feeding natural zooplankton (mainly various stages of copepods) adjusted to larval size by filter systems. Rearing took place outdoor under natural light conditions, and the larvae were weaned to the formulated feed AgloNorse in the bags before collected and transferred to outdoor tanks at temperatures between 10 and 12 °C. The juveniles were further fed Danex-1362 and transferred to tanks at IMR Austevoll (September 2004, 8 °C), before put together in a 125 m^3 netcage at sea (May 2005, 8 °C) with their intensive sibling group reared at this facility. At this stage all fish were examined, and individuals with external signs of skeletal deformities removed. At IMR Austevoll, both the intensive and semi-intensive groups were fed Ewos Marine before stocked in the net cages where diet was shifted to Danex-1562. During the year between the radiological examinations, temperature rose to 16 °C during summer 2005, reached a minimum of 3 °C in March 2006, and increased again to 16 °C in June.

2.2. Radiography

Radiographs were taken using a portable X-ray apparatus (HI-Ray 100, Eickenmeyer Medizintechnik für Tierärzte e.K., Tuttlingen, Germany) and 30×40 cm film (AGFA). During radiography of dead fish AGFA D4 DW films were used, whereas during radiography of live fish AGFA D7 DW films were used. Live fish was anesthetized with Finquel MS222 (Argent Chemical Laboratories Inc., Redmond, WA, USA) before photography. The D4 films were exposed twice with 50 mAs and 72 kV, and the D7 films were exposed once with 50 mAs and 72 kV. The films were developed using a manual developer (Cofar Cemat C56D) with Kodak Professional manual fixer and developer. The pictures were digitalised by scanning (Epson Expression 10000 XL, Seiko Epson Corp., Japan). The vertebral column of each fish was thoroughly examined (Photoshop version 6.0). The number of the affected vertebrae, and the type of deformity were determined. Adjacent vertebrae that were compressed in the anterior–posterior direction and lacked intervertebral spaces were classified as ankylosis and compression, lateral dislocation between adjacent normal vertebrae that lacked intervertebral spaces was classified as ankylosis and dislocation, and vertebrae that were compressed in the anterior–posterior direction with intervertebral spaces were classified as compression (Fig. 1A–D). Individuals with one or more deformed vertebral bodies were classified as deformed.

2.3. Statistics

Chi-square tests were used to test (i) group differences in the prevalence of deformed fish between intensive and extensive cultured cod, and wild cod (level of significance Bonferroni adjusted to $P<0.0083$, Sokal and Rohlf, 1995); (ii) if there was a significant increase over time in the prevalence of deformed fish or deformed

Table 1
The prevalence (%) of individuals with curvatures in the cranial, trunk or tail region of the vertebral column, and individuals with one or more deformed vertebrae, in cultured and wild cod

Group	Time of sampling	Origin[1]	Status at X-ray	Length (cm, mean±S.D.)	Age (yrs)	Deformed fish (%)				N[2]
						Cranial Region	Trunk Region	Tail Region	Deformed Vertebrae[3]	
Intensive	Nov. 2004	A	Dead	15.8±1.6	0	27.5	0	12.5	45.0[a]	40
Intensive	Sept. 2005	CF	Dead	14.4±1.5	0	55.2	3.4	17.2	44.8[a]	29
Extensive	Oct. 2005	P	Dead	13.9±1.9	0	3.7	0	14.8	37.0[a]	27
Extensive	Oct. 2006	P	Dead	16.3±1.6	0	0	5.0	5.0	30.0[a]	40
Wild	Oct. 2005	M	Dead	23.6±7.7	0–2	0	0	0	5.8[b]	69
Wild	Oct. 2006	M	Dead	17.2±3.4	0–1	0	0	0	6.1[b]	49
Semi-intensive	June 2005	P and A	Alive	36.7±2.6	1	0	0	0	13.8	29
Semi-intensive	June 2006	P and A	Alive	51.0±3.1	2	0	0	0	20.7	29
Intensive	June 2005	A	Alive	35.1±1.9	1	52.8	2.8	0	75.0	36
Intensive	June 2006	A	Alive	48.4±3.0	2	52.8	2.8	0	75.0	36

The table also contains basic information on group characteristics at sampling.
[1] A = Austevoll, CF = commercial farm, P = Parisvatn, M = Masfjorden.
[2] The number of fish examined in each group.
[3] Different letters indicate statistical differences, with 'a' as the highest value, within the fish that were dead at X-ray.