

Comparative *Diplectanum aequans* (Monogenea) infestations in cultured European seabass (*Dicentrarchus labrax*) in the Black Sea and the Aegean Sea

Karadeniz ve Ege Denizi'ndeki kültür levrek balıklarında (*Dicentrarchus labrax*) karşılaştırmalı *Diplectanum aequans* (Monogenea) enfestasyonları

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Received date: 22.02.2019

Accepted date: 22.03.2019

How to cite this paper:

Özer, A., Okkay, S., Öztürk, T., Baki, B., Acar, G., Güven, A. & Öztürk, D.K. (2019). Comparative *Diplectanum aequans* (Monogenea) infestations in cultured European seabass (*Dicentrarchus labrax*) in the Black Sea and the Aegean Sea. *Ege Journal of Fisheries and Aquatic Sciences*, 36(2), 163-169. DOI: 10.12714/egejfas.2019.36.2.08

Abstract: In the present study, monogenean infestations were investigated on the gills of the European seabass *Dicentrarchus labrax* grown in culture cages located at Yakakent coast of the Black Sea and Milas coast of the Aegean Sea. Parasitological investigations were conducted simultaneously at both localities during September 2014, February and April 2015. While a total of 100 fish specimens were examined from Yakakent samples whose average length was 19.37 cm, a total of 88 fish specimens were examined from Milas samples whose length was 22.67 cm in average. Only one monogenean species *Diplectanum aequans* was recovered from investigated fish specimens at both sampling localities. Infestation prevalence (%) and mean intensity values of *D. aequans* were 89.0% and 9.6 ± 0.8 in Yakakent samples while those infestation indices were 90.9% and 13.3 ± 1.2 in Milas samples, respectively. The difference between the infestation prevalence (%) values of *D. aequans* at both sampling localities was not statistically significant, on the other hand, the difference was statistically significant in the mean intensity values at both locations. *Diplectanum aequans* showed a homogeneous distribution in all gill arches of fish without any statistically significant difference. This is the first comparative study on the infestation of *D. aequans* on the gills of European sea bass *D. labrax* cultured in the Black Sea and the Aegean Sea.

Keywords: *Diplectanum aequans*, *Dicentrarchus labrax*, Black Sea, Aegean Sea

Öz: Bu çalışmada, Karadeniz'in Yakakent ve Ege Denizi'nin Milas kıyılarındaki ağ kafeslerde yetiştiriciliği yapılan Avrupa levrek balığının (*Dicentrarchus labrax*) solungaçlarındaki monogen parazitlerin neden olduğu enfestasyonlar araştırıldı. Parazitolojik inceleme eş zamanlı olarak Eylül 2014, Şubat ve Nisan 2015 tarihlerinde alınan balık örneklerinde gerçekleştirildi. Yakakent'teki kafeslerden ortalama boyları 19.37 cm olan toplam 100 adet balık incelenirken, Milas'taki kafeslerden ortalama boyları 22.67 cm olan 88 adet balık incelendi. Her iki örneklem alanından incelenen balık türünde sadece bir monogen parazit olan *Diplectanum aequans* türünün varlığı belirlendi. *Diplectanum aequans* türü monogen parazitin enfestasyon oranı (%) ve enfeste balık başına ortalama sayıları Yakakent örneklerinde sırasıyla %89.0 ve 9.6 ± 0.8 olarak belirlenmişken, enfestasyon parametreleri Milas örneklerinde sırasıyla %90.9 ve 13.3 ± 1.2 olarak belirlendi. Her iki örneklem alanından yakalanan balıklarda belirlenen *D. aequans* enfestasyon oranları (%) arasındaki fark istatistik olarak önemli bulunmazken, her iki örneklem alanındaki balıklardaki enfeste balık başına ortalama *D. aequans* değerleri arasındaki fark istatistik olarak önemli bulundu. *Diplectanum aequans* türü parazit incelenen balıkların tüm solungaç yapraklarında homojen bir dağılım gösterdi ve enfestasyon parametrelerinde istatistik bir fark belirlenmedi. Bu araştırma Karadeniz ve Ege Denizi'nde kültürü yapılan Avrupa levrek balığının (*D. labrax*) solungaçlarındaki *D. aequans* türü monogen parazit enfestasyonları üzerine gerçekleştirilen ilk karşılaştırma çalışmasıdır.

Anahtar kelimeler: *Diplectanum aequans*, *Dicentrarchus labrax*, Karadeniz, Ege Denizi

INTRODUCTION

Monogeneans are widespread parasites throughout freshwater, marine and brackish habitats and commonly found on fishes and lower aquatic invertebrates (Özer and Öztürk, 2005). These parasites are located on the gills, fins and skin of their hosts and feed on mucus and epithelial cells of the skin and gills. This nutritional activity is irritant and stimulates productions of excessive mucus, epithelial hyperplasia, haemorrhage, focal redness or deep maturation and, moreover, create openings for bacterial invasion (Noga, 1995; Reed et al., 2009). The Diplectanidae Monticelli, 1903 is a family comprising approximately 48 genera and approximately 523 described species of a wide diversity of marine and freshwater fishes. The genus *Diplectanum* Diesing 1858 is the largest in the family Diplectanidae. *Diplectanum aequans* (Wagener, 1857) is considered a generalist parasite and has been reported from the gills of either cultured or wild *Dicentrarchus labrax* (European seabass), *Dicentrarchus punctatus* (spotted seabass) and *Sparus aurata* (gilthead seabream) from the Aegean Sea, the Mediterranean Sea, the Adriatic Sea and the Black Sea (Oliver, 1987; Tokşen, 1999, 2007; Cecchini et al., 2001; Mladineo, 2006; Akmırza, 2010; Antonelli and Marchand, 2012; Yardımcı and Pekmezci, 2012; Ögüt and Uzun, 2014).

This parasite can lead to diseases such as Diplectanosis and it is considered to be the one of the most significant ectoparasitic diseases of European seabass *Dicentrarchus labrax* (Whittington and Chisholm, 2008). Silan and Maillard (1989) reported mortalities in seabass with a possible cause by the thousands of *Diplectanum aequans* individuals and they mentioned that the parasite became more proliferated at the beginning of warming in water temperature. Dezfuli et al. (2007) reported annual stock losses of juvenile *D. labrax* caused by *D. aequans* range between 5 – 10% in Italy. Ögüt and Uzun (2014) also reported negative influence of *D. aequans* on the fitness of cultured juvenile seabass *D. labrax* in the central Black Sea. *Diplectanum* parasites are oviparous and produce eggs on the gill of seabass and then, followed by a larval stage (oncomiracidium), post larval stage, II. post-larval stage, intermediate stage and adult stage in its life cycle (Silan and Maillard, 1989) and host switch by this monogenean parasite may occur at any stage of this developmental cycle. All developmental stages of this parasite are also temperature driven (Cecchini et al., 2001). Thus, considering wild fish and culture fish as vectors for disease propagation, it is important to investigate and determine their current status in some species of fish such as seabass which is an intensively cultured species in Turkey. Moreover, histopathological gill damages caused by Diplectanid parasites mainly

consist of hyperplasia of the epithelium, fusion of branchial lamellae and the presence of haemorrhagic and inflammatory foci with leucocytic infiltration and these pathological negative impacts on fish hosts indicate their restricting importance in the cultivation of seabass (Gonzalez-Lanza et al., 1991).

Dicentrarchus labrax has been an important species in the Mediterranean, the Aegean and the Black Sea fish culture in sea cages and Turkey is one of the largest *D. labrax* producers in Europe (FAO, 2016). The present study aimed to investigate infestations by a monogenean parasite *Diplectanum aequans* on the gills of *D. labrax* collected simultaneously in the culture cages located in the Black Sea and the Aegean Sea for the first time.

MATERIALS AND METHODS

Monogenean parasites were investigated on the gills of the European seabass *Dicentrarchus labrax* grown in culture cages located at Yakakent coast of the Black Sea (41°40'53.12"N 35°28'22.48"E) and Milas coast of the Aegean Sea (37°17'32"N 27°26'43"E) (Figure 1). Parasitological investigations were conducted simultaneously at both sampling locations during September 2014, February and April 2015. A total of 100 from Yakakent samples and 88 from Milas samples were examined at the Faculty of Fisheries and Aquatic Sciences in Sinop according to the methods specified by Öztürk and Özer (2014). Fish samples from both localities were transferred in boxes containing dried ice and then gills were examined using a light microscope at x100 and x200 magnification. Each gill arches from both sides of all fish samples were carefully examined to determine their exact locations on the gills and total number of parasite individuals per gill arch were counted and noted. Calculation of infection prevalence (%) and mean intensity follow the definitions by Bush et al. (1997). Parasite species was identified using a phase contrast Olympus microscope (BX53) equipped with a digital camera (DP50) and drawing attachment. Morphological identification was conducted with the definitions by Oliver (1968) and Mounira (2008). Measurements were made on 30 fresh parasite individuals and are given in micrometers as mean and range. Some water parameters were measured at a depth of 5 meters using YSI 556 MPS model field type multiparameter device.

The Kruskal-Wallis test (nonparametric ANOVA) was performed to find out the preference of *D. aequans* for particular gill arches of host fish as well as seasons. The differences in parasite loading on the combined 4 left and 4 right gill arches were tested statistically by the Mann-Whitney U test. All statistical analyses were performed at the significance level of 5% using the program GraphPad InStat 3.00.



Figure 1. Map of sampling locations around the Yakakent coasts of the Black Sea and the Milas coasts of the Aegean Sea. * indicates exact location of sampling localities Yakakent and Milas.

RESULTS

Diplectanum aequans (Wagener, 1857) (Figure 2) was the only monogenean species identified on the gills of *Dicentrarchus labrax* in the present study. Morphometric data of this parasite species are as follows; total length of body 1250 μm (1200-1280 μm), width of body 350 μm (310-380 μm), diameter of haptor 196 μm (110-300 μm) and length of marginal hooks 11 μm (11-12 μm).

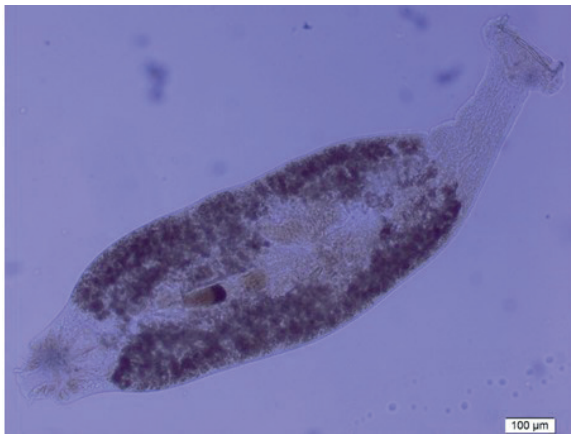


Figure 2. An individual of *Diplectanum aequans* (Wagener, 1857)

The infestation prevalence (%) and mean intensity values of each sampling months from both localities were calculated and presented in Table 1.

The difference between overall mean intensity values of *D. aequans* from both sampling localities was statistically significant ($p < 0.05$). On the other hand, *D. aequans* individuals showed a homogeneous distribution on all gill arches of examined fish without

any statistically significant difference in the infestation indices ($p > 0.05$). Some water parameters at Milas and Yakakent farming sites were measured during the sampling months and presented in Table 2.

DISCUSSION

Various species of Monogenea have been identified to be pathogens that can cause diseases in fishes either directly or by secondary infection (Thoney and Hargis, 1991). *Diplectanum* spp. are the gill parasites which have been associated with high mortalities of host fish as a result of Diplectanosis, the name of the disease they cause. These parasites can be transmitted from fish to fish in wild and in captivity, and from wild to captive fish in culture cages (Özer et al., 2015). During the investigation period in the present study, *Diplectanum aequans* was the only monogenean species identified on the gills of European seabass *Dicentrarchus labrax* and this parasite species has already been reported from this host fish in wild and culture conditions from the Aegean Sea, the Mediterranean Sea, the Adriatic Sea, the Black Sea and Portuguese coasts of the Atlantic Ocean (Oliver, 1987; Silan and Maillard, 1989; Cognetti-Variale et al., 1992; Tokşen, 1999, 2007; Santos, 1996; Mladineo, 2006; Whittington and Chisholm, 2008; Akmirza, 2010; Antonelli and Marchand, 2012; Yardımcı and Pekmezci, 2012; Ögüt and Uzun, 2014). However, this is the first simultaneous study comparing *D. aequans* infestations at two distinct sampling localities at the Black Sea and the Aegean Sea. Prevalence of infestation (%) found in the present study is quite similar (between 50 - 99%) to that reported by the above mentioned authors for the Mediterranean Sea, the Baltic Sea and the Black Sea.

Table 1. Infestation prevalence (%) and mean intensity values of *Diplectanum aequans* determined from European seabass (*Dicentrarchus labrax*) collected from culture cages in two sampling localities in the Black Sea and Aegean Sea and its distribution on the gill arches of the host fish

	Fish Length ± S.E	Infestation Prevalence (%)	Mean Intensity ± S.E
Sampling months			
Yakakent (Black Sea)			
September 2014 (n=39)	16.9 ± 0.2	87.2	6.3 ± 0.9
February 2015 (n=31)	22.4 ± 0.2	96.8	16.4 ± 1.5
April 2015 (n=30)	22.5 ± 0.2	83.3	5.8 ± 0.8
Overall (n=100)		89.0	9.6 ± 0.8^a
Milas (Aegean Sea)			
September 2014 (n=30)	16.0 ± 0.2	96.7	6.9 ± 0.8
February 2015 (n=36)	25.4 ± 0.3	91.7	17.5 ± 1.6
April 2015 (n=22)	26.7 ± 0.5	81.8	15.9 ± 3.6
Overall (n=88)		90.9	13.3 ± 1.2^b
Gill arches			
Yakakent (Black Sea)			
Left 1		54.0	2.1 ± 0.2
Left 2		56.0	2.0 ± 0.2
Left 3		46.0	2.3 ± 0.2
Left 4		44.0	2.0 ± 0.2
Total left		82.0	5.1 ± 0.5^a
Right 1		45.0	2.1 ± 0.2
Right 2		56.0	2.3 ± 0.2
Right 3		45.0	2.3 ± 0.2
Right 4		51.0	2.1 ± 0.2
Total right		84.0	5.3 ± 0.5^a
Milas (Aegean Sea)			
Left 1		51.1	2.8 ± 0.4
Left 2		54.5	2.8 ± 0.3
Left 3		56.8	2.6 ± 0.3
Left 4		46.6	2.9 ± 0.3
Total left		84.1	6.9 ± 0.8^a
Right 1		62.5	2.8 ± 0.3
Right 2		59.1	3.0 ± 0.3
Right 3		54.5	2.5 ± 0.3
Right 4		59.1	2.5 ± 0.2
Total right		78.4	8.1 ± 0.7^a

*Different superscript letters in any statistical comparison show significant differences (p<0.05)

Table 2. Water temperature (°C), dissolved oxygen (mg/l), salinity (ppt) and pH values measured at both sampling localities and three sampling months

Environmental parameters	September		February		April	
	Milas	Yakakent	Milas	Yakakent	Milas	Yakakent
Temperature	25.40	23.00	16.54	15.00	16.90	9.90
Oxygen	9.23	7.80	8.77	8.70	9.99	9.00
Salinity	35.00	16.31	35.50	16.97	35.00	15.90
Ph	8.55	8.20	8.41	8.00	8.50	8.63

Moreover, monthly prevalence (%) of infestation at both sampling localities showed similar pattern at both sampling localities without any significant difference ($p > 0.05$), even though there were fluctuations in the infestation indices. Environmental temperature is known to be one of the important variable both for the host and the parasite (McCarty, 2001; Xenopoulos et al., 2005). Higher values of both infestation prevalence (%) and mean intensities in February at both sampling localities may reflect the stimulating impact of temperature on the spawning of *D. aequans* along with the decrease in fish resistance to parasitic infestations in lower temperatures. Moreover, Cecchini et al. (2001) showed experimentally that eggs of *D. aequans* were able to survive at 5°C and egg hatching could be observed for temperatures ranging from 10°C to 30°C. Similar to our results, Gonzalez-Lanza et al. (1991) also reported a seasonal variations, with maximal infection levels of *D. aequans* occurring in winter, suggesting continuous parasites recruitment in cultured seabass from the Spanish Mediterranean coasts. Thus, following the coincidence of above mentioned both host and parasite factors, we can assume that European seabass exposed to increased *D. aequans* infestations when they are more susceptible in winter. Antonelli and Marchand (2012) indicated that significantly higher *D. aequans* infestations in winter along with negligible infestation levels in summer on the host fish *D. labrax* were resulted from the adaptation of this parasite to cold temperature. Further to this indication, Lambert and Maillard (1974) and Gonzalez-Lanza et al. (1991) also suggested continuous recruitment and the persistence of this parasite species throughout the year.

In the present study, the overall mean intensity values of *D. aequans* at the Aegean Sea samples (13.3 ± 1.2) is higher than that of the Black Sea samples (9.6 ± 0.8) with statistically significant difference ($p < 0.05$). However, our data on intensity values were always lower than those reported from Coscica coasts of the Mediterranean Sea (Antonelli and Marchand, 2012), Tuscany coasts of the Mediterranean Sea (Cognetti-

Varriale et al. 1992), Spanish Mediterranean Sea (Gonzalez-Lanza et al. 1991), the northern coast of the Adriatic Sea (Dezfuli et al. 2007), Lion Gulf of the Mediterranean Sea (Oliver, 1987), Yardımcı and Pekmezci (2012), but higher than those of the eastern Black Sea (Öğüt and Uzun, 2014) and the eastern Adriatic Sea (Mladineo, 2006). Above mentioned sampling areas for *D. aequans* infestations on *D. labrax* have different water salinity values ranging from 17‰ to 38‰ and it is clear that *D. aequans* has the ability of survival as well as hatching success within this range of salinity. Seasonal intensity values at both sampling localities in the present study revealed seasonal fluctuations, winter having the highest infestation values. According to Antonelli and Marchand (2012) these variations in rates of infestation through the seasons were attributed to the changes in the fish behaviour and peak abundances recorded in winter in the present study coincided with an immunodeficiency of fish linked to brutal changes in temperature during the transition from autumn to winter as was determined by Faliex et al. (2008). Moreover, these periods of decreasing water temperature resulted in a stress response of the European seabass (Hadj Kacem et al., 1987) reflecting a weakened immune system of fish, and therefore a greater vulnerability, leaving the opportunity for parasites to increase their populations rapidly (Oliver, 1982).

Host and, to some extent, site specificity is a well-known phenomenon in monogenean parasites of fish. This study provided a comparative data on the distribution of *D. aequans* on the gill arches of host fish *D. labrax* collected from two distinct sampling localities with different salinities, 17‰ in the Black Sea and 35‰ in the Aegean Sea. A homogenous distribution of this parasite without any significant difference ($p > 0.05$) over the gill arches of fish at both sampling localities were determined in the present study. However, some contradictory results on the distribution of *D. aequans* on the gill arches of host fish *D. labrax* have been reported in a limited number of previous studies.

Cognetti-Varielle et al. (1992) reported a non-significant distribution of this parasite's adult and juvenile forms over the gill arches at both left and right side of host fish but, noted that the location of juvenile and adult forms of this parasite at the posterior or anterior parts of each hemibranch of any given gill arches may be different. They attributed these results to the difference in the inlet-outlet water flow through the branchial chambers. Özer and Öztürk (2005) reported no significant preference of another monogenean *Dactylogyrus cornu* for the left and right sets of the gills of host fish *Vimba vimba tenella* although, statistically significant numbers occurred on the filaments of the second gill arches of both sides. Several authors suggested that monogenean distribution to be influenced by the hydrostatic pressure of the branchial pump, coughing action and water current over the gill surface during the respiratory cycle (Hughes and Shelton, 1958; Paling, 1968; Wootten, 1974).

In conclusion, the present study provided the first simultaneous and comparable infestation details of *D. aequans*, a previously known monogenean parasite of

D. labrax collected from two distinct localities in the Black Sea and the Aegean Sea, and results provided here are of practical importance on the determination of control strategies to be developed against this significant pathogen.

ACKNOWLEDGEMENTS

This study (Project Number: 114O736) was supported financially by the Scientific and Technological Research Council of Turkey (TUBITAK). The authors are grateful for this valuable support. It is also acknowledged that this study was previously delivered in the 18th National Fisheries Symposium, 1-4 September 2015, İzmir, Turkey as a poster presentation.

Author contributions

All authors conceived, designed and conducted field and laboratory work. A.Ö. analysed the data and wrote the initial version of the manuscript; all other authors provided editorial advice.

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