

Gill histopathology in cultured sea bass (*Dicentrarchus labrax* (L.) co-infected by *Diplectanum aequans* (Wagener, 1857) and *Lernanthropus kroyeri* (van Beneden, 1851)

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Summary: Because of a 1.5% daily mortality was recorded in sea bass (*Dicentrarchus labrax*) reared in floating-cage farm in Aegean Sea coast of Turkey, 15 sea bass were examined parasitologically and pathologically, in March 2010. *Diplectanum aequans* (Wagener, 1857) and *Lernanthropus kroyeri* (van Beneden, 1851) were detected in all fish. Of the 15 sea bass dissected, 100% were infected by *Diplectanum aequans* (Wagener, 1857), and mean intensity of infection was 31.9, 100% were infected by *Lernanthropus kroyeri* (van Beneden, 1851) and mean intensity of infection was 11.4, respectively. In the pathological examination of the gills, anemia, whitish mucoid exudate and greenish egg sac belong to female *Lernanthropus* were observed. Histologic examinations indicated, both *Diplectanum aequans* and *Lernanthropus kroyeri* were present on branchial lamellae in the gills of all fishes. Lamellary edema formation, fusion of the secondary lamellae and necrosis in both primary and secondary lamellae due to parasitic irritation were determined. As a result, it was concluded that fish deaths were occurred due to the lesions formed after dense infestations of *Diplectanum aequans* and *Lernanthropus kroyeri*.

Key words: *Dicentrarchus labrax*, *Diplectanum aequans*, *Lernanthropus kroyeri*, histopathology

Kültür levreklerinde (*Dicentrarchus labrax*, L.) *Diplectanum aequans* (Wagener, 1857) ve *Lernanthropus kroyeri* (van Beneden, 1851) miks enfeksiyonunda solungaç histopatolojisi

Özet: 2010 yılı Mart ayında, günlük %1.5 mortalite gözlenen, Ege Bölgesi'nde yüzer ağ kafeslerde yetiştiriciliği yapılan kültür levrek çiftliğinden alınan 15 levreğin parazitolojik ve patolojik incelemesi yapıldı. Balıkların tamamında *Diplectanum aequans* (Wagener, 1857) ve *Lernanthropus kroyeri* (van Beneden, 1851) parazitleri saptandı. İncelenen 15 levrek balığının sırasıyla; *Diplectanum aequans* (Wagener, 1857) ile %100 oranında enfekte ve ortalama enfeksiyon yoğunluğunun 31.9, *Lernanthropus kroyeri* (van Beneden, 1851) ile ise %100 oranında enfekte ve ortalama enfeksiyon yoğunluğunun 11.4 olduğu belirlendi. Patolojik incelemede, solungaçlarda anemi, beyaz mukoid eksudat ve dişi *Lernanthropus*'a ait yeşilimsi renkte yumurta keselerine rastlandı. Mikroskopik incelemede, tüm balıkların solungaç lamelleri üzerinde hem *Diplectanum aequans*'a hem de *Lernanthropus kroyeri*'ne rastlandı. Parazitlerin etkisine bağlı olarak lamellar ödem, sekonder lamellerde füzyon ile primer ve sekonder lamellerde nekroz gözlemlendi. Sonuç olarak, balıklardaki ölümlerin, *Diplectanum aequans* ve *Lernanthropus kroyeri* parazitlerinin yoğun enfestasyonu sonucu oluşan lezyonlardan kaynaklandığı kanısına varıldı.

Anahtar sözcükler: *Dicentrarchus labrax*, *Diplectanum aequans*, *Lernanthropus kroyeri*, histopatoloji

Introduction

Dicentrarchus labrax was the first non-salmonid species to be cultivated commercially in Europe and even now remains the most important commercially cultured fish species grown in sea cages in the Mediterranean. The largest producers of *D. labrax* are Greece, Turkey, Italy, Spain, Croatia and Egypt (3). The *Diplectanidae* is a family comprising approximately 20 genera and more than 250 described species parasitizing the gill of a wide diversity of marine and some freshwater teleost fishes. Diplectanosis caused by *Diplectanum aequans* (and *D. laubieri*) is one of the most significant ectoparasitic diseases in the culture of sea bass *D. labrax* (20).

Prevalence and mean intensity of 73.6 to 100% and 3.45 to 112 were recorded for *D. aequans* from cultured *D. labrax* in the Mediterranean, Aegean Sea and Black Sea area (2,4,10).

Serious monogenean infections through their attachment and feeding can induce of range of histopathological changes to the epithelium, which can facilitate the invasion and establishment of a range of secondary fungal, bacterial and viral infections (1,18). The *Diplectanidae* showed definite and different patterns of gill distribution. *D. aequans* penetrates deep into the basement membrane and connective tissue of primary lamellae causing destruction to the secondary lamellae.

The worm's attachment to the host's gill epithelium is ensured by the dorsal and ventral spines of the opisthaptor squamodiscs and by the hamuli, which penetrate deep into the epithelial cells (2). Histopathological gill damage mainly consists of hyperplasia of the epithelium, fusion of branchial lamellae and the presence of hemorrhagic and inflammatory foci with leucocyte infiltration (4).

The largest genus of *Lernanthropus* Blainville, 1822 is among the most common genera of parasitic copepods, and its members are parasitic on the gills of marine teleost fishes. *Lernanthropus kroyeri* van Beneden, 1851 has been frequently observed in sea bass culture from many localities along the coast of Europe, from the Adriatic Sea to the Southern North Sea (6). Prevalence and mean intensity of 35 to 100% and 10.8 to 50.3 were recorded for *L. kroyeri* from cultured *D. labrax* in the Mediterranean and Aegean Sea (9,19).

Some species of *Lernanthropus* are strictly host specific, whereas many are parasitic on several species of fish within one or several genera (8,17). *Lernanthropus* is known to cause such pathological changes as, necrosis in epithelial tissue and ligament, increased mucus secretion, narrowing in capillary veins meanwhile *Lernanthropus* attaches to the gill filaments with third leg (7).

The aim of present study is to report the presence of the parasites on the gills and the pathological changes caused by them in a floating-cage sea bass farm in South Aegean region.

Materials and Methods

In March 2010, a routine examination for ectoparasites of 15 specimens of sea bass (*D. labrax*) 26.15±5 cm in total length (mean±S.E.) and weighing 300±50 g were randomly sampled from a floating-cage farm in Aegean Sea coast of Bodrum, Turkey. In the sampling area, mean annual temperature was 18 °C. Body surface, mouth and gills were examined under light and stereo microscope for ectoparasites. Gill filaments with attached monogeneans and copepods were removed. Monogenean specimens were mounted temporarily in ammonium-picrate glycerine to study the reproductive organs and other sclerotized parts, such as the squamodiscs, anchors, connecting bars, and marginal hooklets. Copepods were fixed in 70% alcohol and identification was based on morphological features. The monogeneans and copepods were classified according to Oliver (11,12) and Kabata (6) respectively.

Following the necropsy, gills were fixed in 10% neutral formaldehyde solution for pathologic examination. Tissue samples were routinely processed and embedded in paraffin. Tissue sections 4-6 µ in width were stained with haematoxyline-eosin (HxE) and examined under light microscope.

Digital micrographs of the parasites were taken. Prevalence (number of parasitized fish/number of fish observed) and mean intensity of infection (number of parasites/number of infected fish) were calculated and presented with 95% confidence intervals (CI) and 95% bootstrap confidence intervals (BCI), by following (16) and by using Quantitative Parasitology 3.0 programme (15).

Results

Daily mortality of 1.5% was recorded in floating-cage in a farm. Of the 15 sea bass dissected, 100% (CI 77.7–100%) were infected by *D. aequans* (Wagener, 1857), and mean intensity of infection was 31.9 (BCI 24.6–39.9), 100% (CI 77.7–100%) were infected by *L. kroyeri* (van Beneden, 1851) and mean intensity of infection was 11.4 (BCI 9.4–13.2) (Figure A-C).

In the macroscopic examination, the gills were anemic and also showed linear hemorrhagic areas. Greenish stripes between the lamellae were diagnosed as egg sac of female *Lernanthropus* according to microscopic examination. Also the mucus covering the gills was seen to be increased (Figure D).

In the microscopic examination, both *D. aequans* and *L. kroyeri* were observed in the branchial lamellae of all fishes. *D. aequans* was seen to attach deep into the basal membrane and fibrous tissue of the primary lamellae with opisthaptor squamodiscs and caused destruction in secondary lamellae. Primary lamellae were destructed where *L. kroyeri* was penetrated to the gills. There were also lamellary edema, fusion of the secondary lamellae due to significant epithelial proliferation, mucus cell proliferation, vacuolar degeneration of the lamellary epithelium, and necrosis in tips of the primary lamellae where the parasites penetrate (Figure E-G).

Discussion and Conclusion

Two diplectanid monogeneans are commonly found on sea bass in the Mediterranean: *D. aequans* and *D. laubieri*. Both species, and especially *D. aequans*, are potentially harmful in intensive sea bass farming (4). According to the result of parasitologic and pathologic examinations in present study, the identified diplectanid monogenean was *D. aequans*, which accompanied significant lesions.

The prevalence of *D. aequans* (Wagener, 1857) was 100% and mean intensity of infection was 31.9 in cultured sea bass within this study. Furthermore, González-Lanza et al (4) detected prevalence as 80.64% and mean intensity of infection as 112 in their study. On the other hand, Dezfuli et al. (2) reported the prevalence as 73.6% and mean intensity of infection as 34.61. Moreover, Oktener et al. (10) were found prevalence as %100 and mean intensity of infection as 3.45 in their study.

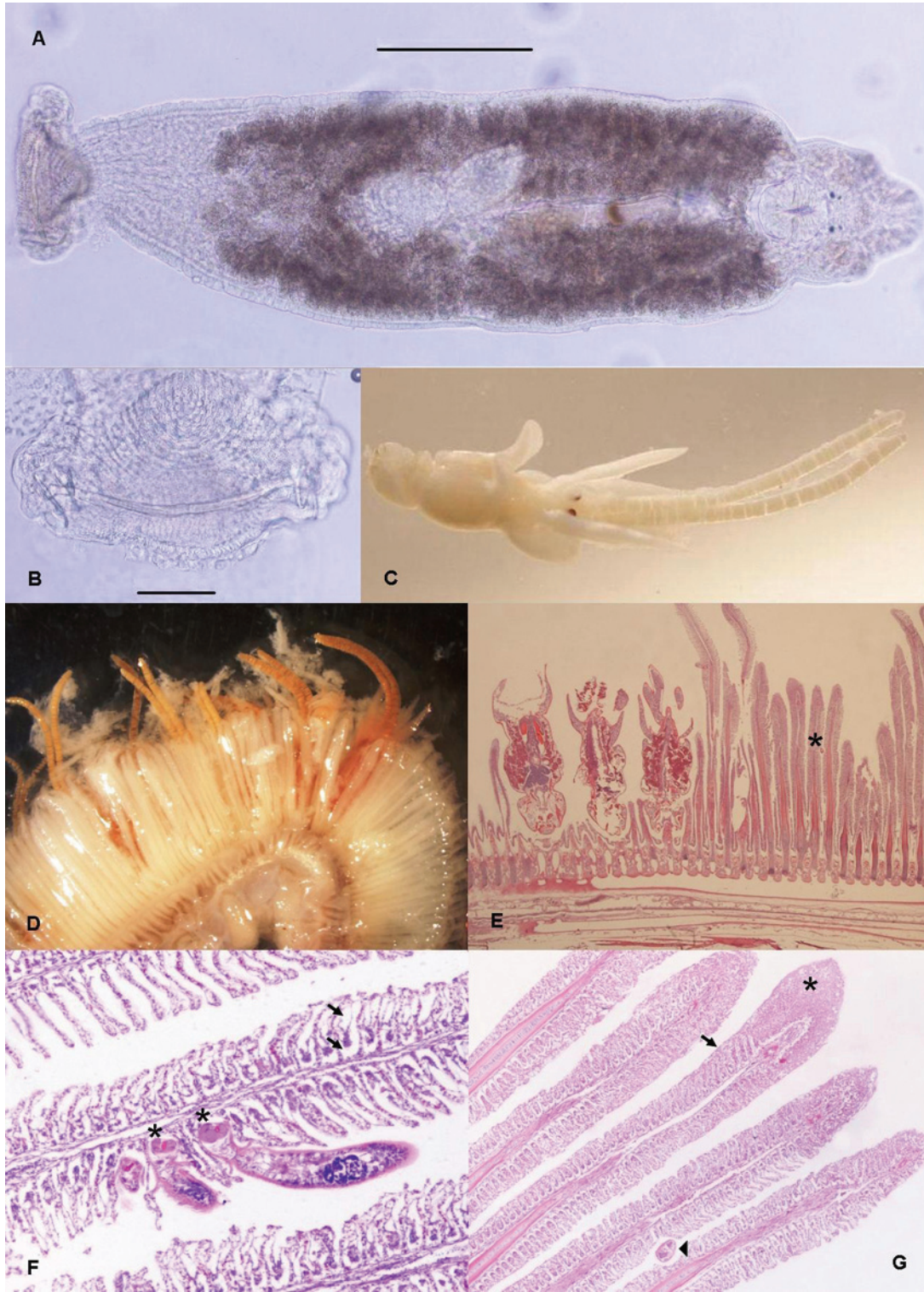


Figure. A. Microscopic appearance of *D. aequans* (Wagener 1857), entire body. (scala 200µm). B. Microscopical appearance of *D. aequans*, haptor (scala 50 µm). C. Microscopic appearance of mature female *L. kroyeri* (van Beneden, 1851), entire body. D. Microscopic appearance of mature female specimens of *L. kroyeri* attached between the gill hemibranchs. E. Female *L. kroyeri* (van Beneden, 1851) and *D. aequans* (Wagener 1857) attached between the gill hemibranchs. F. Female *L. kroyeri* (van Beneden, 1851) and *D. aequans* (Wagener 1857) in contact with the primary lamellae (asterisks) and lamellary edema (arrows), HE, X100. G. Hyperplastic area rich in secretory cells in the primary lamellae (asterisk), fusion of the secondary lamellae (arrow) and section of *D. aequans* (Wagener 1857) (arrow head), HE, X 40. Şekil. A. *D. aequans* (Wagener 1857)'in mikroskobik görünümü (scala 200µm). B. *D. aequans* (Wagener 1857)'in haptor yapısının mikroskobik görünümü (scala 50 µm). C. Ergin dişi *L. kroyeri* (van Beneden, 1851)'nin mikroskobik görünümü. D. *L. kroyeri* (van Beneden, 1851) ergin dişisinin solungaç hemibransları arasındaki mikroskobik görünümü. E. Dişi *L. kroyeri* (van Beneden, 1851) ve *D. aequans* (Wagener 1857) (yıldız), HE, X10. F. *D. aequans* (Wagener 1857)'in opisthaptorunun primer lamel ile temas bölgesi (yıldız) ve lamellar ödem (oklar), HE, X100. G. Primer lamelde salgı hücrelerinden zengin hiperplastik bölge (yıldız), sekonder lamellerde füzyon (ok) ve *D. aequans* (Wagener 1857) kesiti (ok başı), HE, X 40.

Hyperplasia and hemorrhages of affected gills were reported by Oliver (13) as the main pathological effect of *D. aequans*. The leucocyte infiltration was also reported by González-Lanza et al. (4). In present study, although hyperplasia of the affected gills was seen neither hemorrhage nor inflammatory cell infiltration was observed in contrast with previous studies.

Parasitic copepods have worldwide distribution and are economically important parasites in marine aquaculture (5). Disease outbreaks and mortalities caused by *L. kroyeri* are frequently observed in sea bass culture and economic losses occur as the result of reduced feed conversion, growth reduction, mortality, loss of product value and treatment costs (9). In present study the prevalence of *L. kroyeri* was 100% and the mean intensity of infection was 11.4 in the investigated sea bass. Similarly, Toksen (19) found prevalence as 100% and mean intensity of infection as 50.3. Manera & Dezfuli (9) were found prevalence as 35% and mean intensity of infection as 10.86 in their study.

Parasitic copepods feed on host mucus, epithelial tissue and blood, and their attachment and feeding activities seem to be responsible for disease development. Generally, the relationship between the number of parasitic copepods and the severity of the disease is dependent on; fish size and age, the general stage of fish health and the species of copepod and the developmental stages (14). In present study, hosting more than one parasite per gill was associated with large size of the fishes and their branchial arches. Also it was concluded that existence of *D. aequans* seriously harms the general status of the fish and that facilitates the attachment of *Lernanthropus* to the branchial lamellae.

Kabata (5) listed 3 types of local cause that crustaceans can effect on the gills of fish; occlusion of the branchial circulation, destruction due to the pressure of feeding and hypertrophy. In this study, occlusion of the branchial circulation and destruction due to the pressure of feeding were observed as previously reported.

As a result; it was concluded that serious lesions and necrosis of the primary and secondary lamellae of the gills are interpreted as mixed parasitism of *D. aequans* and *L. kroyeri* causes more severe destruction and clinical signs.

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