Available online at www.sciencedirect.com
ScienceDirect
Aquaculture 258 (2006) 164–172
Aquaculture
www.elsevier.com/locate/aqua-online

Vertebrae fusion in Atlantic salmon (*Salmo salar*): Development, aggravation and pathways of containment

P. Eckhard Witten [a,b,*], Alex Obach [c], Ann Huysseune [d], Grete Baeverfjord [a]

[a] *AKVAFORSK, The Institute of Aquaculture Research, N-6600 Sunndalsøra, Norway*
[b] *Department of Biology, Dalhousie University, 1355 Oxford Street, Halifax NS, Canada B3H4J1*
[c] *Marine Harvest Technical Centre, Sjøhagen 3, 4016 Stavanger, P.O. Box 48, N-4001 Stavanger, Norway*
[d] *Ghent University, Biology Department, Ledeganckstraat 35, B-9000 Ghent Belgium*

Received 11 December 2005; received in revised form 28 April 2006; accepted 2 May 2006

Abstract

Fusion of vertebral bodies characterises many types of spine deformities in farmed Atlantic salmon and other bony fish. Little is known about development and progress of this condition in individual fish, knowledge that is required for prevention and control of spine malformations.

To clarify the pathogenesis of vertebrae fusion, we describe the development and progress of the disease in farmed Atlantic salmon based on (a) tracing vertebrae fusion in individual fish at three different stages, (b) analyzing vertebrae fusion in animals 12 months after seawater transfer, and (c) histological examination of the fusion process.

Vertebrae fusion was observed to develop both prior to and after smoltification; early and late fusion stages were detected in pre-smolts and in animals 12 months after seawater transfer. The process involves transformation of intervertebral notochord tissue into cartilage, shape alterations of vertebral body endplates, mineralisation of the intervertebral cartilage, and finally replacement of intervertebral cartilage by bone. Two fused vertebrae can develop into a centre of severe malformation through the continuous amalgamation of neighbouring vertebrae. Alternatively, animals have the capacity to contain the problem through reshaping and remodelling of two fused vertebral bodies into a single, regularly structured and jointed vertebra. Successfully reshaped vertebrae apparently do not inflict further spine malformations. We here demonstrate for the first time that the onset of vertebrae fusion must not inevitably lead to fish with deformed vertebral columns. Defining conditions that favour repair and prevent the spread of vertebrae fusion is a future task that could make a significant contribution to the control of spine deformities in farmed salmon.

Keywords: Spinal deformities; Vertebral column; Bone remodelling; Skeleton

1. Introduction

Vertebral deformities are a recurring problem in Atlantic salmon and other farmed teleost species that surface externally as spine curvatures (lordotic, kyphotic and scoliotic malformations), anterior/posterior (a/p) shortening of the animal's body, or combinations of curvatures and a/p shortening (Gill and Fisk, 1966; McKay and Gjerde, 1986; Dedi et al., 1995; Kvellestad et al., 2000; Gavaia et al., 2002; Witten et al., 2005). Related to many cases of spine malformation are flattening (platyspondyly) and fusion of vertebrae (Kvellestad et al., 2000). Less severe cases involve only few vertebrae, and vertebrae fusion may occur

* Corresponding author. AKVAFORSK, The Institute of Aquaculture Research, N-6600 Sunndalsøra, Norway. Tel.: +47 71 69 53 30; fax: +47 71 60 53 01.
E-mail address: eckhard.witten@akvaforsk.no (P.E. Witten).

0044-8486/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2006.05.005

without obvious external signs. Severe cases with numerous affected vertebrae involve a visible a/p shortening of the vertebral column with or without a nodal thickening of the spine, and subsequently cause a phenotype that is usually referred to as "short tail" (Gil-Martens et al., 2005).

Generally, changes of internal bone structures occur long before deformities become visible externally. Thus, bone deformities are usually diagnosed late and it is difficult to obtain information about the onset and the cause of the malformation (Witten et al., 2005). Consequently, fundamental questions about the pathogenesis of vertebrae fusion are still unanswered. Whether the development of vertebral body flattening and fusion is determined during early life stages, or if spine shortening relates to late developmental disorders, or both, is a matter of discussion. Several studies demonstrate that early developmental disorders account for some cases of shortening of the spine that surface later in life (Wargelius et al., 2005). The delicacy of early salmon developmental stages, due to the patterning of the vertebral column structures prior and after hatching, was described by Nordvik et al. (2005). Reports confirm that elevated incubation temperatures, both applied as heat shock and as long term exposure increased the prevalence of spinal deformities (Baeverfjord et al., 1999; Ørnsrud et al., 2004; Wargelius et al., 2005). These cases could be comparable to congenital block vertebrae known from humans and other mammalian species (Bagley et al., 1993; Leivseth et al., 2005). The fact that heat shock does not cause spinal deformities in all embryos (Wargelius et al., 2005) and findings that not only elevated egg incubation temperatures but also elevated temperatures during the animal's subsequent development in freshwater cause skeletal deformities (Baeverfjord and Wibe, 2003) indicate that certain spine deformities are caused by incidents in later life stages. Handling stress, vaccination, sub-optimal environmental conditions, and malnutrition have been considered as causes (Baeverfjord et al., 1998; Vågsholm and Djupvik, 1998), and the importance of genetic factors in susceptibility to environmental stressors has been demonstrated by Gjerde et al. (2005). A recent analysis of vertebral column shortening in salmon showed vertebral malformations that developed late, perhaps not before smoltification (Witten et al., 2005). Deformities that start late in life and after a period of healthy vertebral column growth suggest that the early developmental conditions of these animals may not have been adverse to regular spine growth in these cases. Consequently, in the search for etiological factors, attention is shifted to rearing conditions of later life stages. Knowledge about the start and the course of skeletal deformities, induced later in life, is required to be able to adjust rearing conditions within a certain time window in order to prevent the development of skeletal deformities.

The present study was conducted to learn more about the start and the aetiology of vertebral body fusion as one of the most common manifestations of spinal malformation in farmed Atlantic salmon. We present data showing that vertebral body fusions can start either early or late in the life of a salmon, but usually increase in severity with time. Unexpectedly, we found that deformed vertebrae must not necessarily be the starting point of an aggravating spine malformation. Salmon has mechanisms to reshape fused vertebral bodies and to contain the problem. Consequently, determining rearing conditions that favour containment and prevent aggravation of skeletal lesions could help to control spinal deformities in farmed salmon.

2. Material and methods

2.1. Selection of material

Atlantic salmon (*Salmo salar*) was farmed under standard commercial conditions by Akvaforsk (Sunndalsøra, Norway) and by Marine Harvest (Stavanger, Norway). The study was subdivided into three parts. Part one consisted of the radiological tracing of the development of vertebral body fusion in different life phases. Part two, also based on radiology, examined the degree of vertebrae fusion in animals of a different group 12 months after seawater transfer. Part three analyzed the histopathology of vertebrae fusion. For part one, 200 animals were individually tagged by intraperitoneal injection of glass encapsulated ID tags (Trovan®, Trovan Ltd., UK) and individually X-rayed: the first time prior to smoltification (mean weight 100 g), the second time after 6 months of seawater rearing (mean weight 1.1 kg) and finally after 12 months in seawater (mean weight 2.3 kg). For part two, 100 individuals (mean weight 1.5 kg) were randomly selected 12 months after seawater transfer and X-rayed. Based on these X-rays, spine sections with fused vertebrae below the dorsal fin were selected from five animals for histological examination (part three). Non-deformed spine sections from five other animals served as controls.

2.2. Radiology

X-rays from all fish were taken using a portable 'Mini X-ray HF80+' machine (Mini X-ray) and 'Kodak Industrex M Film Ready Pack II' (Kodak Industry). No screens for increasing the strength of the X-ray beam were used. The settings of the X-ray unit were 70 kV, 15 mA, 2 s exposure time, and a distance of 40 cm

between the beam source and the X-ray film was used. Radiographs were developed with Kodak chemicals according to the protocol of the manufacturer (Witten and Hall, 2002).

2.3. Histological procedures

Vertebrae were fixed in 10% neutral buffered paraformaldehyde for 24 h, rinsed in tap water for 24 h, and decalcified for 72 h in a 10% EDTA solution buffered with 0.1 M Tris base at pH 7.0. After decalcification, samples were stepwise dehydrated and embedded in Paraplast. Serial sections (10 μm) were prepared in the sagittal plane of the vertebral columns, starting at the periphery and ending in the mediosagittal plane of the vertebrae. Sections were stained with Masson's trichrome as the basic analytical procedure and finally mounted with DPX (Fluka), described in detail by Witten and Hall (2003).

Based on the stained sections, the quality of skeletal and other connective tissues and cells was determined. The sections also served for the determination of possible inflammatory processes indicated by the presence of cells such as lymphocytes, granulocytes, monocytes, or macrophages (Witten et al., 1998; Pressley et al., 2005).

3. Results

3.1. Prevalence of skeletal deformities in the examined fish groups

3.1.1. Animals examined at three different life stages

From 200 individually tagged animals a total of 183 fish were X-rayed on three different occasions. Of these, 22 fish (12%) displayed fusion-related shape alterations in vertebrae on the third and final examination. Of these 22 fish, vertebral deformities were detected in 14 fish at the initial examination, in four additional fish at the second examination, and in the remaining four fish at the final examination. The average number of vertebrae that were fused or displayed shape changes next to a fusion centre affected was 3 (±2.5) in the final examination. The total number of vertebrae per individual ranged between 57 and 61. Of the 22 fish showing vertebral alterations, 16 individuals presented a single focus with two affected vertebrae. In the remaining six fish, four to eleven vertebrae were affected, and three individuals displayed fusion at two distinct locations. In two fish, shape changes of vertebral endplates, indicative of an onset of a fusion process, were observed at first examination, but no pathology was found at later examination.

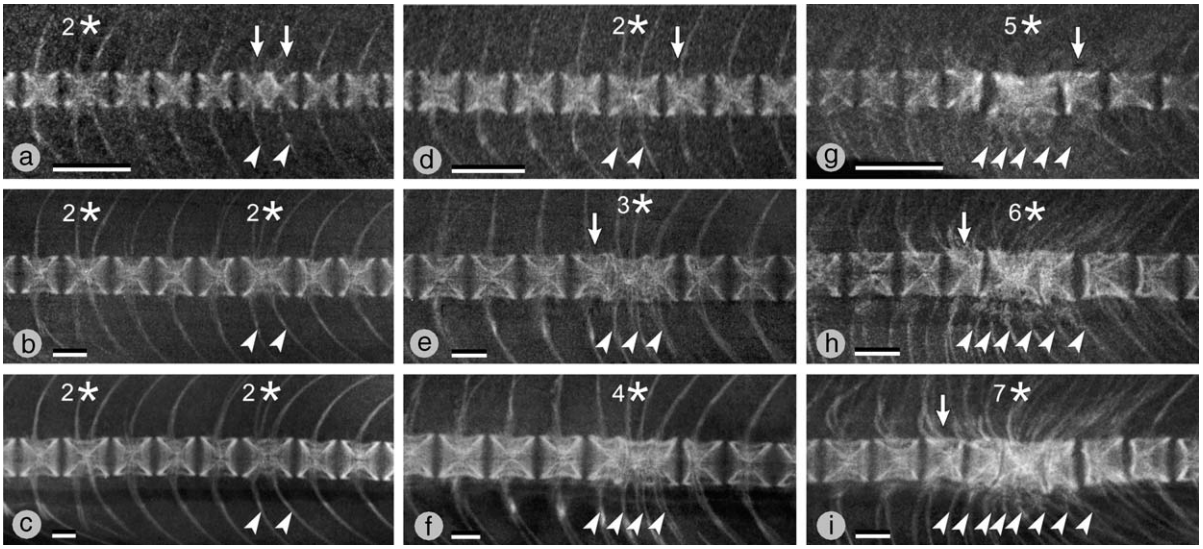

Fig. 1. Development of vertebrae fusion in three different animals (animal one: a–c, animal two: d–f, animal three: g–i). Vertebral columns from each animal are shown in the same size at different scales to visualise shape alterations, scale bars equal 500 μm. White stars indicate fused vertebrae, the number in front of the star refers to the number of fused vertebrae. White arrows label vertebrae in the process of fusion. White arrowheads label haemal arches. The X-rays represent the animals at pre-smolt stage (a, d, g), 6 months after seawater transfer (b, e, h), and 12 months after seawater transfer (c, f, i). X-rays of pre-smolts (a, d, g) show that animals of the same age group display different degrees of vertebral body fusion ranging from the starting fusion (a), fully fused and largely remodelled vertebrae (d), to multiple vertebrae fusion with amalgamation of adjacent vertebral bodies (g). Seawater stages show fused and completely remodelled vertebrae that became a regular part of the spine (b–c, left), the almost complete reshaping of fused vertebrae (b–c, right), and the failure of reshaping with continuing incorporation of adjacent vertebral bodies (e, f and h, i).

3.1.2. Animals examined after 12 months in seawater

From the 100 animals that were examined for the occurrence of vertebral body deformations, 32 animals showed fusion of vertebrae. The average number of vertebrae that were fused or displayed shape changes next to a fusion centre was 5.76 (±4.04) out of 57–61 vertebrae. The number of deformed vertebrae ranged from 2 to a maximum of 19 in one individual. Twenty-five out of the 32 animals with fused vertebrae showed a single focus with two vertebrae affected, typically in the spine segment below the dorsal fin. Seven animals showed vertebrae fusion at two or three locations.

3.2. Course of vertebrae fusion

Animals X-rayed prior to smoltification displayed various degrees of vertebral body fusion, ranging from initial contact between vertebral endplates (Fig. 1a) to the complete amalgamation of vertebral bodies (Fig. 1d). In animals of all groups, initial stages of vertebral fusion involved two vertebral bodies (Figs. 1a and 2a, b). A cluster of three vertebrae is already in a progressed stage of fusion (Fig. 2c). Starting apparently from two fused vertebrae, multiple fusion (Fig. 1d–h) develops through incorporation of neighbouring vertebral bodies (Fig. 2g). The first sign of vertebral body fusion on X-rays is the flattening of the funnel-shaped vertebral endplates of two adjacent vertebrae, followed by the reduction and subsequent disappearance of the intervertebral space. Finally, vertebral body endplates disappear and fused vertebral bodies reshape into a single vertebra (Fig. 1a–c) or become part of a multiple vertebrae fusion centre (Fig. 1d–i).

3.3. Timing of vertebrae fusion

Animals examined prior to smoltification (Fig. 1a, d, g) and animals examined 12 months after seawater transfer (Fig. 2a–g) display comparable fusion stages, ranging from early signs of fusion to fully fused vertebral bodies. Comparing individuals prior to smoltification, 6 and 12 months after seawater transfer provides rough data about the speed of the fusion process. Pronounced morphological alterations were observed between the pre-smolt stage and at 6 months after seawater transfer. The comparison of individuals after 6 and after 12 months in seawater revealed

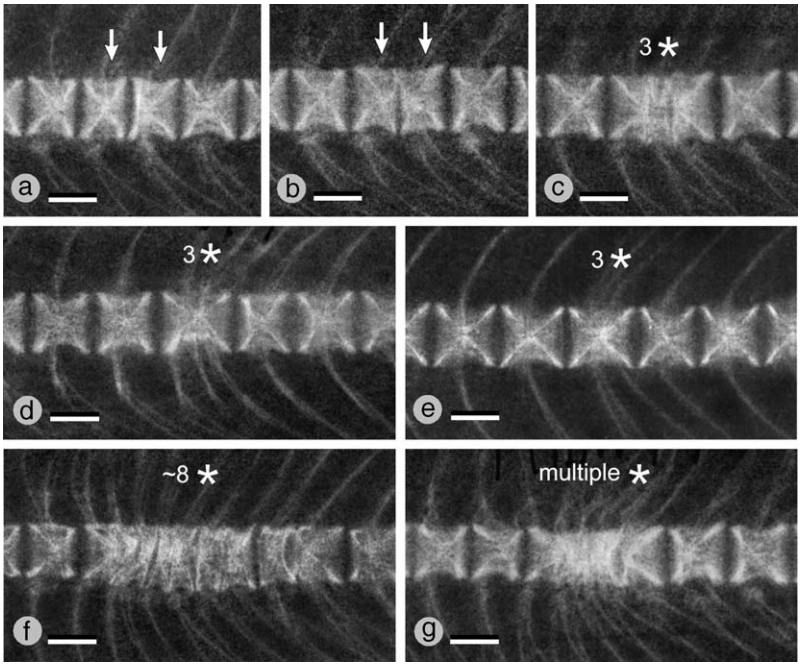

Fig. 2. Stages of vertebrae fusion in seven individuals, X-rayed 12 months after seawater transfer. White arrows label vertebrae prior to or in the process of fusion. White stars indicate fused vertebrae, the number in front of the star refers to the number of fused vertebrae. This group of grown animals displays all stages of vertebrae fusion, similar to those that are observed in different developmental stages (see Fig. 1). Malformations displayed in the first row range from initial fusion (a, b) to the onset of remodelling of fused vertebrae (c). The figures of the second row exhibit less (d) and more (e) successful attempts to remodel fused vertebrae into normal shaped vertebral bodies. The third row shows examples for the aggravation of the problem; multiple vertebral body fusion with the apparent continuous amalgamation of adjacent vertebrae (f, g). Scale bars equal 500 μm.

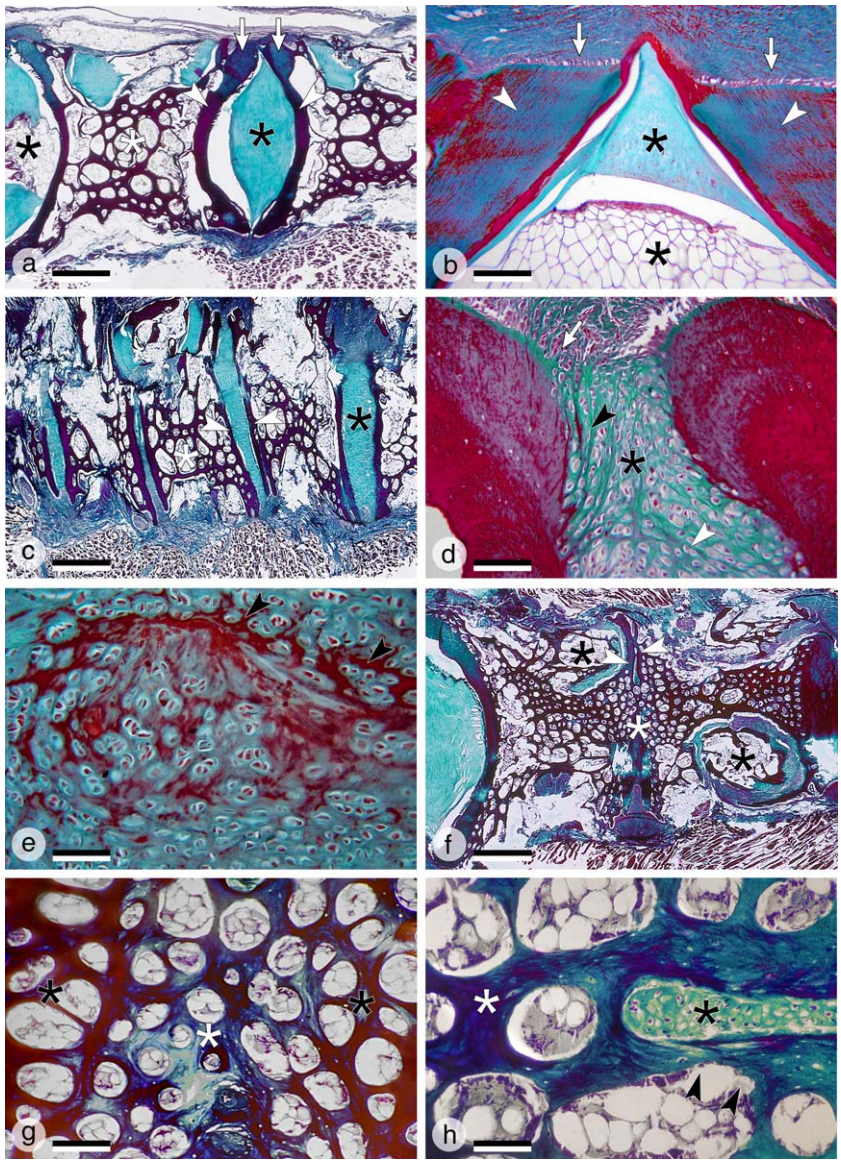

Fig. 3. Microstructure and histology of normal shaped vertebrae (a, b), of vertebrae with changes that precede the fusion of vertebral bodies (c, d, e), and of vertebrae in the process of fusion and reshaping (f, g, h), visualised by Masson's trichrome staining. (a) Normal shaped vertebral bodies are jointed by notochord tissues (the left black asterisk indicates notochord cells, the right black asterisk indicates the notochord sheath) and consist of funnel-shaped bony endplates (white arrowheads) and spongiosa-like intravertebral bone. Growth in length and in diameter takes place adjacent to the articulation area of two vertebrae (white arrows). Bar=2 mm. (b) Enlarged view of the articulation area between two vertebral body endplates. White arrowheads label the bone of the vertebral body endplates, white arrows point to the bone growth zone and the black asterisks label the notochord sheath (top) and notochord cells (below). Bar=250 μm. (c) Prior to the fusion of deformed vertebrae, intervertebral notochord tissue is replaced by fibrocartilage and hyaline cartilage (black asterisk). Vertebral body endplates display signs of atrophy (white arrowhead) whereas intravertebral bone structures remain unchanged (white asterisk). Bar=2 mm. (d) A higher magnification of the growth zones of two deformed vertebrae shows the replacement of notochord tissue by cartilage (black asterisk) and the change from osteogenesis to chondrogenesis towards the intervertebral space (white arrow). Cartilage in the intervertebral space that replaces the notochord tissue displays signs of mineralisation, starting at the collagen fibre bundles in fibrocartilage (black arrowhead) and in the matrix that surrounds chondrocytes in hyaline cartilage (white arrowhead). Bar=80 μm. (e) Higher magnification of mineralising intervertebral hyaline cartilage (black arrowheads). Bar=50 μm. (f) Final stages of vertebrae fusion. Vertebral endplates (white arrowheads) and the cartilage of the intervertebral space are being replaced by intravertebral bone spongiosa (white asterisk). The basis of haemal arches and neural arches are still present (black asterisks). The new bone spongiosa that develops in the location of the former joint space (g, h) resembles the initial spongiosa of the vertebral body. Bar=2 mm. (g) Final stages of vertebrae fusion. New bone spongiosa that develops in the location of the former joint space (white asterisk) resembles the initial spongiosa of the vertebral body (black asterisks). Bar=400 μm. (h) Final stage of vertebrae fusion, dorsal to the left. Residues of former intervertebral cartilage (black asterisk) are surrounded by new spongiosa (white asterisk). Eroded bone surfaces (black arrowheads) indicate the resorption of the bone of the former vertebral body endplates. Bar=80 μm.

differences in the progression of the disease, ranging from near stabilisation (Fig. 1b, c), over slow continuation (Fig.1e, f) to uninhibited progression (Fig. 1h, i). Furthermore, the comparison of the stages of vertebrae fusion in the individuals shown in Fig. 1d–e and h–i indicates that the incorporation of a vertebra into a fusion centre can be completed within 6 months.

3.4. Two scenarios of fusion progression

Examining vertebral body fusion through different life stages and analyzing fusion stages in animals 12 months after seawater transfer reveals that the fusion of vertebral bodies proceeds according to one of two different scenarios.

In the first scenario, fusion is restricted to two (Fig. 1a–c) or maximal three (Fig. 2c–e) vertebral bodies. The length of fused vertebral bodies decreases and the fused vertebrae finally acquire the external shape of a normal non-fused vertebral body (Figs. 1a–c and 2c–e). Completely fused and reshaped vertebrae can only be detected with certainty based on the presence of supernumerary haemal and neural arches that do not fuse (Figs. 1c and 2e). Non-fused vertebral bodies adjacent to completely fused and reshaped vertebrae do not show early signs of fusion such as flattened endplates or reduced intervertebral space. This indicates that the process of vertebrae fusion has stopped.

In the second scenario, fusion is not restricted to two or three vertebral bodies. Similar to the first scenario, the length of fused vertebral bodies decreases (Fig. 2d–f). Yet, the amalgamation of vertebral bodies located next to the fusion centre (Figs. 1d–i and 2f–g) coincides with a failure of fused vertebrae to reshape into a normal shaped element. Vertebral bodies located cranial and caudal to a centre of deformation show signs of early vertebrae fusion (Figs. 1d–i and 2f–g), indicating continuation of the fusion process.

3.5. Restoration of fused vertebral bodies

Radiographs of vertebral bodies in different stages of fusion show shortening and restoration into the shape of non-fused vertebra (see above). The histological examination of non-fused and fused vertebrae provides further insight into the fusion process. The flattening of the funnel-shaped vertebral endplates of adjacent vertebrae coincides with the replacement of notochord tissues (Fig. 3a, b) by cartilaginous tissues (Fig. 3c). Cartilage that occupies the intervertebral space has features of fibrocartilage and of hyaline cartilage (Fig. 3d). Compared to non-deformed vertebrae (Fig. 3a) the flattened vertebral body endplates of deformed vertebrae appear to be less solid (Fig. 3c). Internal vertebral bone structures show no significant alterations (Fig. 3c). In stages of advanced vertebral body fusion we observed the calcification of the intervertebral cartilage. In areas with fibrocartilage calcification starts along the collagen fibres (Fig. 3d), whereas in areas with hyaline cartilage calcification starts in the cartilage matrix that surrounds the chondrocytes (Fig. 3e). Finally, the cartilage and the vertebral body endplates are being replaced by regular internal vertebral bone structures (Fig. 3f–h). Unlike the vertebral body endplates and the intervertebral cartilage, the bases of haemal and neural arches do not disappear (Fig. 3f).

4. Discussion

This study shows that the fusion of vertebral bodies can progress according to two different scenarios, which we will refer to as “aggravation” and “containment”. In the aggravation scenario the initial fusion of two vertebral bodies leads to a centre of vertebrae compression through the continuous amalgamation of further vertebrae. This process of vertebrae fusion causes spine shortening and has obvious implications for the animal’s performance. This type of malformation is well known for salmon and other fish species (Madsen and Dalsgaard, 1999; Afonso et al., 2000; Gavaia et al., 2002; Kvellestad et al., 2000; Lewis et al., 2004). A similar process has been described in humans presenting fused vertebrae that inflict deformities in adjacent spine segments (Leivseth et al., 2005). To our surprise, and reported here for the first time, we also found that salmon is obviously capable of containing the progress of vertebral body fusion through remodelling of fused vertebrae into one non-deformed vertebra (i.e., the second, or “containment” scenario). Terminally remodelled and reshaped vertebrae may only be identified with certainty on radiographs based on the presence of supernumerary neural and haemal arches. Completely reshaped vertebral centra apparently do not inflict further spinal deformities and appear to be stable units within a non-deformed vertebral column. Reshaping of fused vertebrae into one vertebral body with normal contours nevertheless appears to be restricted to a maximum of two or three fusing vertebrae. Although signs of reshaping can also be observed in multiple vertebrae fusion, it is conceivable that the complicated compression of muscle segments, haemal and neural arches causes insurmountable mechanical disturbances, which prevent the restoration of the vertebral body shape.

Spinal deformities underlying the short tail phenotype have been described as platyspondyly and range from localised single or multiple fusions of vertebrae to

the homogenous compression of the entire vertebral column. Also a mixture of both malformations may occur, with fused vertebrae in parts of the spine and homogeneously compressed but not fused vertebrae in other parts (Gill and Fisk, 1966; Kvellestad et al., 2000). All variants of shortening (whether through fusion and/or compression) start with the development of cartilaginous tissue in the intervertebral space that replaces the intervertebral notochord tissue and the osteogenic tissue of the vertebral endplate growth zone (Witten et al., 2005). The example of salmon that grow to an adult size with cartilage instead of notochord tissue connecting vertebrae reveals, however, the functionality of this amphiarthrotic mammalian-like articulation (Gil-Martens et al., 2005; Witten et al., 2005; Lories and Luyten, 2005). The mineralisation of the intervertebral cartilage obviously terminates the tissues' joint function: directly, through the stiffening of the cartilage, and because, except in sharks, mineralisation of cartilage makes it susceptible to resorption and subsequent endochondral bone formation (Witten and Huysseune, 2007).

The histopathological examination cannot provide conclusive evidence that bone alterations induce the replacement of notochord tissue by cartilage or that alterations in the notochord itself initiate cartilage differentiation. Current knowledge about tissue interactions during vertebral column development, however, suggests that alterations of the notochord tissue precede bone alterations. It is established for tetrapods that the notochord plays an important role in the induction of vertebral bodies and in maintaining vertebrae morphogenesis and growth (Patt and Patt, 1969; Hall, 1977, 2005; Hunter et al., 2003; Smits and Lefebvre, 2003). Compression of vertebrae in tetrapods has been linked to the failure of notochord cells to maintain proper vertebral development (Hall, 1977, 2005; Oegema, 2002). Recent findings have shown that also the salmon notochord regulates vertebrae formation in early ontogeny (Grotmol et al., 2003, 2005; Wargelius et al., 2005). This strongly suggests that, similar to tetrapods, the failure of the notochord to fulfil its regulating role in salmon can lead to vertebrae compression. In mammals, the notochord ceases its regulating role for vertebral development as part of normal ontogeny, followed by the replacement of notochord tissue by cartilage and fibrocartilage (Oegema, 2002; Hunter et al., 2003). This transformation is thought to be induced by pressure, a general chondrogenic factor that also converts osteogenic tissue into chondrogenic tissue (Beresford, 1981; Smits and Lefebvre, 2003; de la Fuente and Helms, 2005). Pressure elicits the upregulation of Sox9 (a master transcription factor for chondrogenic differentiation) and collagen type II and downregulates the synthesis of collagen type I (de la Fuente and Helms, 2005; Wenger et al., 2005; Hall and Witten, in press). At the intervertebral disk, pressure also upregulates Sox5 and Sox6, transcription factors that control chondrogenesis and the maturation of notochord cells (Smits and Lefebvre, 2003). In salmon, and other teleosts, chondrogenesis and osteogenesis are not necessarily strictly separated. Switching from osteogenesis to chondrogenesis is part of normal skeletal development, and intermediate tissues are commonly found (Huysseune and Sire, 1990; Huysseune and Verraes, 1990; Huysseune, 2000; Witten and Hall, 2002, 2003; Gillis et al., 2006). Farmed fish build up a high muscle mass and these muscles may exert strong mechanical forces on the tissues of the intervertebral joints. Thus a combination of elevated muscle mass and pressure could interrupt the function of salmon notochord cells and trigger the development of cartilage in the intervertebral space (Gil-Martens et al., 2005; Witten et al., 2005). In this context it is interesting that the replacement of intervertebral notochord tissue by cartilage has been reported from ageing fish (Schaffer, 1930). Like other degenerative joint diseases, fusion of the vertebral column in salmon could be a natural process in old fish but is a pathological process if it occurs too early in life.

To prevent spine malformations it is important to know when and under which conditions deformities develop (Koumoundouros et al., 2001). Considering the observation that fused vertebrae can be successfully reshaped, it will also be important to know which developmental stages and farming/environmental conditions can favour containment and prevent aggravation. Our data indicate that the fusion of vertebrae may occur at any time in the life of a salmon, since pre-smolts display already the entire variety of vertebral body fusions, ranging from the initial stages of fusion, to progressive multiple fusions, to successfully remodelled vertebrae. The same variety of pathological stages can be observed in animals 12 months after seawater transfer. Apparently, vertebrae fusion and related deformities such as platyspondyly (Kvellestad et al., 2000) are not necessarily the result of early developmental disorders but can also develop late in life (Witten et al., 2005). One could, nevertheless, speculate that vertebral body fusions which surface earlier in life are more likely to be contained than damage that occur in later life stages, also keeping in mind that early vertebral body fusion in the caudal region of fish can be a part of normal skeletal development (Witten and Huysseune, 2007). The spine of the pre-smolt shown in Fig. 1a displays a perfectly reshaped vertebrae fusion (left) but a fusion at an earlier stage of development (right) is not completely reshaped – even after 12 months in seawater – and may cause a problem later in life. In the other pre-smolts that

display fusion, reshaping is unsuccessful and leads to aggravation. In line with this thought, we cannot exclude the possibility that successfully reshaped vertebrae, observed in animals 12 months after seawater transfer, have been reshaped prior to smoltification. Obviously, we need more information about the possible time windows in which the decision is made to restore or to aggravate vertebrae fusion. The same applies for farming/environmental conditions that might facilitate containment or favour the aggravation of the problem. The ongoing discussions about causes of spine malformation often relate to factors that influence the animal's bone metabolism (Roy et al., 2002; Fjelldal et al., 2004; Toften and Jobling, 1996; Roberts et al., 2001; Graff et al., 2002). The histopathological examination of vertebrae fusion and related malformations, however, suggests that vertebrae fusion *per se* is primarily a joint related problem and bone alterations are a secondary event. Irrespective of the ongoing discussion about the primary causes for vertebrae fusion, the existence of developmental pathways that allow the restoration of normal shaped vertebral bodies may stimulate the search for farming/environmental conditions that favour containment and prevent the aggravation.

Acknowledgements

We thank Laura Gil-Martens (Stavanger) and our colleagues form the Norwegian School of Veterinary Science (Oslo) for their intellectual support and for assistance in X-raying the fish.

References

Afonso, J.M., Montero, D., Robaina, L., Astorga, N., Izquierdo, M.S., Ginés, R., 2000. Association of a lordosis–scoliosis–kyphosis deformity in gilthead seabream (*Sparus aurata*) with family structure. Fish Physiol. Biochem. 22, 159–163.

Baeverfjord, G., Wibe, Å., 2003. Short tail deformities in Atlantic salmon —effect of freshwater production temperature. Beyond Monoculture. Abstracts at Aquaculture Europe 2003, Trondheim. EAS special publication, vol. 33, pp. 121–122.

Baeverfjord, G., Åsgaård, T., Shearer, K.D., 1998. Development and detection of phosphorous deficiency in Atlantic salmon, *Salmo salar* L., parr and post-smolts. Aquac. Nutr. 4, 1–11.

Baeverfjord, G., Lein, I., Asgård, T., Rye, M., Storset, A., Siikavuopi, S.I., 1999. Vertebral deformations induced by high temperatures during embryogenesis in Atlantic salmon (*Salmo salar*). Toward predictable quality. Abstracts at Aquaculture Europe 99, Trondheim. EAS special publication, vol. 27, pp. 6–7.

Bagley, R.S., Forrest, L.J., Cauzinille, L., Hopkins, A.L., Kornegay, J.N., 1993. Cervical vertebral fusion and concurrent intervertebral disc extrusion in 4 dogs. Vet. Radiol. Ultrasound 34, 336–339.

Beresford, W.A., 1981. Chondroid Bone, Secondary Cartilage and Metaplasia. Urban & Schwarzenberg, Baltimore-Munich. 454 pp.

de la Fuente, L., Helms, J.A., 2005. Head, shoulders, knees, and toes. Dev. Biol. 282, 294–330.

Dedi, J., Takeuchi, T., Seikai, T., Watanabe, T., 1995. Hypervitaminosis and safe levels of vitamin for larval flounder (*Paralichthys olivaceus*) fed *Artemia* nauplii. Aquaculture 133, 135–146.

Fjelldal, P.G., Grotmol, S., Kryvi, H., Gjerdet, N.R., Taranger, G.L., Hansen, T., Porter, M.J.R., Totland, G.K., 2004. Pinealectomy induces malformation of the spine and reduces the mechanical strength of the vertebrae in Atlantic salmon, *Salmo salar*. J. Pineal Res. 36, 132–139.

Gavaia, P.J., Dinis, M.T., Cancela, M.L., 2002. Osteological development and abnormalities of the vertebral column and caudal skeleton in larval and juvenile stages of hatchery-reared Senegale sole (*Solea senegalensis*). Aquaculture 211, 305–323.

Gill, C.D., Fisk, D.M., 1966. Vertebral abnormalities in sockeye, pink and chum salmon. Trans. Am. Fish. Soc. 95, 177–182.

Gillis, J.A., Witten, P.E., Hall, B.K., 2006. Chondroid bone and secondary cartilage contribute to apical dentary growth in juvenile Atlantic salmon (*Salmo salar* Linnaeus., 1758). J. Fish Biol. 68, 1133–1143.

Gil-Martens, L., Obach, A., Ritchie, G., Witten, P.E., 2005. Analysis of a short tail type in farmed Atlantic salmon (*Salmo salar*). Fish Vet. J. 8, 71–79.

Gjerde, B., Pante, M.J.R., Baeverfjord, G., 2005. Genetic variation for a vertebral deformity in Atlantic salmon. Aquaculture 244, 77–87.

Graff, I.E., Hoie, S., Totland, G.K., Lie, Ø., 2002. Three different levels of dietary vitamin D-3 fed to first-feeding fry of Atlantic salmon (*Salmo salar* L.): effect on growth, mortality, calcium content and bone formation. Aquac. Nutr. 8, 103–111.

Grotmol, S., Kryvi, H., Nordvik, K., Totland, G.K., 2003. Notochord segmentation may lay down the pathway for the development of the vertebral bodies in the Atlantic salmon. Anat. Embryol. 207, 263–272.

Grotmol, S., Nordvik, K., Kryvi, H., Totland, G.K., 2005. A segmental pattern of alkaline phosphatase (ALP) activity within the notochord coincides with the formation of the vertebral primordia. J. Anat. 206, 427–436.

Hall, B.K., 1977. Chondrogenesis of the somitic mesoderm. Adv. Anat. Embryol. Cell Biol. 53, 1–50.

Hall, B.K., 2005. Bones and Cartilage: Developmental Skeletal Biology. Elsevier, London. 760 pp.

Hall, B.K., Witten, P.E., in press. The origin and plasticity of skeletal tissues in vertebrate evolution and development. In: Anderson, J.S., Sues, H.D. (Eds.), Major Transitions in Vertebrate Evolution. Indiana University Press, Bloomington.

Hunter, C.J., Matyas, J.R., Duncan, N.A., 2003. The three-dimensional architecture of the notochordal nucleus pulposus: novel observations on cell structures in the canine intervertebral disc. J. Anat. 202, 279–291.

Huysseune, A., 2000. Skeletal system. In: Ostrander, G.K. (Ed.), The Laboratory Fish. Part 4 Microscopic Functional Anatomy. Academic Press, San Diego, pp. 307–317.

Huysseune, A., Sire, J.Y., 1990. Ultrastructural observations on chondroid bone in *Hemichromis bimaculatus* (Teleostei, Cichlidae). Tissue Cell 22, 371–383.

Huysseune, A., Verraes, W., 1990. Carbohydrate histochemistry of mature chondroid bone in *Astatotilapia elegans* (Teleostei: Cichlidae) with a comparison to acellular bone and cartilage. Ann. Sci. Nat., Zool. Paris 13(11), 29–43.

Koumoundouros, G., Divanach, P., Kentouri, M., 2001. Osteological development of the *Dentex dentex* (Osteichthyes: Sparidae): dorsal, anal, paired fins and squamation. Mar. Biol. 138, 399–406.

Kvellestad, A., Høie, S., Thorud, K., Tørud, B., Lyngøy, A., 2000. Platyspondyly and shortness of vertebral column in farmed Atlantic salmon *Salmo salar* in Norway—description and interpretation of pathological changes. Dis. Aquat. Org. 39, 97–108.

Leivseth, G., Frobin, W., Brinckmann, P., 2005. Congenital cervical block vertebrae are associated with caudally adjacent discs. Clin. Biomech. 20, 669–674.

Lewis, L.M., Lall, S.P., Witten, P.E., 2004. Morphological descriptions of the early stages of spine and vertebral development in hatchery-reared larval and juvenile Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 241, 47–59.

Lories, R.J.U., Luyten, F.P., 2005. Bone morphogenetic protein signalling in joint homeostasis and disease. Cytokine Growth Factor Rev. 16, 287–298.

Madsen, L., Dalsgaard, I., 1999. Vertebral column deformities in farmed rainbow trout (*Oncorhynchus mykiss*). Aquaculture 171, 41–48.

McKay, L.R., Gjerde, B., 1986. Genetic variation for a spinal deformity in Atlantic salmon, *Salmo salar.* Aquaculture 52, 263–272.

Nordvik, K., Kryvi, H., Totland, G.K., Grotmol, S., 2005. The salmon vertebral body develops through mineralization of two preformed tissues that are encompassed by two layers of bone. J. Anat. 206, 103–114.

Oegema, T.R., 2002. The role of disc cell heterogeneity in determining disc biochemistry: a speculation. Biochem. Soc. Trans. 30, 839–844.

Ørnsrud, R., Gil, L., Waagbø, R., 2004. Teratogenicity of elevated egg incubation temperature and egg vitamin A status in Atlantic salmon, *Salmo salar* L. J. Fish Dis. 27, 213–223.

Patt, D.I., Patt, G.R., 1969. Comparative Vertebrate Histology. Harper & Row Publishers, New York. 438 pp.

Pressley, M.E., Phelan, P.E., Witten, P.E., Mellon, M.T., Kim, C.H., 2005. Pathogenesis and inflammatory response to *Edwardsiella tarda* infection in the zebrafish. Dev. Comp. Immunol. 29, 501–513.

Roberts, R.J., Hardy, R.E., Sugiura, S.H., 2001. Screamer disease in Atlantic salmon, *Salmo salar* L., in Chile. J. Fish Dis. 24, 543–549.

Roy, P.K., Witten, P.E., Hall, B.K., Lall, S.P., 2002. Effects of dietary phosphorus on bone growth and mineralisation of vertebrae in haddock (*Melanogrammus aeglefinus* L.). Fish Physiol. Biochem. 27, 35–48.

Schaffer, J., 1930. Die Stützgewebe. In: von Möllendorf, W. (Ed.), Handbuch der mikroskopischen Anatomie des Menschen, zweiter Teil. Julius Springer Verlag, Berlin, pp. 1–54.

Smits, P., Lefebvre, V., 2003. *Sox5* and *Sox6* are required for notochord extracellular matrix sheath formation, notochord cell survival and development of the nucleus pulposus of intervertebral discs. Development 130, 1135–1148.

Toften, H., Jobling, M., 1996. Development of spinal deformities in Atlantic salmon and Arctic charr fed diets supplemented with oxytetracycline. J. Fish Biol. 49, 668–677.

Vågsholm, I., Djupvik, H.O., 1998. Risk factors for spinal deformities in Atlantic salmon, *Salmo salar* L. J. Fish Dis. 21, 47–53.

Wargelius, A., Fjelldal, P.G., Hansen, T., 2005. Heat shock during early somitogenesis induces caudal vertebral column defects in Atlantic salmon (*Salmo salar*). Dev. Genes Evol. 215, 350–357.

Wenger, K.H., Woods, J.A., Holecek, A., Eckstein, E.C., Robertson, J.T., Hasty, K.A., 2005. Matrix remodeling expression in annulus cells subjected to increased compressive load. Spine 30, 1122–1126.

Witten, P.E., Hall, B.K., 2002. Differentiation and growth of kype skeletal tissues in anadromous male Atlantic Salmon (*Salmo salar*). Int. J. Dev. Biol. 46, 719–730.

Witten, P.E., Hall, B.K., 2003. Seasonal changes in the lower jaw skeleton in male Atlantic salmon (*Salmo salar* L.): remodelling and regression of the kype after spawning. J. Anat. 203, 435–450.

Witten, P.E., Huysseune, A., 2007. Mechanisms of chondrogenesis and osteogenesis in fins. In: Hall, B.K. (Ed.) Fins and Limbs; Development, Evolution and Transformation. Chicago University Press, Chicago, 79–92.

Witten, P.E., Villwock, W., Renwrantz, L., 1998. Haematogram of the Tilapia *Oreochromis niloticus* (Cichlidae, Teleostei) and application of a putative phenoloxidase for differentiation between neutrophilic granulocytes and monocytes. Can. J. Zool. 76, 310–319.

Witten, P.E., Gil-Martens, L., Hall, B.K., Huysseune, A., Obach, A., 2005. Compressed vertebrae in Atlantic salmon (*Salmo salar*): evidence for metaplastic chondrogenesis as a skeletogenic response late in ontogeny. Dis. Aquat. Org. 64, 237–246.