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Occurrence of eye abnormality in cultured red seabream (*Pagrus major*) and induced hybrid, red seabream *Pagrus major* (♀) × black seabream *Acanthopagrus schlegeli* (♂)

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Red seabream, *Pagrus major*, is a valuable aquaculture species in Korea, but spontaneous eye abnormality occurred in cultured individuals. The incidence of eye abnormality was 4% in the group of cultured red seabream. The abnormality was characterized by unilateral and bilateral exophthalmos, opacity and lens pathology. Lense prolapse was found in two cases. Lenses in diseased fishes was considerably small in diameter and eyes were deformed as a whole. In the group of induced hybrid red seabream *Pagrus major* (♀) × black seabream *Acanthopagrus schlegeli* (♂), the incidence of eye abnormality was the same 4%, but only opacity was registered. Opacity was also found in two of sixteen examined wild-caught red seabream. Histopathological changes of lenses in cultured red seabream included vacuolated cytoplasm of lens fibers, necrosis of fibers in central part of lens, folding and increase in thickness of lens capsule, and epithelial proliferation beneath the anterior lens capsule. In affected eyes no parasites or gas bubbles were found.

Key words : Red seabream (*Pagrus major*), Hybrid (female *Pagrus major* × male *Acanthopagrus schlegeli*), Eye, Pathology, Lens abnormality, Cataracts

Introduction

Marine aquaculture has been developing rapidly in Asia and Korea (Choi, 1997). Annual production of cultured marine fish has constantly increased. Red seabream, *Pagrus major* Temminck et Schlegel, belonging to Sparidae is widely distributed in the coastal waters in Korea and is a valuable aquaculture species. A variety of parasites, bacteria and viruses are recognized as serious pathogens of this species including *Pasteurella piscicida* (Yasunaga *et al.*, 1983), *Flexibacter maritimus* (Wakabayashi *et al.*, 1984), *Vibrio* sp. (Yasunobu *et al.*, 1988), *Edwardsiella* sp. (Choi, 1997). Red seabream iridoviral disease caused great losses in cultured red seabream in Japan (Tanaka, 2000). This disease also is registered for fishes from the coastal waters of Korea (Jung and Oh, 2000; Kim *et al.*, 2002). As with infective and invasive diseases deformities such as body shortening, pugheadness and lordosis put an essential loss to cultivating red seabream (Kumai *et al.*, 2001; Kihara *et al.*, 2002).

Eye lesions of fish are numerous and varied in their aetiology (Roberts, 1978). Most common are exophthalmos, cataracts, keratopathy, various retinopathies, and uveitis (choroids and iris system lesions) (Hargis, 1991). Cataracts or lens opacities in cultured fish have been shown to occur as a result of sub-optimal nutrition, toxic agents, parasites, variation in temperature, rapid growth and exposure

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to sunlight (Hargis, 1991). The purpose of the present study was to examine eye abnormality in cultured red seabream *P. major* and hybrid between female red seabream and male black seabream *Acanthopagrus schlegeli* and describe histopathological changes in abnormal eyes.

Materials and Methods

Fish

The description of eye pathology was based on observation in three groups of both wild-caught and captive-bred fish over a 6-month period November 2002 to April 2003. The first group comprised of 1 tank of wild-caught red seabream (n=16), the second group 4 tanks of captive-bread red seabream (n=100), and third group 2 tanks of induced hybrid fish, *Pagrus major* (♀) × *Acanthopagrus schlegeli* (♂) (n=50). Adult red seabreams *P. major* were collected using set-nets from the coastal water of Busan Bay. Adult induced hybrid were given from National Fisheries and Development Agency and transported to the laboratory aquarium at Korea Maritime University and cultured for 3 months prior to study. Fish were held in 2000 L tanks, each containing about 25 fish, and cultured in ambient water temperature condition. The water exchange rate was 30 L min^{-1}, and tanks were illuminated for 10 h daily. Fish were fed by commercial marine fish diet (Agiribrand Korea).

Observations were carried out daily and clinical signs of diseases, mortalities and any comments relevant to the appearance of fish were recorded; only sporadic mortalities have been detected during the period of study. Before examination the fish were killed with an overdose of lidocaine-HCl/$NaHCO_3$. Freshly sampled eyes were dissected out, measured and fixed 10% neutral formalin. Four indices of eye were measured using Vernier caliper: diameter of lens (D_{lens}) , diameter of eye in frontal (D_1) and dorsal (D_2) planes and thickness of eye in cm. External eye morphology of fish was taken using digital camera (Nikon, Japan) under lightening condition.

Histological techniques

Formalin fixed eyes were fixed again in Bouin's solution and embedded in paraffin. Sections of 5㎛ thickness were stained with Mayer's hematoxylin and eosin (HE) and examined.

Results

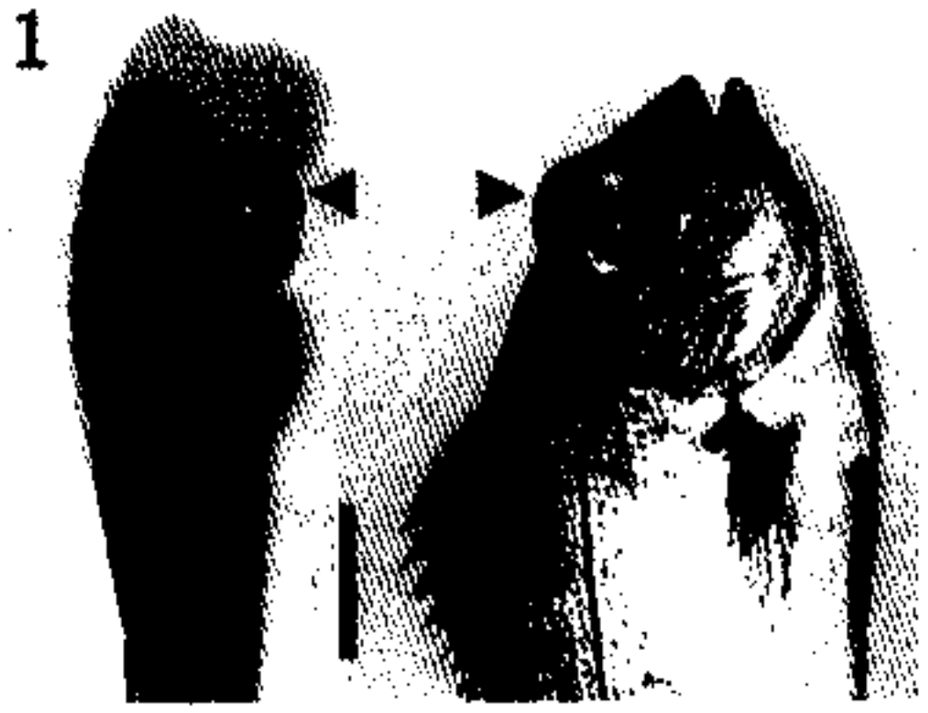
Gross pathology

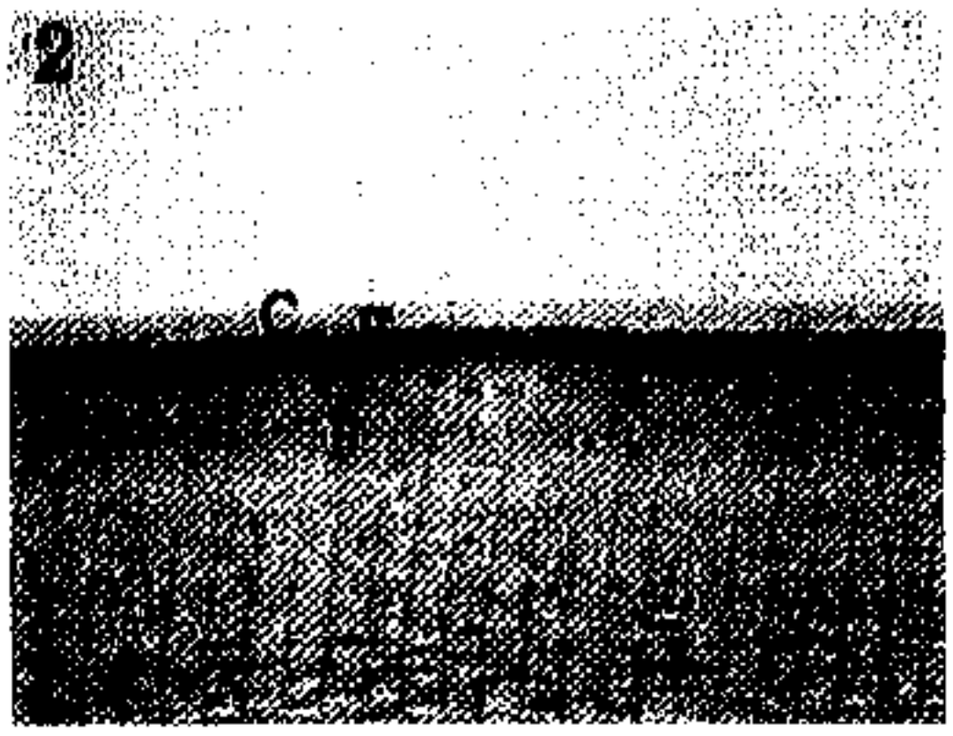
The incidence of eye abnormality was 4% in the group of cultured red seabream. Changes were characterized by unilateral (3 cases from 100 individuals) (Fig. 1) and bilateral (1 case) exophthalmos, lens pathology (2 cases). After dissection of eyes, lense prolapse in the interior of eyeball was found. As shown in Table 1, lens in control red seabream was 0.46 cm in diameter but 0.20 and 0.24 cm in respective 2 diseased cases. Diseased eyes were deformed as a whole because diameters of eye in frontal and dorsal planes were unequall, compared with control fish. In the group of hybrid fish, eye abnormality was same 4% (2 cases from 50), but only opacity was registered. Opacity was

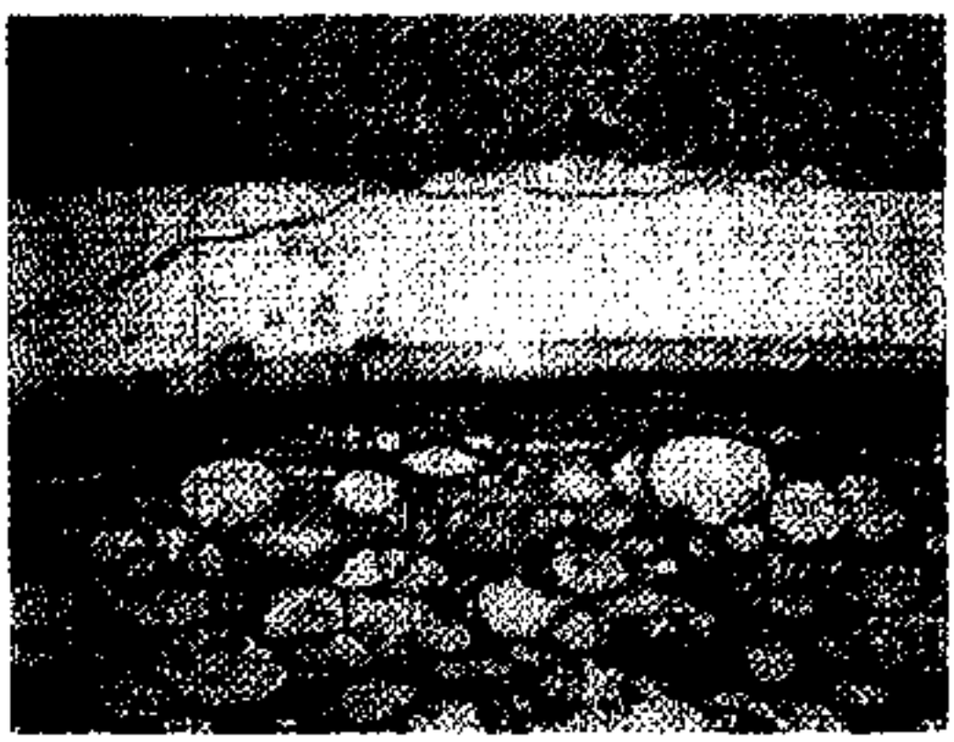
Table 1. Diameter of lens and eye in normal and diseased red seabream, *Pagrus major*

Indices*	Normal eye (cm)	Diseased eyes (cm)	
		Case 1	Case 2
D_{lens}	0.46	0.24	0.20
$D_{1\ lens}$	1.16	1.40	1.33
$D_{2\ lens}$	1.16	1.26	1.15
Thickness of eye	0.95	1.10	0.83

* D_{lens}: diameter of lens; $D_{1\ eye}$: diameter of eye in frontal plane; $D_{2\ eye}$: diameter of eye in dorsal plane.







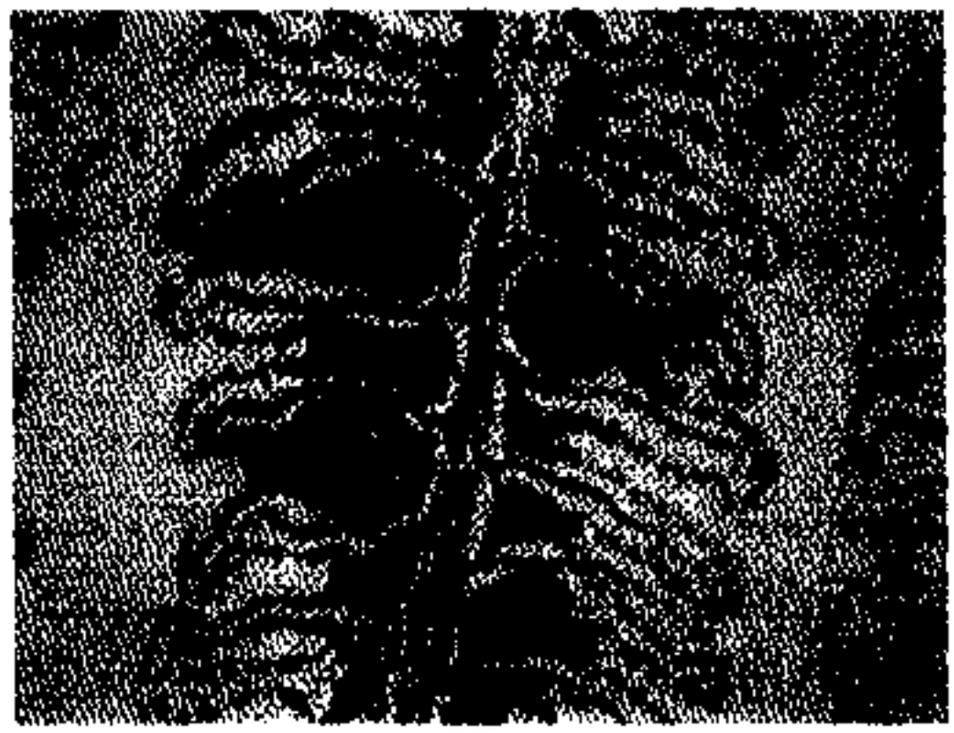


Fig. 1. A cultured red seabream, *Pagrus major* with unilateral exophthalmos (arrow heads). Left : frontal view; Right : dorsal view. Bar indicate 2 cm.

Fig. 2. Histological section of lens of control fish (HE, × 200). C : capsule; E : epithelium; F : fibers, differentiated subcapsular cells.

Fig. 3. Histological section of lens of diseased fish (HE, × 200). Note severe vacuolization of lens fibers and flattened shape of subcapsular epithelial cells. Designation the same as in Fig. 2.

Fig. 4. Necrosis in the central part of lens in diseased eye (HE, × 200).

Fig. 5. The increase in size of lens capsule and its folding. Note the epithelial hyperplasia (arrow) near capsule foldings (HE, × 200).

Fig. 6. Histological section of gill of cultured diseased red seabream. Note the cysts located in the interlamellar spaces which have pseudocapsule formed by fish cells (arrows). Cysts caused a tissue reaction, i.e. the hyperplasia and the lamellar fusion (HE, × 560).

also found in two wild-caught red seabream (2 cases of 16). In diseased fish gills were pale.

Histopathology of eye

It was histologically revealed that structure of lenses in affected fish had many alterations. In control fish lens had uniformed capsule and subcapsular epithelium consisting a layer of cuboidal cells (Fig. 2). In diseased fish the epithelium was slightly flattened. Lens fibers (differentiated subcapsular cells) closer to surface of lens were severely vascuolated (Fig. 3). Fibers in central part of lens were necrotic (Fig. 4). The lens capsule has many foldings and their thickness was increased (Fig. 5). Epithelial proliferation beneath the anterior lens capsule was found in some places more often near capsule folding. Such pathological changes are characteristic of cataractous lenses.

Under normal condition the retina is composed of three layers of cells: photoreceptor cells, bipolar neurons, and ganglion cells; layers are distinctly seen. In diseased eyes these three layers in some places were not seen clearly because layers were dislocated. A severe haemorrhagic lesion was noted in choroids. No parasitic evidences or gas bubbles were seen in association with eyes lesions.

Histological study of gills of effected fishes revealed the presence of numerous basophilic inclusions or 'cysts' in interlamellar regions and at lamellar tips. All cysts exhibited the same amorphous appearance. Proliferative cell response and lamellar fusion were observed in association with cysts (Fig. 6).

Discussion

Exophthalmos occurs as in wild and cultured fishes (Stephens *et al.*, 2001). Sometimes exophthalmos are associated with gas in the eyes. In the west Australian dhufish, *Glaucosoma hebraicum*, from coastal waters, exophthalmos was associated with the presence of gas bubbles in the anterior chamber and heamorrhages in the choroids (Stephens *et al.*, 2001). Stephens *et al.* (2001) also reported that exophthalmos was occasionally seen in snapper, *Pagrus auratus*, but not in black bream, *Acanthopagrus butcheri*, at the same facility as dhufish. In present study, no gas bubbles were found in red seabream.

Exophthalmos was very often developed in experimental dhufish *Glaucosoma hebraicum*. Prevalence varied 26 to 68% in first-generation offspring of wild-caught fish over 1 year of age. In experimental fish mortality was associated with bacterial septicaemia, epitheliocystis, *Caligus* sp., and bronchitis and hyperplasia of branchial epithelial cells, because of infestation with monogenean *Haliotrema* sp., but were not associated with the development of exophthalmos (Stephens *et al.*, 2001). No mortality of fish with eye abnormality occurred during the present study.

The lens is affected by a number of pathological processes, all leading to progressive degenerative cataract formation. There have been increasing numbers of reports on cataracts or lens opacities in farmed fish. In smolting Atlantic salmon, *Salmo salar* L., farmed in northern Norway, the mean incidences of cataracts in February, April and May were 12, 46 and 84%, respectively (Waagbø *et al.*, 1996). In present study occurrence of cataracts in red sea bream were much more rare, about 2%, but we studied more grown-up fish.

Cataracts in cultured fish have been shown to occur as a result of sub-optimal nutrition, such as deficiencies of zinc (Richardson *et al.*, 1986), riboflavin (Hughes *et al.*, 1981), thiamine (Hughes, 1985), methionine (Poston *et al.*, 1977) and tryptophan (Poston and Rumsey, 1983).

The pathological changes of cataractous lenses,

including the pathology following nutritional related cataracts, include hydropic swelling and lysis of lens fibers, and attempted regeneration resulting in epithelial hyperplasia and sometimes capsular reduplication (Wilcock and Dukes, 1989; Hargis, 1991). In the present study, we found such pathological changes of lens as thickening of lens capsule, epithelial proliferation at the anterior pole, hydropic vacuolization of lens fibers and necrosis. This is in agreements with previously reported histopathological alterations in Atlantic salmon (Waagbø *et al.*, 1996).

Waagbø *et al.* (1996) have proposed a special method for cataract severity assessment. According to that method the severity of cataract were graded from 0 representing healthy fish to 3 representing fish with severe cataract. Two cases of cataract occurring during this study were classified as 1 grade.

Histological study of gills of examined fish with eye abnormality revealed the presence of numerous basophilic inclusions or 'cysts'. Perhaps affected fish additionally suffered from infectious gill disease, epitheliocystis, induced by prokaryotic intracellular organisms.

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