

## **Health status of wild and cultured sea bass in the northern Adriatic Sea**

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**ABSTRACT:** A complex survey has been conducted in the northern Adriatic Sea over a period of one year that included comparative parasitological, bacteriological, virological, histological and biochemical studies of the cultured and wild sea bass (*Dicentrarchus labrax* L.). The results show that parasite infestations were due mainly to ectoparasitic monogenea *Diplectanum* sp. in both cultured and wild sea bass. *Philometra* sp. and *Lernaea* sp. were detected in wild sea bass while *Triaenophorus* sp. and *Eimeria* sp. were found in reared sea bass. Bacterial pathogens isolated from both reared and wild sea bass belong to Pseudomonadaceae (*Pseudomonas* sp., *P. fluorescens*) and unknown Gram-negative bacteria. Moraxellaceae (*Acinetobacter* sp.), Vibrionaceae (*Shewanella putrefaciens*), Enterobacteriaceae (*Pantoea agglomerans*) and *Flavobacterium* sp. were isolated from reared fish only. Virological examinations were negative. Histological analysis revealed “fatty liver” (fatty infiltration and degeneration) in the cultured fish. Triglyceride, cholesterol and glucose levels were higher in cultured sea bass ( $2.55 \pm 1.77$  mmol/l,  $3.68 \pm 1.43$  mmol/l and  $9.97 \pm 3.33$  mmol/l, respectively) than in wild fish ( $0.80 \pm 0.57$  mmol/l,  $2.95 \pm 0.77$  mmol/l and  $4.79 \pm 3.29$  mmol/l, respectively). The present paper contributes to establishing a relationship between disease and pathophysiological conditions in wild and cultured fish.

**Keywords:** Adriatic Sea; sea bass; bacteria; parasite; triglyceride; cholesterol; glucose

The Adriatic Sea is a gulf in the Mediterranean. The northern Adriatic is the northernmost part of the Mediterranean (it extends to 45°47'N). It is a moderately warm sea. Sea temperatures are mild and during the winter their range is between 10 and 13°C while summer range is between 22 and 26°C (Sarusic, 2000). Average salinity is about  $38.3 \times 10^{-3}$  but in some years there is a more powerful influx of the more salty eastern Mediterranean water into the Adriatic which intensifies the salinity of the sea so that the values are higher than  $39 \times 10^{-3}$  (Jardas, 1996).

The farming of marine organisms has a long tradition on the eastern Adriatic coast, but vigorous development of mariculture started in the seven-

ties of the last century and continues there with increasing intensity. With increasing importance of marine aquaculture, fish diseases are becoming a more serious problem. Being under constant veterinarian supervision those problems are recognized and treated, but the health status of the wild fish population remains largely unexplored. Sea bass (*Dicentrarchus labrax* L.) is one of the most popular marine fish raised in the Adriatic Sea. It is intensively cultured in floating cages. Since cage culture is an open system exposed to cross dissemination of infection between cultured and wild fish (Paperna, 1984), the aim of the study was to investigate the difference in the health condition of cage reared versus wild sea bass population.

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## MATERIAL AND METHODS

The study included 98 sea basses *Dicentrarchus labrax* L. ( $253.3 \pm 102.8$  g weight;  $267.2 \pm 35.9$  mm length) from two fish farms (Porto Budava and Limski Channel) reared in floating cages and 30 wild, free living sea basses ( $460.6 \pm 193.92$  g weight,  $343.2 \pm 46.5$  mm length) netted in Tarska Bay (belong to the same water body and microclimate as the farms in question). Samplings were undertaken in 1999/2000. After capture, fish were held in containers filled with seawater for no more than 4h before processing. During the investigation the sea temperature (UC-12 Digital DO/O2 Temp.meter-Central Kagaku) ranged from 11.5 to 23.2°C and salinity (SPR-N refractometer Atago, Japan) ranged from 16 to  $40 \times 10^{-3}$ . Several diagnostic procedures were done as a part of complete necropsy and subsequent analyses on each fish. Blood was collected from the caudal vein/artery into 2 ml syringes for smears, biochemical analysis and electron microscopy. Thin smears were stained with the May-Grünwald/Giemsa (M-G). One thousand erythrocytes per slide were examined under light microscope (1 000×) for the presence of cytoplasmic inclusions that are characteristic of viral erythrocytic infections (VEI). For electron microscopy, 1 ml of blood samples was fixed in 2% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated with ethanol and embedded in Epon-Araldite resin (Sigma). Ultrathin sections of the samples were stained in uranyl acetate and lead citrate and examined at 60kV with electron microscope Zeiss, EM 10A. Remaining blood was centrifuged at  $11\ 200 \times g$  for 95 seconds (StatSpin MP, USA) and resultant plasma was frozen for storage until analysis. Plasma triglyceride, cholesterol and glucose levels were determined by a biochemical analyser (VETTEST 8008, USA). For parasitological assay wet mounts of gills and skin were examined by light microscopy for the presence of ectoparasites. All suspected materials were fixed in 70% ethanol until the identification. Identification was done according to Bykhovskaya-Pavlovskaya *et al.* (1962). Samples for bacteriological examination were obtained from the spleen, kidney and liver aseptically inoculated onto Tryptic soy agar (Biolife, Italy) supplemented with 2% NaCl. Plates were incubated at 22°C for up to 5 days. Representative colonies of the different morphological bacterial types were isolated and restreaked on fresh medium until purity was attained. Pure cultures of the

isolated bacteria were subjected to morphological, physiological and biochemical tests. The taxonomic position of isolates was determined following the schemes of Austin and Austin (1987) and Krieg and Holt (1984). Also, API-20E systems (Biomerieux, France) modified for marine isolates were applied. Virological assay was performed according to OIE (1997). *Epithelioma papillosum cyprini* (EPC), fathead minnow (FHM) and chinook salmon embryo (CHSE-214) cell cultures were inoculated with homogenates of liver, spleen and kidney and incubated at 15–20°C. Cells were examined for the presence of cytopathic effect (CPE). If no CPE developed in the inoculated cultures, the inoculated cultures were subcultured for further 7 days. For a histological examination, samples of liver, spleen, kidney and brain were fixed in 4% neutral buffered formalin, dehydrated through a graded ethanol-xylene series and embedded in paraplast. Sagittal and transverse sections 3–5 µm thick were stained with haematoxylin/eosin (H&E).

## RESULTS AND DISCUSSION

In the present paper, the pathological findings of cultured and wild fish from the northern Adriatic Sea are described.

Clinical signs observed in the examined cultured sea bass included skin reddening and fine base reddening only in one fish (0.98%). Internally, liver haemorrhages were observed in 3 fishes (2.94%), white nodules (1 mm of diameter) on the kidney surface in 3 fishes (2.94%) and spleen enlargement (splenomegaly) in 7 fishes (6.86%). In the wild population of sea bass examined no macroscopic changes were found. In the internal organs, white nodules (1 mm of diameter) on spleen were recorded only in one wild sea bass (0.6%). Despite the number of pathogens isolated, there was not always a link between isolated bacteria and post-mortem findings on respective specimens. The pathological conditions found in the cultured and wild fish and their overall prevalence are shown in Table 1. Bacterial pathogens isolated from reared sea bass belong to Pseudomonadaceae (*Pseudomonas* sp., *P. fluorescens*), Moraxellaceae (*Acinetobacter* sp.), Vibrionaceae (*Shewanella putrefaciens*), Enterobacteriaceae (*Pantoea agglomerans*), *Flavobacterium* sp., and unknown Gram-negative, facultatively anaerobic, non-motile bacteria that were cytochrome oxidase positive. The API-20

Table 1. Infectious agents and pathological conditions of cultured and wild *Dicentrarchus labrax* L. in the northern Adriatic Sea

Infectious agents	Overall prevalence (%)		Organ
	Cultured fish	Wild fish	
<b>Bacteria</b>			
Enterobacteriaceae ( <i>Pantoea agglomerans</i> )	2.04	0	Sp
Vibrionaceae ( <i>Shewanella putrefaciens</i> )	3.06	0	L, Sp
Moraxellaceae ( <i>Acinetobacter</i> sp.)	3.92	0	L, Sp
Pseudomonadaceae ( <i>Pseudomonas</i> sp., <i>P. fluorescens</i> )	7.84	20	G, L, K, Sp
Unknown Gram-negative bacteria	3.92	3.33	Sp
<i>Flavobacterium</i> sp.	2.04	0	L
<b>Parasites</b>			
<i>Diplectanum</i> sp.	50	16.66	G
<i>Philometra</i> sp.	0	3.33	G
<i>Lernae</i> sp.	0	3.33	Sk, M
<i>Triaenophorus</i> sp.	1.02	0	L
<i>Eimeria</i> sp.	2.04	0	I, L

Explanations: L = liver, K = kidney, Sp = spleen, G = gills, Sk = skin, M = muscle, I = intestine

systems showed a doubtful profile for those bacteria and placed them with 71% confidence in the *Pasteurella multocida* group. However, the API strip biochemical profile of all 32 reactions had only a positive reaction of ornithine decarboxylase, which is unlikely for the *P. multocida*. Since these profiles differ from those expected for *Aeromonas salmonicida* and *Photobacterium damsela*, these bacteria were classified as unknown. The pathogenic microorganisms isolated from wild population also belong to those unknown Gram-negative bacteria and Pseudomonadaceae. Most of the isolates were morphologically and biochemically identical. Majority of bacteria that infect sea bass, with several exceptions, are saprophytic, facultative and opportunistic organisms that often cause debilitating infections following some predisposing factors. *P. fluorescens* is one cause of haemorrhagic septicemia of marine fish (Austin and Austin, 1987; Frerichs and Roberts, 1989). High temperatures and crowding are also contributing factors. *Acinetobacter* sp. are common inhabitants of marine ecosystem and populate skin, gills and digestive tract. Any break of the integument of the host may lead to colonization of the other organs and start a disease cycle,

although the pathogenic mechanism of the organism is unknown. *Shewanella putrefaciens* is probably a part of the normal microflora in fish (Gillespie, 1981) but it can cause disease with high mortalities in rabbitfish *Siganus rivulatus* farmed in sea cages (Saeed *et al.*, 1987).

Among the parasitic infections shown in Table 1, the monogenea *Diplectanum* sp. was the most frequent in both groups. Prevalence was higher in cultured (50%) than in wild fish (16.66%), which may be a consequence of rearing conditions (high stocking density). *Diplectanum* sp. is a monogenean gill parasite that causes proliferative reactions of epithelial cells in the infected gills, but fish under investigation had no such extent of parasite invasion. Wild sea bass were infested with the nematode *Philometra* sp. and crustacea *Lernaea* spp. In different developmental stages *Philometra* sp. migrates through visceral organs. The consequence of this migration is inflammation and ulceration of the infested organs. Crustacea *Lernaea* sp. are encountered in fish species in both fresh and salt water. This is a highly adapted crustacean that penetrates the host's skin to form an extremely strong and damaging attachment. The

stage usually seen is the adult female, attached to the surface of the fish. Wounds caused by the parasite are prone to secondary infections. It is a problem in warm water and during the summer months in temperate regions (Southgate, 1993). *Triaenophorus* sp. and *Eimeria* sp. were found only in cultured fish. Plerocercoids of *Triaenophorus* sp. are the most commonly found in the viscera and musculature of fish where encysted cause extensive damage (Southgate, 1993). Coccidia *Eimeria* sp. can cause nodular coccidiosis in the submucosa of the gut. In our investigation *Eimeria* sp. was found in the gut wall and liver. Virological examinations on EPC, FHM and CHSE-214 cell cultures were negative and there was no cytopathic effect even after 2 blind passages. Brain examinations did not reveal viral lesions connected to nodavirus infection. Pinto *et al.* (1989) described the occurrence of a viral erythrocytic infection with cytoplasmic inclusion bodies and nuclear degeneration in the Mediterranean wild and cultured sea bass. While analysing blood smears under light microscope, erythrocyte nuclei with irregular shape were observed (in both wild and reared sea bass) but no intracytoplasmic inclusions were seen. Also, an extensive survey of blood samples under electron microscope did not reveal any suspected virus particles. Such nuclear abnormalities could be a consequence of toxicities or nutritional deficiencies such as folic acid deficiency (Hibiya, 1985). Histopathological analysis revealed "fatty liver" (fatty infiltration and degeneration) in all examined cultured sea bass, possibly as a consequence of intensive manufactured food intake. This syndrome is caused by over-storage of lipids in the hepatocytes, which leads to vacuolization, atrophy and degeneration of these cells with partial displacement of the liver by adipose tissue (Christoflogiannis, 1993). The disease may be transferred from farmed to wild fish stocks if the wild fish have contact with farmed ones, especially in the months when the natural feed is not abundant and farms become a rich source of manufactured food for free-living fish. As the diet of sea bass in farms (consisting of extruded commercial pellets) is high in fat content, intensive food intake can lead to changes in the plasma parameters such as triglycerides, cholesterol and glucose. So, triglyceride, cholesterol and glucose levels were higher in cultured sea bass ( $2.55 \pm 1.77$  mmol/l,  $3.68 \pm 1.43$  mmol/l and  $9.97 \pm 3.33$  mmol/l, respectively) than in wild fish ( $0.80$

$\pm 0.57$  mmol/l,  $2.95 \pm 0.77$  mmol/l and  $4.79 \pm 3.29$  mmol/l, respectively).

The results show similarities (bacterial and parasitic diseases) and differences (biochemical parameters) between wild and cultured fish but only continued monitoring (especially wild fish) during several years will show the real epizootological situation.

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