Praca oryginalna *Original paper*

Gill disease in a gilthead sea bream (Sparus aurata L.)

DAMIR KAPETANOVIĆ, BOŽIDAR KURTOVIĆ, IRENA VARDIĆ, EMIN TESKEREDŽIĆ, ZLATICA TESKEREDŽIĆ

Laboratory for aquaculture, Division for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička 54, 10 000 Zagreb, Croatia

Kapetanović D., Kurtović B., Vardić I., Teskeredžić E., Teskeredžić Z.

Gill disease in a gilthead sea bream (Sparus aurata L.)

Summary

This paper describes the first report of gill disease in gilthead sea bream (Sparus aurata L.), cultured in the Northern Adriatic near the island of Cres in Croatia. The pathological findings of cultured gilthead sea bream were examined in detail, using prompt clinical and necropsy examination, bacterial and hematological analysis. Beside yellowish-brown, swollen and clumped gills, there were no other apparent changes in the diseased fish. Bacterial analysis of these fish revealed the presence of mixed infections. The predominant bacterium from affected gills was Stenotrophomonas maltophilia. Furthermore Aeromonas salmonicida masoucida/achromogenes and Empedobacter brevis were also identified. Fish with affected gills had significantly decreased serum proteins, specific weight and haematocrit compared to fish without changes of the gills.

Keywords: gilthead sea bream, gill damage

The etiology of gill damage varies and can be attributed to vitamin deficiency, toxic metals, poor water quality, bacteria and parasites (4). The following bacteria have been associated with gill damage (1): *Bacillus* spp., *Chryseobacterium scophthalmum*, *Escherichia vulneris*, *Flavobacterium* spp., *Janthinobacterium lividum*, *Miccrococcus luteus*, *Mycoplasma mobile*, *Myxococcus piscicola*, *Piscirickettsia salmonis*, *Planococcus* sp., *Vibrio* spp. Gill damage has been widely reported in other fish species, however not in gilthead sea bream so far.

Several bacterial species are known as pathogenic for gilthead sea bream such as *Pseudomonas*, *Photobacterium damselae* spp. *piscicida*, *Aeromonas salmonicida* and *Vibrio* spp. (8). On the other hand bacterial problems in the Adriatic are mainly due to *Vibrio anguillarum*, *Pasteurella piscicida* and *Myxobacteria* spp. (6).

Stenotrophomonas maltophilia is a bacterium prevalent in aquatic environment and can be a potential opportunistic pathogen in fish, domestic animals and humans (3). Treatment of *S. maltophilia* infections presents a significant challenge since this organism is typically resistant to the most commonly used antimicrobial drugs (5).

The present paper describe some of the clinical and pathological findings of gill disease in gilthead sea bream cultured on a Croatian marine farm located in Northern Adriatic, including identification and antibacterial characterization of bacteria isolated from the affected gills.

Material and methods

Fish were sampled four times as a part of one-year monitoring program on the marine fish farm located near the island Cres in the Northern Adriatic. During one sampling an outbreak of gill disease was recognized in intensively cultured gilthead sea bream. Gilthead sea bream were held together with european sea bass (*Dicentrarchus labrax*), common sea bream (*Pagrus pagrus*), sharpsnout sea bream (*Diplodus puntazzo*) and common dentex (*Dentex dentex*). None of these species were affected. During disease outbreak water temperature was 15.5°C and water quality parameters (total ammonia, nitrogen, pH, salinity, heterotrophic plate count (IDEXX laboratories, Inc.), dissolved oxygen, chemical oxygen demand and conductivity) were within acceptable ranges.

Ten fish ranging between 10.0 g and 313.0 g were randomly selected for diagnostic processing. Four of them demonstrated the external signs on the gills common to the clinical presentation. Blood was collected by caudal puncture. Haematocrit was measured with StatSpin-mp multipurpose centrifuge (Norwood, Massachusets 02062 USA). Stat Spin micro hematocrit capillary tube reader was used for haematocrit values. Quantity of serum proteins, refraction index, and specific gravity of serum was measured with refract meter (Atago, Japan).

Initial external abnormalities were grossly examined followed by sampling of gills for bacteriological analysis and microscopic examination of skin scrapings and gill cuts (normal and abnormal areas). After external examination the abdominal cavity was aseptically opened and spleen, liver and kidney were sampled for bacteriological analysis. Samples were plated onto Tryptic soy agar (BBL) supple-

Tab. 1. Results of the hematological analysis

Sample No.	Serum prot.	Refraction index	Spec. gravity	Haematocrit
1*	1.8	1.3400	1.021	10
2*	2.8	1.3415	1.026	12
3*	2.6	1.3415	1.025	16
4*	4.6	1.3445	1.030	16
Mean value ± st. dev.	2.95 ± 1.18	1.342 ± 0.002	1.025 ± 0.004	13.50 ± 3.00
5	NA	NA	NA	8
6	4.4	1.3440	1.0340	28
7	5.4	1.3460	1.0239	46
8	7.2	1.3485	1.0490	46
9	6.0	1.3470	1.0420	36
10	5.8	1.3465	1.0410	38
Mean value ± st. dev.	5.76 ± 1.01	1.346 ± 0.002	1.038 ± 0.009	33.67 ± 14.28

Explanations: * – gilthead sea bream affected by gill damage; NA – not analyzed

mented with 1% NaCl (Kemika). Plates were incubated at 22°C for 2-5 days. Bacterial characterization of the isolated pure cultures was performed by the means of standard morphological, phenotypic tests. Isolated strains were further characterized with the API kits (bioMérieux, France) and identified with the APILAB Plus identification software (bioMérieux, France).

The drug sensitivity of the isolated strains was determined with disc diffusion method on Mueller Hinton agar (BBL) using the discs obtained from BD-BBL. The following antimicrobial discs were tested: ampicillin, chloramphenicol, erythromycin, nitrofurantion, norfloxacin, novobiocin, oxytetracycline, penicillin, piperacillin, sulfamethoxazole/trimethoprim, tetracycline and trimethoprim.

All data were expressed as means ± SEM. The significance between data was evaluated with Student's t-test.

Results and discussion

The only external signs observed on individual fish were brown to yellowish-brown, swollen and clumped areas of gills (fig. 1), whereas diagnostic necropsy was negative. Microscopically only weak infestation with *Dactylogyrus* spp. was found on the gills of two gilthead sea breams without gill damage.

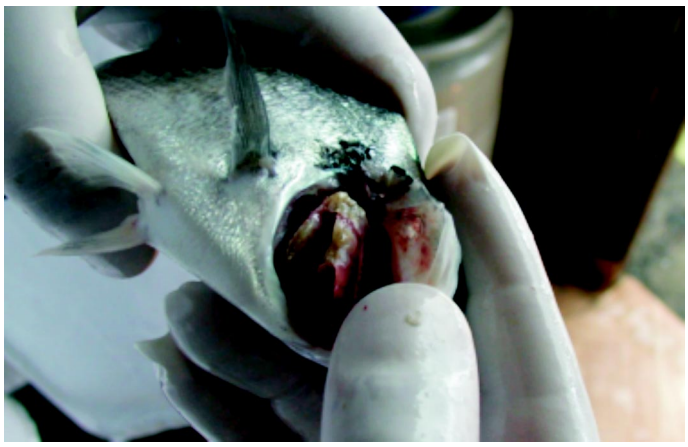

Fig. 1. Gilthead sea bream affected by gill damage: swollen and clumped gill areas

The hematological analysis of gilthead sea bream showed differences between fish with and without changes on gills (tab. 1). The serum protein of affected and unaffected fish was 2.95 ± 1.18 and 5.76 ± 1.01 respectively, with significant difference ($p \leq 0.05$). The refraction index was lower in affected fish (1.342 ± 0.002) than in unaffected fish (1.346 ± 0.002) however not significantly. The specific gravity was lower in affected fish (1.025 ± 0.004) than in unaffected fish (1.038 ± 0.009) with significant difference ($p \leq 0.05$). The haematorcrit of affected fish and unaffected fish was 13.5 ± 3 and 33.67 ± 14.28 respectively, with significant difference ($p \leq 0.05$).

The bacteria isolated from the gills of the affected gilthead sea bream were identified as: *Stenotrophomonas maltophilia*, *Aeromonas salmonicida masoucida/achromogenes* and *Empedobacter brevis.* In two fishes *S. maltophilia* and *E. brevis* were isolated as pure culture, while in the third case, both *S. maltophilia* and *Aer. salmonicida masoucida/achromogenes* were isolated as mixed culture. Bacterial analysis of liver and kidney was negative. In spleen of two gilthead sea bream without abnormalities *Staphylococcus epidermidis* was identified, using API Staph with probability of 97.7%. *E. brevis* was isolated in pure culture from the gills of one affected sea bream and identified by the API 20NE system, with probability of 83.1%. The average probability of *S. maltophilia* from the pure culture of gills in one affected gilthead sea bream using API 20NE and API 20E system was 99.9%, and 99.3% respectively. Another strain of *S. maltophilia* from the other affected fish was isolated in mixed culture and identified by the API 20E system, with probability of 65.6%, whereas *Aer. salmonicida* was isolated from the same gills using API 20NE system, with probability of 76.9%.

According to the results of the antibiotic susceptibility test *E. brevis* was resistant only to novobiocin and sulfamethoxazole/trimethoprim, whereas *Aer. salmonicida* is susceptible to norfloxacin, erythromycin, tetracycline and chloramphenicol. *Staph. epidermidis* strains were sensitive to sulfamethoxazole/trimethoprim, chloramphenicol, oxytetracycline and tetracycline. All tested strains of *S. maltophilia*, showed the same drug resistance pattern. They were resistant to most of drugs and susceptible to sulfamethoxazole/trimethoprim.

The etiology of gill damage in gilthead sea bream is not sufficiently understood and cannot be attributed to only one bacterial species. At least three different pathogens *Stenotrophomonas maltophilia*, *Aeromonas salmonicida* and *Empedobacter brevis* contributed to

etiology. The most consistent bacterium recovered from the diseased gills was *S. maltophilia*, which showed high antimicrobial resistance. This is in accordance with findings of other investigators (5). *Aeromonas salmonicida* is a common bacterial pathogen of fish, including gilthead sea bream (8). There was no evidence of systemic infection, and bacteria found in the gills were not recovered from the internal organs of affected fish, except for *Staphylococcus epidermidis* from spleen (1).

Results of the hematological analysis showed evidence of systemic changes in fish with gill disease. These results confirmed previous findings (7, 2) that the hematopoiesis is severely affected in bacterial diseases.

References

1. *Austin B., Austin D. A.*: Bacterial fish pathogens, [in:] Disease of Farmed and Wild Fish. Springer-Praxis, Chichester 1999, p. 155.
2. *Barham W. T., Smith G. L., Schonbee H. J.*: The haematological assessment of bacterial infection in rainbow trout, Salmo gairdneri. J. Fish Biol. 1980, 17, 275-281.
3. *Ben-Gigirey B., Vieites J. M., Kim S. H., An H., Villa T.G., Barros--Velázquez J.*: Specific detection of Stenotrophomonas maltophilia strains in albacore tuna (Thunnus alalunga) by reverse dot-blot hybridization. Food Control 2002, 13, 293-299.
4. *Govett P. D., Rotstein D. S., Lewbart G. A.*: Gill metaplasia in a goldfish, Carassius auratus auratus (L.). J. Fish Dis. 2004, 27, 419-423.
5. *Krueger T. S., Clark E. A., Nix D. E.*: In vitro susceptibility of Stenotrophomonas maltophilia to various antimicrobial combinations. Diag. Microbiol. Inf. Dis. 2001, 41, 71-78.
6. *Oraić D., Zrnčić S., Šoštarić B.*: The most prevelent diseases in cultivated sea bass (Dicentrarchus labrax) and sea bream (Sparus aurata) in fish farms along the Croatian coast. Proc. third Internat. Symp. Aquatic Animal Health, Baltimore, Maryland, USA 1998, p. 208.
7. *Uzbilek M. K., Yildiz H. J.*: A report on spontaneous diseases in the culture of grass carp (Ctenopharyngodon idella Val. 1884), Turkey. Turkish J. Vet. Anim. Sci. 2002, 26, 407-410.
8. *Zorrilla I., Chabrillón M., Arijo S., Díaz-Rosales P., Martínez-Manzanares E., Balebona M. C., Moriñigo M. A.*: Bacteria recovered from diseased cultured gilthead sea bream (Sparus aurata L.) in southwestern Spain. Aquaculture 2003, 218, 11-20.

Author's address: Damir Kapetanović DVM, Laboratory for aquaculture, Department for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička 54, Zagreb, Croatia; e-mail: kada@irb.hr