

## Pathological Investigations of Winter-like Disease in Cultured Sea Breams (*Sparus aurata*)

S. Serap BİRİNCİOĞLU \* Ahmet AYDOĞAN \*\*  Hamdi AVCI \*

\* Department of Pathology, Faculty of Veterinary Medicine, Adnan Menderes University, TR-09016 Aydın - TURKEY

\*\* Department of Pathology, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, TR-15030 Burdur - TURKEY

Makale Kodu (Article Code): KVFD-2012-7847

### Summary

During the January 2008, an outbreak occurred in 15 sea bream farms located in Güllük gulf of Bodrum, Turkey and approximately two million sea breams (*Sparus aurata*) were died in three weeks. When the outbreak was took place, surface temperature and dissolved oxygen of sea water was measured as 10°C and 7.9 mg/l on January 10, 2008. For pathological examinations, a total of 65 fish (8-9-month-old) were collected from 9 of 15 farms. At necropsy, extreme abdominal distention, milk-like discharge around the anus and depigmentation of skin were observed in all fish. The liver was swollen and yellowish-white in color. Microscopically, degeneration, severe fatty changes and multifocal necrotic areas were observed in the liver. The similar necrotic areas were also noted in the kidney and spleen. Severe verminous enteritis due to *Myxidium spp.* was seen in two fish farms. The gills were generally edematous and hyperemic. According to result of bacteriological examinations, *Vibrio spp.* (farm no: 1) and *Staphylococcus spp.* (farms no: 1, 8) were isolated and identified in some farms. In conclusion, it is thought that the outbreak occurred in sea breams might be winter disease associated with sudden decreases in water temperature together with induced fatty liver.

**Keywords:** Winter disease, Sea bream, Histopathology, Liver

## Kültür Çipuralarında (*Sparus aurata*) Winter-benzeri Hastalık Üzerinde Patolojik İncelemeler

### Özet

Bodrum Güllük körfezinde bulunan 15 farklı balık çiftliğinde, 10 Ocak 2008 döneminde, kültür çipuralarında (*Sparus aurata*) görülen bir salgın sonucu üç hafta içinde yaklaşık 2 milyon balık ölümü meydana geldi. Ölümün meydana geldiği dönemde, deniz suyunun yüzey sıcaklığı 10°C ve çözünmüş oksijen miktarı 7.9 mg/l olarak ölçüldü. Çalışmada, salgının görüldüğü 15 işletmenin dokuzundan alınan toplam 65 adet çipura (8-9 aylık) incelendi. Dış bakıda, tüm balıklarda karında aşırı şişkinlik, anus etrafında süt benzeri akıntı ve deride depigmentasyon görüldü. Karaciğerler şişkin ve sarımsı-beyaz renkteydi. Mikroskopik incelemede, karaciğerde dejenerasyon, şiddetli yağlanma ve multifokal nekroz alanları dikkati çekti. Benzer nekroz alanlarına böbrek ve dalaklarda da rastlandı. İki işletmede barsaklarda *Myxidium spp.*ye bağlı şiddetli paraziter enteritis saptandı. Solungaçların sekonder lamellerinde ödem ve hiperemi oldukça belirgindi. Bakteriolojik inceleme sonucunda, bir işletmede *Vibrio spp.* (işletme no: 1), iki işletmede ise *Staphylococcus spp.* (işletme no: 1, 8) izole ve tanımlandı. Sonuç olarak, çipuralarda görülen bu ölümlerin, deniz suyu sıcaklığında meydana gelen ani düşüşün neden olduğu karaciğer yağlanmasıyla karakterize Winter hastalığına bağlı olarak oluşabileceği düşünüldü.

**Anahtar sözcükler:** Winter hastalığı, Çipura, Histopatoloji, Karaciğer

### INTRODUCTION

"Winter syndrome" or "winter disease" (WD) is a seasonal, multifactorial disease and found primarily in sea breams (*Sparus aurata*)<sup>1-3</sup>. In recent years, it has caused significant mortality rates in sea bream farms along the Mediterranean coast such as Spain, Italy, Greece, Croatia, Malta and Portugal, during the winter months<sup>2,4</sup>. Etiology of WD has not been

certain because of determining association between the disease and any specific etiological agent<sup>4,6</sup>. Whereas, some environmental stress factors such as decrease in salinity of sea water, low water temperature (<13°C), fish density, immunosuppression of fish and nutritional factors can be effective on occurrence of WD and particularly the



İletişim (Correspondence)



+90 248 2132173



aaydogan@mehmetakif.edu.tr

lower water temperature may be initial reason of all these factors <sup>4,5,7,8</sup>. The purpose of this study was to investigate pathologic changes of an outbreak of winter-like disease affecting cultured sea breams in Turkey.

## MATERIAL and METHODS

The total of 65 dead sea breams (8-9-month-old) was collected from 9 of 15 farms (Farms no: 1 - 9). However, healthy 10 sea breams were sampled and used as control group. These control fish were euthanased by overdose of anesthesia. Necropsies of all fish were performed. Tissue samples of liver, kidney, heart, gill, pyloric caeca, stomach, intestines, swim-bladder, brain, skin and skeletal muscle of all fish were fixed in 10% neutral-buffer formalin solution. Then, they were embedded in paraffin, sectioned at 4 to 5 µm and stained with haematoxylin and eosin (HE), Brown-Brenn and PAS stains for routine light microscopic examination. In addition to determine the presence of lipid in the liver, Oil Red O staining method was used in the frozen liver sections <sup>9</sup>. Bacteriological analysis was performed in the University of Ege, Faculty of Fisheries and Bornova Veterinary Control and Research Institute, Izmir, Turkey.

## RESULTS

On January 2008, an outbreak was detected on the Güllük gulf of Bodrum which affected 15 fish farms. Approximately two million sea breams died because of this outbreak. But death was not seen in sea bass (*Dicentrarchus labrax*) in these farms. Salinity rate, surface temperature, pH and dissolved oxygen of sea water of Güllük Gulf were measured as 0.1‰, 10°C, 7.77, 7.9 mg/l respectively on January 10, 2008. For protection to outbreak, sea bream cages of many fish farms transported from Güllük Gulf to open sea and Akbük Gulf (surface water temperature of Akbük gulf: 16-17°C). So, the fish deaths were stopped after the moving of these fish cages.

**Macroscopic Findings:** Macroscopically, extreme abdominal distention, milk-like discharge around the anus and depigmentation of skin were generally observed in fish. At necropsy, abdominal cavity was completely filled with testicles and enlarged swim-bladders. The stomach was greatly enlarged and their lumens contained many undigested foods. The liver was swollen, yellowish-white in color and soft to touch with blunt edges in all fish (Fig. 1). In the some fish, the gills were pale, swollen and covered by amount of mucus. Petechial hemorrhages were also observed in the gills. At four fish farms, white spots with different sizes were seen on the liver, kidney and spleen. At two fish farms, distention of the stomach and intestines was observed. Stomachs were filled with white fibrin content and intestines were filled with clear green content.

**Microscopic Findings:** The most common microscopic finding observed in all fish was severe and diffuse lipid



**Fig 1.** Macroscopic appearance of liver was swollen and yellowish-white in color (arrows)

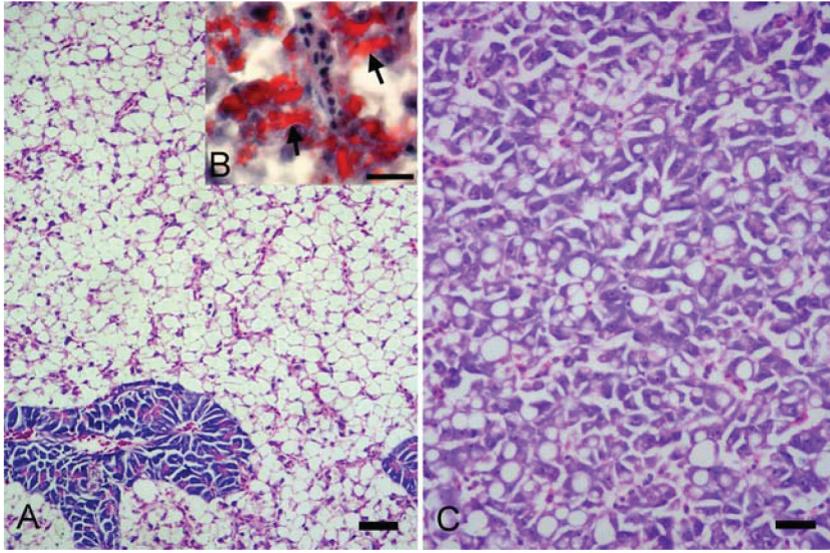
**Şekil 1.** Sarımsı-beyaz renkte ve şişkin karaciğerin makroskopik görünümü (oklar)

infiltration and degeneration in the liver (hepatic steatosis). The hepatocytes were swollen and contained varying size of lipid droplets (Fig. 2A). These droplets were detected as fat droplets with Oil Red O stain method (Fig. 2B). In the control fish, slight lipid infiltration in the liver was detected (Fig. 2C). At four farms (farms no: 2, 4, 6, 7) multifocal well encapsulated necrotic areas were diagnosed in the macroscopically white spotted areas of liver, kidney (Fig. 3A) and spleen (Fig. 3B). Any causative agent was not found in the necrosis areas with *special staining* techniques such as Brown-Brenn and PAS. Degeneration together with hyaline droplet accumulation was detected in some renal tubules of the kidneys. At two farms (farms no: 1, 2), *verminous enteritis due to Myxidium spp.* was observed (Fig. 3C). In the intestines of these fish, abundant intraepithelial myxosporeans with inflammatory cells infiltrations were seen in propria mucosa. The observed main microscopical lesions in the gills were marked edema, hyperemia, telangiectasia, adhesions in the secondary lamellae and increased numbers of eosinophilic granular cells (EGC). In addition, at farm no- 4, there were eggs of *Sanguinicolid* trematodes in the lumens of arterioles of fish (Fig. 4A-B) and at three farms (farms no: 3, 4, 5), *Monogenean* parasites were detected in gills of fish. Vascular and cellular reactions were more severe in the gills of these fish.

At the bacteriological examinations, bacterial agents such as *Vibrios spp.* (farm no: 1) and *Staphylococcus spp.* (farms no: 1, 8) were isolated and identified in some affected fish.

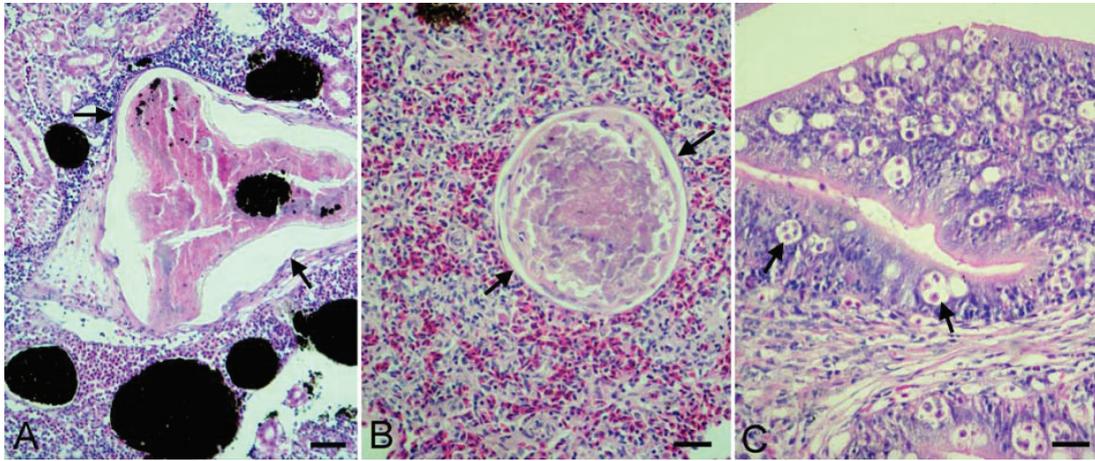
## DISCUSSION

Outbreaks of WD have been occurred along the coasts of Spain, Italy, Greece, Croatia, Malta and Portugal in sea breams <sup>2,4</sup>. In some outbreaks, mortality rates reached as high as 30-50% and it caused severe economic losses <sup>2,10</sup>. In this study, during the outbreak, approximately two million dead fish were found and it was resulted from serious economic losses in all affected farms.



**Fig 2.** Severe and diffuse infiltration of lipid in the liver of diseased fish. A- HE. Bar, 100  $\mu$ m, B- Oil Red O (arrows), Bar, 10  $\mu$ m, C- Mild infiltration of lipid in the liver of control fish. HE, Bar, 50  $\mu$ m

**Şekil 2.** Karaciğerde şiddetli ve yaygın lipid infiltrasyonu. A- HE. Bar, 100  $\mu$ m, B- Oil Red O (oklar), Bar, 10  $\mu$ m, C- Karaciğerde hepatosit sitoplazmalarında az sayıda bulunan yağ damlaları, kontrol grubu. HE. Bar, 50  $\mu$ m

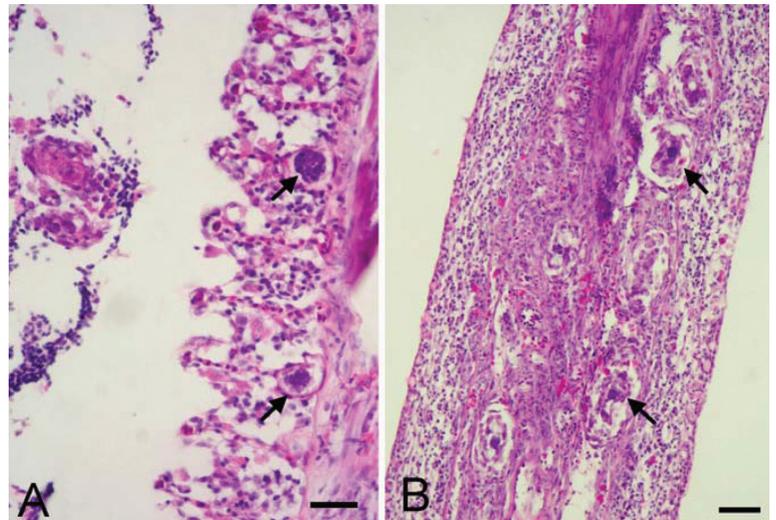


**Fig 3.** A- Necrotic area in the kidney of diseased fish (arrows). HE. Bar, 100  $\mu$ m, B- Necrotic area in the spleen of diseased fish (arrows), HE. Bar, 50  $\mu$ m, C- Verminous enteritis of diseased fish due to *Myxidium* spp. (arrows), HE. Bar, 50  $\mu$ m

**Şekil 3.** A- Böbrekte fokal koagülasyon nekroz alanı (oklar). HE. Bar, 100  $\mu$ m, B- Dalakta fokal koagülasyon nekroz alanı (oklar). HE. Bar, 50  $\mu$ m, C- Hasta balıklarda *Myxidium* spp.'in neden olduğu verminöz enteritis (oklar), HE. Bar, 50  $\mu$ m

**Fig 4.** Eggs of *Sanguinicolid* trematodes in the lumens of arteries of gills (arrows), HE. A- Bar, 50  $\mu$ m, B- Bar, 100  $\mu$ m

**Şekil 4.** Solungaç damar lümenlerinde *Sanguinicolid* trematod yumurtaları (oklar), HE. A- Bar, 50  $\mu$ m, B- Bar, 100  $\mu$ m



The controversial etiology of WD is still unknown because there is no correlation between the disease and any

specific etiological agent <sup>5,6,8</sup>. However, in previous studies, some bacterial agents such as *Aeromonas hydrophila*,

*Enterobacteriaceae* <sup>5,6</sup>, and *Pseudomonas anguilliseptica* <sup>11-13</sup>, have been isolated and some parasites such as *Sanguinicolid* trematodes <sup>14</sup> have been reported during the outbreaks of WD. In the present study, according to bacteriological examinations, bacterial agents such as *Vibrio spp.* and *Staphylococcus spp.* were isolated in three farms. In addition, eggs of *Sanguinicolid* trematode and *Monogenean* parasites were seen in the gills of some fish. It is thought that these isolated agents and detected parasites are not responsible for outbreak because of these etiologic factors is not seen in all affected farms.

It has been reported that WD outbreaks are appeared during the winter seasons and several environmental stress factors such as decrease in salinity and temperature (<13°C) of sea water influenced the formation of WD <sup>5,8,10,13</sup>. Hepatic steatosis is accentuated in sea bream affected by WD and a polifactorial, metabolic-based pathology which causes high mortality when the water temperature drops below 11-13°C <sup>2,4</sup>. In this study, in the winter of 2008, some environmental stress factors such as sudden decreases in salinity and water temperature were occurred in Güllük gulf of Bodrum, Turkey. Salinity rate and temperature of sea water were measured at 0, 1‰ and 10°C respectively.

Clinically and pathologically, hyposensitivity, erratic swimming, atrophy of the exocrine pancreas, pale and friable liver related to hepatic steatosis and necrosis in the muscles are reported as characteristic findings of WD <sup>2,4,8</sup>. In the present study, necrosis in the muscles and atrophy of the exocrine pancreas were not seen. Pathological changes may occur in direct response to the stress factors and this state may lead to increased respiration, oxygen consumption and depletion of stored body lipids <sup>15</sup>. Previous studies on WD indicated that macroscopically friable and whitish or yellowish liver associated with hepatic steatosis is noted in all fish <sup>3,8,16</sup>. It has been argued that lipid infiltration in the liver is a rapid lipid accumulation and an adaptive mechanism for the response to decrease in water temperature <sup>3,17</sup>. It was thought that, in this study, hepatic steatosis in many fish was occurred in liver tissue for the response to decrease in water temperature as an adaptive mechanism. In addition, there has been no previous report about multifocal well encapsulated necrotic areas in the liver, kidney and spleen for WD. But, these necrotic areas were observed at four farms (farms no: 2, 4, 6, 7) in this study. Furthermore, in many experimental and natural fish disease, pathological findings of gills have been reported <sup>18-20</sup>. In the present study, lesions were found in gills consisted of edema, hyperemia, telangiectasia, adhesions in the secondary lamellae and increased numbers of EGC in accordance to literatures, and they were recorded more severe in fish with gill parasites. Although, these gill findings have not been reported in WD, it was considered that they could be nonspecific and could not relate to WD.

In the present study, a common etiologic pathogen agent was not detected in affected fish farms. In addition,

reductions in fish deaths with carriage of the affected fish cages where the water temperature was measured as 16-17°C in the gulf supported that cause of outbreak in sea breams might be WD associated with decreases in water temperature.

## REFERENCES

- Lemly AD:** Winter stress syndrome: An important consideration for hazard assessment of aquatic pollutants. *Ecotoxicol Environ Saf*, 34, 223-227, 1996.
- Sarusic G:** Clinical signs of the Winter Disease phenomenon in sea bream (*Sparus aurata*, L.). *Bull Eur Ass Fish Pathol*, 19, 113, 1999.
- Tort L, Rotllant J, Rovira L:** Immunological suppression in gilthead sea bream (*Sparus aurata*) of the North-West Mediterranean at low temperatures. *Comp Biochem Physiol- A: Mol Integr Physiol*, 120, 175-179, 1998.
- Tort L, Padros F, Rotllant J:** Winter syndrome in the gilthead sea bream *Sparus aurata*. Immunological and histopathological features. *Fish Shellfish Immunol*, 8, 37-47, 1998.
- Bovo GF, Borghesan F, Comuzzi M, Ceschias G, Giorgetti G:** Winter disease in orati di allevamento: Osservazioni preliminary. *Boll-Soc Ital Patol Ittica*, 17, 2-11, 1995.
- Doimi M:** A new Winter Disease in sea bream (*Sparus aurata*): A preliminary report. *Bull Eur Ass Fish Pathol*, 16, 17-18, 1996.
- Lemly AD:** Metabolic stress during winter increases the toxicity of selenium to fish. *Aquat Toxicol*, 27, 133-158, 1993.
- Ibarz A, Padros F, Gallardo MA, Fernandez-Borras J, Blasco J, Tort L:** Low- temperature challenges to gilthead sea bream culture: Review of cold-induced alterations and Winter Syndrome. *Rev Fish Biol Fisheries*, 20, 539-556, 2010.
- Luna GL:** Histologic Staining Methods of the Armed Forces Institute of Pathology. 3<sup>rd</sup> ed., Mcgraw Hill Book Company, US, 1968.
- Contessi B, Volpatti D, Gusmani L, Galeotti M:** Evaluation of immunological parameters in farmed gilthead sea bream, *Sparus aurata* L., before and during outbreaks of Winter Syndrome. *J Fish Dis*, 29, 683-690, 2006.
- Berthe FCJ, Michel C, Bernardet JF:** Identification of *Pseudomonas anguilliseptica* isolated from several fish species in France. *Dis Aquat Org*, 21, 151-155, 1995.
- Domenech A, Fernandez-Garayzabal JF, Lawson P, Garcia JA, Cutili MT, Blanco M, Gibello A, Moreno MA, Collins MD, Dominguez L:** Winter disease outbreak in sea-bream (*Sparus aurata*) associated with *Pseudomonas anguilliseptica* infection. *Aquaculture*, 156, 317-326, 1997.
- Domenech A, Fernandez-Garayzabal JF, Garcia JA, Cutili MT, Blanco M, Gibello A, Moreno MA, Dominguez L:** Association of *Pseudomonas anguilliseptica* infection with "winter disease" in sea bream, *Sparus aurata* L. *J Fish Dis*, 22: 69-71, 1999.
- Padros F, Zarza C, Crespo S:** Histopathology of cultured sea bream *Sparus aurata* infected with sanguinicolid trematodes. *Dis Aquat Org*, 44, 47-52, 2001.
- Lemly AD, Esch GW:** Effects of the trematode *Uvulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis macrochirus*: Ecological implications. *J Parasitol*, 70, 475-492, 1984.
- Gallardo MA, Sala-Rabanal M, Ibarz A, Padros F, Blasco J, Fernandez J, Sanchez J:** Functional alterations associated with the winter syndrome in gilthead sea bream (*Sparus aurata*). *Aquaculture*, 223, 15-27, 2003.
- Ibarz A, Blasco TJ, Beltran M, Gallardo MA, Sanchez J, Sala R, Fernandez-Borras J:** Cold-induced alterations on proximate composition and fatty acid profiles of several tissues in gilthead sea bream (*Sparus aurata*). *Aquaculture*, 249, 477-486, 2005.
- Roberts RJ:** Fish Pathology. 3<sup>rd</sup> ed., W.B. Saunders, Toronto, 2001.
- Avci H, Aydoğan A, Tanrıkul TT, Birincioğlu SS:** Pathological and microbiological investigations in rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) naturally infected with *Lactococcus garvieae*. *Kafkas Univ Vet Fak Derg*, 16 (Suppl-B): S313-S318, 2010.
- Aydoğan A, Metin N:** Investigation of pathological findings infected with *Aeromonas salmonicida* in rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792). *Kafkas Univ Vet Fak Derg*, 16 (Suppl-B): S325-S328, 2010.