한국어병학회지 제34권 제1호 (2021)
J. Fish Pathol., 34(1) : 117~121

www.ksfp.org
pISSN 1226-0819, eISSN 2233-5412
http://dx.doi.org/10.7847/jfp.2021.34.1.117

단 보

# Notochord opacity in fry ayu, *Plecoglossus altivelis*

**Min Do Huh†, Hyo Eun Lee, Mu Kun Lee* and Bo Sung Kim****

*Department of Aquatic Life Medicine, College Of Fisheries Science, Pukyong National University, Busan, Korea*
**Korean Aquatic Organism Disease Inspector Association, Busan, Korea*
***Aquatic Life Disease Control Division, National Fishery Products Quality Management Service*

An ayu (*Plecoglossus altivelis*) farm in Korea recently experienced an epidemic of vertebral column deformities where about 90% of fry displayed a vertebral column that was grossly opaque along either the cranial part of the column or its entire length. Abnormal fish were lordotic, scoliotic and/or kyphotic about midway down the spine. Examination of serial sections of whole fish showed only histological lesions in the vertebral column and suggested some disturbance in the early development of the vertebral centrum. Such abnormalities included a frayed spinal or notochord sheaths with irregular thickening and compression, mal-absorbed notochord cells, thickening of around cell layer and hypercellularity on both facets of the notochord sheath. No parasites, fungi, or bacteria were detected. While this lesion has only been reported once in the past, this is the first report of histopathological findings.

**Key words:** Fry of ayu, Notochord opacity, Notochord, Spinal deformity

## Introduction

Ayu, *Plecoglossus altivelis*, is one of the most important freshwater food fish and has long been cultured in Korea. In late 2001, about 90% of ayu fry on a commercial farm displayed white discoloration of the spinal column. Here we report the gross and histopathological features and discuss the possible mechanisms for lesion development.

## Materials and Methods

### History

Near the end of September 2001, fry ayu were submitted to our laboratory. The fish had been cultured at an inland ayu hatchery in Kyongnam Province, Korea. Fry were 65-85 days-old and 1.0-1.5 cm in total length. Water temperature was approximately 25 ℃. About 200,000 of fry were housed in polypropylene tanks which were 800cm in diameter and 80cm in depth. The outbreak was first noticed about 40 days after the feed was changed from live *Artemia* to a commercial particulate feed. Fifty days after the feed change, morbidity ranged from 20-90% in various tanks. Water quality was within normal limits. While no mortalities were attributed to the epidemic, all fry were eventually euthanized by the owner. According to the owner, this was his first experience with this disease in his 8 year old career at the ayu hatchery. He had kept the same routine feed and feeding schedule as previous years.

### Gross and histological examination

Six apparently normal and abnormal ayu from these groups were selected and fixed in Bouin's

†Corresponding author: Min Do Huh
Tel: +82-51-629-5942; Fax: +82-51-629-5942
E-mail: mindo60@gmail.com

solution. After fixation, the body was cross-sectioned at about 2-3 mm intervals along the body. The body sections were processed routinely for histology, embedded in paraffin and 5 μm serial sections were stained with hematoxylin and eosin (HE), Gomori methanamine silver and Giemsa. Digital light micrographs (Olympus BX50-32E01, Japan) were taken with an image capture system (Polaroid DMC-3, UK).

## Results and Discussion

Normal ayu were entirely transparent except for melanin pigment in the eye and melanophores in both the ventral or hypaxial and dorsal or epaxial skin. In contrast, abnormal ayu had a vertebral column that was grossly opaque along either the cranial part of the column or its entire length (Fig. 1). Many diseased individuals were lordotic, scoliotic and/or kyphotic about midway down the vertebral column or through its entire length (Fig. 1). The opaque segments of the vertebral column were connected by regularly spaced, round discs. No histological evidences for the association with parasites, fungi, or bacteria were detected.

Microscopic lesions were only observed along the entire length of the vertebral column and its surrounding connective tissue (Fig. 2). Normal individuals showed an orderly microanatomy (Fig. 2A and 2C), with a nearly resorbed notochord, that was surrounded by single-celled primary and secondary notochord sheaths and a single cell thick osteogenic layer. In contrast, all diseased individuals had a severely frayed notochord sheath which was irregularly thick and compact, as well as notochord remnants (Fig. 2B and 2D). In addition, there was hypercellularity on both sides of the notochord sheath (Fig. 2D). Hypercellularity of the inner layer seemed to be due to abnormal resorption of the notochord. Hypercellularity of the periphery extended over the entire surface of developing vertebra. The collagenous stroma of the notochord sheath in diseased individuals was not homogeneously stained and showed a somewhat undulating appearance, suggesting irregular collagen packing.

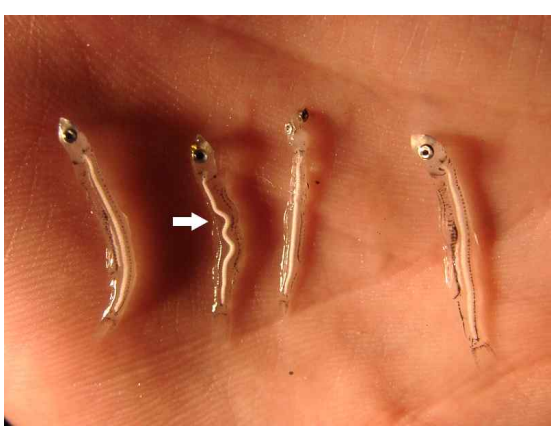

Fig. 1. ayu fry showing an opaque spinal column in sharp contrast to their transparent body. one of them shows a lordotic, kyphotic and scoliotic spinal column (arrow).

We confirmed that the vertebral column and its notochord remnants were opaque by comparing the gross findings with histological findings. Grossly, there was a transparent area between the meningeal melanophores and the area corresponding to the notochord which exactly corresponds to the spinal cord (Fig. 3A). This evidence was histologically supported by the melanophores that were distributed on the dorsal meningeal border of spinal cord (Fig. 3B). The notochord is only a small part of the vertebral column (see comment 13 below).

There is little information about the exact physicochemical mechanism that can account for the maintenance and loss of a fish's transparency. However it is known that ocular corneal transparency is maintained by highly ordered collagen architecture of the corneal stroma (Maurice, 1957). Collagen architecture is also closely associated with the nature of stromal ground substances (Kostyuk et al, 2002, Chakravarti et al, 2000, Conrad & Fundergurgh, 1992).

Fish transparency could also be maintained by an orderly arrangement of the collagen matrix. Hypercellularity of the affected notochord and vertebral col-

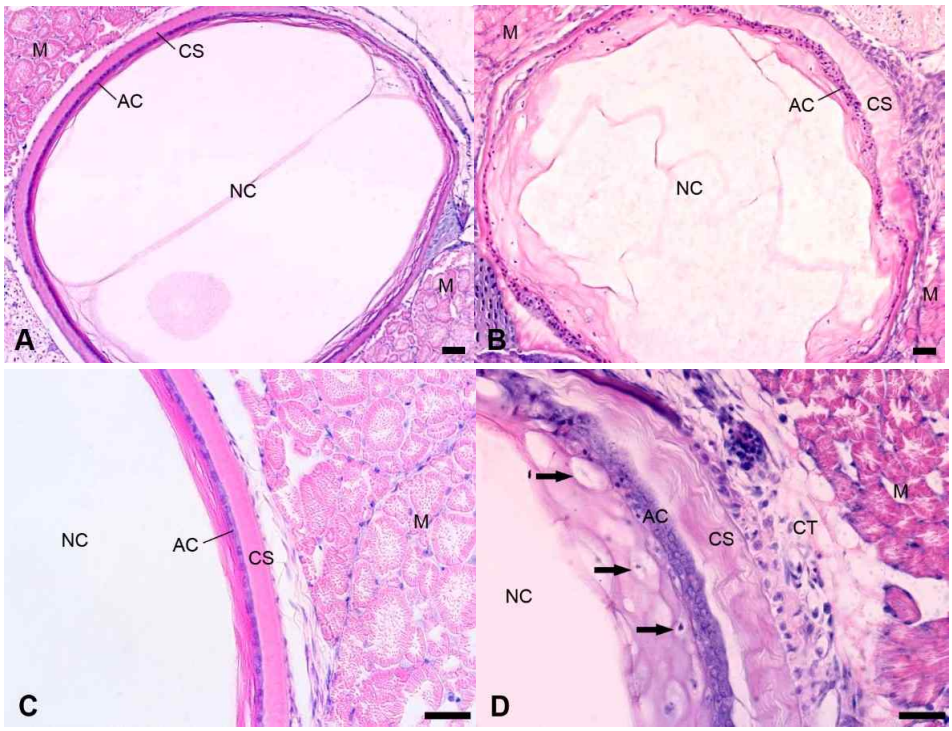


Fig. 2. Histological sections of the spinal column from normal ayu (A, C) and ayu with spinal column opacity (C, D). AC: around cell layer, CS: notochord sheath, CT: connective tissue, M: lateral skeletal muscle, NC: center of the notochord, Arrows: Mal-absorbed degenerative notochord cells, Scales: 20 μm.

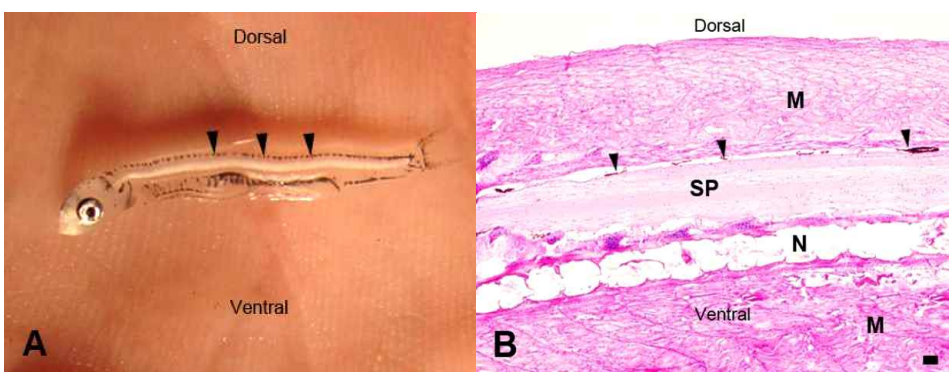


Fig. 3. Gross (A) and histological findings (B) of an affected fish showing notochordal opacity. The transparent spinal cord is located beneath a line of meningeal melanophores (arrow heads) and the opaque vertebral column consisting of notochord remnants (N) and ossified vertebrae is a transparent spinal cord located. SP: spinal cord, M: lateral skeletal muscle, Scales: 20 μm.

umn sheaths might also contribute to the development of this lesion (Jester et al 1999). Hypercellularity of epithelial cells is a common and well-known cause of opacity in fish skin (e.g., such as caused by ecto-parasite infestations).

Regardless of the mechanism the opacification of

the vertebral column could be explained by the histological abnormalities of the vertebral column, especially alterations of the chorda sheath. Backscattering of the light passing through the altered vertebral column could block light transmission (Jester et al 1999).

To our knowledge, the only other report of this type of lesion is that of Sorimach (1994), who described 'whitening disease of notochord' in ayu and considered it to probably be caused by nutritional deficiency. However, no microscopic pathology was presented.

A variety of skeletal deformities, including those affecting the spinal column, have been reported in farmed fish (Silverstone 2002, Roberts 2001, Faustino & Power 1998, Ferguson 1989). Spinal deformities mainly include scoliosis, lordosis, and vertebral fracture. From our histopathological findings, it seems that diseased ayu had some problem during notochord regression and early ossification. This indicates that opacification of the vertebral column in ayu is a type of skeletal deformity which could occur in an early stage of development.

The factors that contribute to skeletal deformity may be genetic, environmental, or both (McKay & Gjerde 1986). The history of this outbreak in ayu suggests that the feed might have been the primary cause, since this was a new batch of feed and everything else on the farm had presumably stayed the same since before and after the epidemic (that is, only the feed batch was different, the environment and genetic source of fish was the same). Deficiencies in phospholipids, highly unsaturated fatty acids, certain amino acids (ex, tryptophan), vitamin A, some peptides (ex, protein hydrolysates) or ascorbate can cause skeletal deformities in young fish (Cahu et al 2003). The histopathological changes in the spinal column strongly suggest that diseased ayu might have had some disturbance in collagen organization. Vitamin C is essential for the formation of hydroxyproline and hydroxylysine, which gives collagen its helical structure, its rigidity and structural strength (Roberts 2001, Jones et al 1997). A high requirement for calcium by fish is also needed for proper development of calcified tissues such as bone and scales (Roberts 2001).

Further studies are needed to confirm whether diet plays a role in this disease. This time we unfortunately missed the chance to examine the hepatic tissue of abnormal ayu. For the complete assimilation of a commercial particulate feed, the integrity of hepatic homeostatic function of ayu should be established. Overfeeding easily committable in farming condition can cause the hepatic dysfunction which resultantly could lead to the failure of proper nutritional assimilation.

## References

Cahu, C., Infante, J.Z., Takeuchi, T. Nutritional components affecting skeletal development in fish larvae, Aquaculture 227:245-258. 2003.

Chakravarti, S., Petroll, W.M., Hassell, J.R., Jester, J.V., Lass, J.H., Paul, J. & Birk, D.E. Corneal opacity in lumican-null mice: defects in collagen fibril structure and packing in the posterior stroma. Invest Ophthalmol Vis Sci, 41:3365-3373. 2000.

Cahu, C., Infante, J.Z., Takeuchi, T. Nutritional components affecting skeletal development in fish larvae, Aquaculture 227:245-258. 2003.

Chakravarti, S., Petroll, W.M., Hassell, J.R., Jester, J.V., Lass, J.H., Paul, J. & Birk, D.E. Corneal opacity in lumican-null mice: defects in collagen fibril structure and packing in the posterior stroma. Invest Ophthalmol Vis Sci, 41:3365-3373. 2000.

Ferguson, H.W. Systemic pathology of fish, Iowa State University Press, pp. 215-229. 1989.

Faustino, M. & Power, D.M. Development of osteological structures in the sea bream vertebral column and caudal fin complex. J Fish Biol, 52: 11-22. 1998.

Gllick, J., Giles, S., Bannigan, S. & Puri, P. Midgut atresia result from abnormal development of the notochord in an adriamycin rat model. J. Pediatric Surgery, 37: 717-722. 2002.

Jester, J.V., Moller-Pederson, T., Huang, J., Sax, C.M., Kays, W.T., Cavangh, H.D., Petroll, W.M. & Piatigorsky, J. The cellular basis of corneal transparency: evidence for 'corneal cystallins'. J Cell Sci, 112: 613-622. 1999.

Jones, T.C., Hunt, R.D., King, N.W. Veterinary pathology, 6th ed, Williams & Wilkins. pp. 781-815. 1997

Kostyuk, O., Nalovina, O., Mubard, T.M., Regini, J.W., Meek, K.M., Quantock, A.J., Elliott, G.F. & Hodson, S.A. Transparency of the bovine corneal stroma at physiological hydration and its dependence on concentration of the ambient anion. J Physiol, 543:633-642. 2002.

Makay, L.R., Gjerde, B. Genetic variation for spinal deformity in Atlantic salmon, Salmo salar. Aquaculture 52:263-272. 1986.

Maurice, D.M. The structure and transparency of the cornea. J Physiol, 136:263-286. 1957.Hardy, R.W. The nutritional pathology of teleosts. In: Fish Pathology, Roberts RJ, 3rd ed, W B Saunders, pp. 347-366. 2001.

Santamaría, J.A., Andrades, J.A., Herráez, P., Fernández-Llebrez, P. & Becerra, J. Perinotochordal connective sheet of gilthead sea bream larvae (Sparus aurata, L.) affected by axial malformations: An histochemical and immunocytochemical study. The Anatomical Record 240: 248-254. 1994.

Silverstone, A.M., Hammell, L. Spinal deformities in farmed Atlantic salmon. Can Vet J, 43:782-784. 2002.

Sorimach, M. A manual for rapid diagnosis of bacterial fish disease (in Japanese). Ministry of Agriculture, Forestry and Fisheries, Zinnou, Japan. 1994.

Manuscript Received : Apr 05, 2021
Revised : Jun 08, 2021
Accepted : Jun 10, 2021