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Prevalence and Intensity of Ectoparasites in Rainbow Trout *(Oncorhynchus mykiss)* from Larvae Stage to Market Size in Turkey

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Abstract

Rainbow trout (*Oncorhynchus mykiss*) from three Turkish farms were sampled monthly from May 2003 to June 2004 to determine ectoparasite seasonality, prevalence, and intensity. Smears of gills, skin, and fins of 1260 larvae to market-size fish were examined. Six protozoans and one monogenean were found: *Ichthyobodo necator, Chilodonella piscicola, Ichthyophthirius multifiliis, Gyrodactylus* sp., *Trichodina claviformis, Apiosoma piscicolumn,* and *Hexamita salmonis*. The first three were found mainly in fry and all except *I. multifiliis* occurred in low temperatures from late fall through early spring. Infections of *C. piscicola* and *Gyrodactylus* sp. were persistent and *T. claviformis* was found for the first time on rainbow trout in the area.

Introduction

The climate and geography of the eastern Black Sea region of Turkey provides a suitable environment for the culture of coldwater fish species, particularly rainbow trout, *Oncorhynchus mykiss*. Since the introduction of rainbow trout in the early 1980s, the area has been closed to fish importation, mainly because of technical and economical limitations. Thus, many parasitic and bacterial diseases were absent in the area. Recently, however, along with the use of high quality feed and techniques, increased production capacity enhanced fish transfers into and out of the area, leading to the spread of new dis-

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eases such as enteric red-mouth disease at alarming rates. The lack of epidemiological data about fish diseases in general makes it difficult to know which diseases were or are present and in which seasons they occur. Therefore, it is currently impossible to follow the spread of infectious diseases into and out of different regions of Turkey.

Ectoparasitic protozoans can cause severe, acute, or chronic problems in aquaculture and may become a limiting factor since they can multiply rapidly and be transmitted directly. If ectoparasites are not controlled, they can lead to secondary bacterial or viral infections (Shimura et al., 1983; Urawa, 1993; Athanassopoulou et al., 2004). If, in fish to be transferred to the sea, the skin is eroded by external parasites, adaptation to sea water is diminished (Urawa, 1996). Some ectoparasites, e.g., *Ichthyophthirius multifiliis,* can cause chronic stress resulting in decreased growth rates (Wolf and Markiw, 1982). Thus, even if the infection level is too low to cause mortality, the ectoparasite can have economic effects by causing low growth rates in their hosts. Attention should be given to ectoparasites to prevent, control, or diminish the economic impact of secondary bacterial infections and related use of antibiotics.

In this study, we carried out an extensive survey to determine monthly occurrence of ectoparasites and related epizootics during the trout life cycle, from larvae stage (2 g) to market size (~250 g), taking pond-to-pond variability into consideration. Results can have significant implications on the transfer of rainbow trout to sea cages for grow-out in the Black Sea.

Materials and Methods

Study site. The Macka River, the largest river system in the eastern Black Sea region, is the location of over 20 rainbow trout farms. Rainbow trout is the only species cultured in the region. Since the main objective of this study was to determine whether any parasite species were present at a given time, three high-capacity farms in close proximity (within 1 km of each other) were selected to increase the chance of detecting ectoparasites in the system. The capacity of the upstream farm is 110 tons, the midstream farm 95 tons, and the downstream farm 65 tons. In addition, each farm contributes 20-50 tons annually to the Black Sea netpen culture. To further increase the chances of detecting target species, the most problematic pool in each farm was selected. The farms are about 700 m above sea level and water temperature fluctuates 4-20ºC. River temperature and oxygen levels are shown in Fig. 1.

Sampling. The three farms were visited monthly from March 2003 to June 2004 to determine the presence, occurrence, prevalence, and mean intensity of external fish parasites following the protocol for parasites in freshwater fish developed for the Ecological Monitoring and Assessment Network (EMAN), Canada.

On the same day, skin, gills, and fin scrapings of thirty-five fish from each pool (total 1260 fish for 12 samplings) were examined microscopically at the farm. Smears of infected skin and gills were fixed for 5 min with absolute ethyl alcohol. External parasites were fixed in 10% buffered formaldehyde with 1 ppt oxytetracycline or 80% ethanol for species determination. In cases of ectoparasitic disease epizootics, whole fish samples were fixed with buffered formaldehyde for further examination. Weight and fork lengths were measured to calculate the condition factor.

Modified Klein's silver impregnation technique (Lom, 1958) was used to stain ciliated protozoans. The smears were dipped into silver-nitrate ($AgNO_3$, 4% w/v) for 15 min, exposed to UV light for 30 min, and digitally photographed. Morphological parameters were measured using Photoshop 6.0 (Adobe Design Inc). Monogeneans were carefully flattened and fixed with alcohol-formalin-ascetic acid (AFA) for observation and photographing.

Identification of parasites. Trichodinid species were identified using the descriptions of Lom (1958) and the key reported by Dobberstein and Palm (2000). *Chilodonella piscicola* and *Ichthyobodo necator* were identified using descriptions of Lom and Dyková

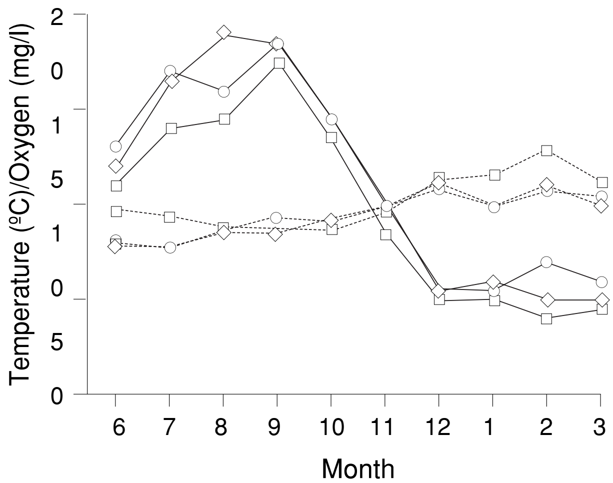

Fig. 1. Monthly temperature (——) and oxygen (- - - - -) at upstream (□), midstream (◇), and downstream (○) farms in which rainbow trout were examined for ectoparasites.

(1992) and monogenean species using the descriptions of Buchmann and Bresciani (1995). The terms mean intensity (the mean number of a given ectoparasite on infected fish in a sample) and prevalence (proportion of infected fish in a sample) follow the definitions of Bush et al. (1997).

Statistical analysis. Non-parametric one-way ANOVA was used to analyze differences in condition factor, mean intensity, and prevalence. Differences were considered significant when $p<0.05$. Statistical analyses were performed using Statistica 6.0 (Statsoft Inc., Cary, NC).

Results

Six ectoparasites were found: *Ichthyobodo necator*, *Gyrodactylus* sp., *Chilodonella piscicola*, *Trichodina claviformis*, *Ichthyophthirius multifiliis*, and *Apiosoma piscicolumn*. Only the first four cause epizootics and were therefore evaluated in this study. Information about *I. multifiliis* is published elsewhere (Ogut et al., 2006) as is information about a protozoan endoparasite, *Hexamita salmonis*, found during this study (Ogut and Akyol, 2005). All the epizootics that occurred during the survey were attributed to these organisms or bacterial diseases and there was no unexplained mortality related to unknown reasons (e.g., viral or toxicants). Mean monthly condition factors of the rainbow trout at the three farms are given in Table 1.

Ichthyobodo necator was observed only on the skin (not on the gills) of fry (>1.5 g) from April to June (Table 2). Diseased fish were lethargic, listless, and swam up to the water surface only to turn quickly back to the water column (making infected ponds identifiable just by looking at the water surface). Heavily infected fish, however, did not always show signs of infection. Disease epidemics with heavy mortality (8-56%) were observed when not controlled. *Ichthyobodo necator* was first observed in the midstream farm which had heavy mortality and high prevalence rates (46%). The epidemic was controlled with formalin (180 ml per ton water for 20 min, three treatments) or with Chloramin-T (10 g per ton water for 40 min, three treatments) and the parasite was not observed again until November. One month after the epidemic in the midstream farm, *I. necator* was observed in the downstream farm where it remained

Table 1. Mean condition factors (CF) of rainbow trout at three farms in the Macka River in Turkey.

	Upstream	*Midstream*	*Downstream*
Jan	1.12±0.02	1.23±0.02	1.14±0.02
Feb	1.04±0.01	1.2±0.02	1.19±0.02
Mar	1.04±0.02	1.16±0.02	1.04±0.01
Apr	1.08±0.00	1.32±0.03	1.09±0.06
May	1.08±0.03	1.27±0.04	1.39±0.04
Jun	1.07±0.02	1.15±0.02	1.00±0.08
Jul	1.03±0.03	1.36±0.02	1.09±0.02
Aug	1.27±0.04	1.26±0.03	1.19±0.03
Sep	1.46±0.03	1.39±0.02	1.22±0.02
Oct	1.39±0.02	1.34±0.02	1.21±0.02
Nov	1.31±0.05	1.25±0.03	1.14±0.03
Dec	1.14±0.01	1.22±0.02	1.32±0.02

from May to August since the farmer did not apply any treatment. The prevalence decreased gradually from 76% to 3% during this period but the mean intensity unexpectedly did not change (31-50%). The protozoan was not detected in the upstream farm at any time.

The only monogenean ectoparasite species was a *Gyrodactylus* sp. not previously described in the literature (Fig. 2). The parasite was present mainly on caudal fins but, during heavier infections, also on the skin and gills. Infected fish were listless, disoriented, and frequently jumped out of the water. *Gyrodactylus* sp. was present throughout the year, except when the oxygen level was 8 mg/l or below. During the cold season, many live parasite offspring were detected inside dead mothers that were hanging on the tail of the host. Mean intensity and parasite prevalence did not correlate (r = 0.12).

Chilodonella piscicola was present in the system mainly in the winter. This species ranged 53-82 µm in length, was asymmetrically oval, had 12 kineties on the right ciliary band, and 12 kineties on the left ciliary band (Fig. 3). It had an oval macronucleous and a micronucleous. Mean intensity and prevalence were high only in the upstream farm. The parasite was detected in the midstream farm at very low levels (mean intensity 2.5-4.67, prevalence 6-9%) and not at all in the downstream farm. The *C. piscicola* level in the upstream farm was controlled by applying formalin (180 ml per ton of water). No treatment was applied in the midstream farm since the parasite level was low. Unexpectedly, there was a single case of *C. piscicola* in August.

The morphometric *T. claviformis* was the only trichodinid species observed during the study. This species is large (79.8±5.6 µm) and the body is laterally flat and cylindrical (Fig. 4). The diameter of the adhesive disc is 63.7±4.2 µm, the border membrane width 5.3±0.3 µm, and the diameter of the denticle ring 44.4±5.8 µm. A large number of various size granules were scattered in the center. There were 28.5±1.7 denticles and 12.5±1.7 radial pins per denticle. Denticle length was 9.5±0.9 µm, blade length 7.3±0.9 µm, ray length 5±0.8 µm, and denticle span 15.4±1.4 µm. The turn of the aboral ciliary wreath was 355-362°. The

Table 2. Mean monthly intensity (MI) and prevalence (P in %) of four ectoparasites on cultured rainbow trout.

	Ichthyobodo necator				*Gyrodactylus sp*					
	Midstream		*Downstream*		*Upstream*		*Midstream*		*Downstream*	
	MI	*P*	*MI*	*P*	*MI*	*P*	*MI*	*P*	*MI*	*P*
Jan	-	-	-	-	1	6	3.00±3.0	3	1.5±0.6	11
Feb	-	-	-	-	1	3	-	-	3.00±2.8	6
Mar	-	-	-	-	-	-	1	3	1.40±0.6	14
Apr	1	46	-	-	1	3	-	-	-	-
May	-	-	35.12±16.4	71	1.88±2.1	23	-	-	-	-
Jun	-	-	34.75±16.3	46	-	-	-	-	-	-
Jul	-	-	31.00±21.9	11	-	-	-	-	-	-
Aug	-	-	50	3	1	3	-	-	-	-
Sep	-	-	-	-	-	-	1	3	-	-
Oct	-	-	-	-	1	9	-	-	1.20±0.5	14
Nov	50	3	-	-	13.75±24.2	11	8.68±9.8	9	-	-
Dec	-	-	-	-	1	6	3.00±3.0	17	1.33±0.5	17

	Chilodonella piscicola				*Trichodina claviformis*					
	Upstream		*Midstream*		*Upstream*		*Midstream*		*Downstream*	
	MI	*P*	*MI*	*P*	*MI*	*P*	*MI*	*P*	*MI*	*P*
Jan	3.25±2.2	12	4.67±5.5	9	2.64±2.6	31	1	14	2.14±1.8	20
Feb	5.50±3.7	17	2.50±2.1	6	1.33±0.5	17	1	3	5.94±5.5	51
Mar	4.64±4.0	32	-	-	2.75±2.9	11	-	-	-	-
Apr	1	6	-	-	1.60±1.3	29	-	-	-	-
May	21.4±33.9	14	-	-	2.00±1.2	31	-	-	-	-
Jun	-	-	-	-	-	-	1	10	-	-
Jul	-	-	-	-	-	-	-	-	-	-
Aug	-	-	-	-	-	-	-	-	-	-
Sep	-	-	1	6	-	-	-	-	1	3
Oct	-	-	-	-	2.08±1.5	34	2	3	2.2±1.4	29
Nov	-	-	-	-	-	-	-	-	-	-
Dec	-	-	-	-	2.29±2.0	20	1	3	6.33±11.2	17

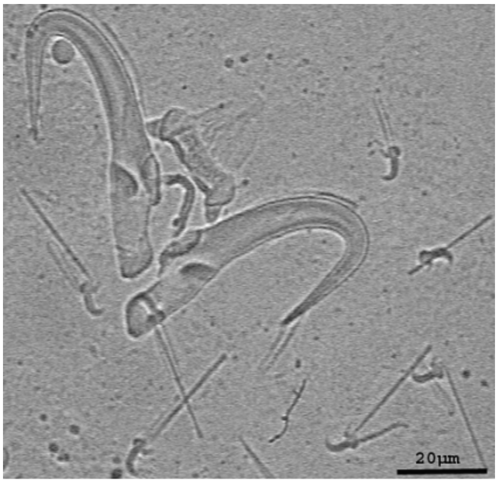

Fig. 2. Marginal hooks, anchors, and ventral bars of the *Gyrodactylus* sp.

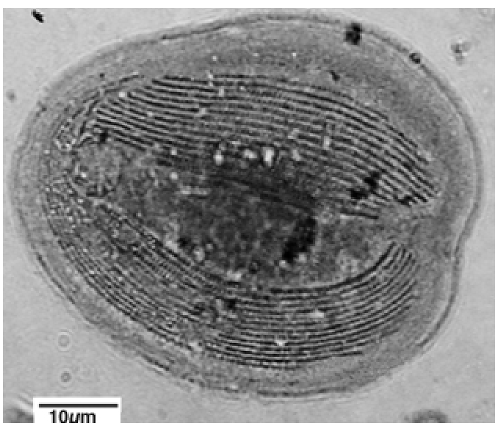

Fig. 3. *Chilodonella piscicola.*

macronucleous was horseshoe-shaped with a diameter of 51±2.3 µm. Of the 1260 examined trout, 122 were infected (mean intensity 2.9±0.4, prevalence 9.7%). Generally, *T. claviformis* was present on fish longer than 7 cm. The mean intensity of this species did not correlate with prevalence (r = 0.03, $p<0.05$) and was low despite prevalence as high as 40%. This ciliate was present in the river system from the late fall to mid spring.

Gyrodactylus sp. was the only species found together with *I. multifiliis, C. piscicola*, and *T. claviformis* (Table 3). Two fish were infected by three ectoparasites: *T. claviformis*, *C. piscicola*, and *Gyrodactylus* sp. The cases of parasite coexistence were too few to determine any trend.

Discussion

The ectoparasitic fauna predominantly comprised *Ichthyobodo necator*, *Gyrodactylus* sp., *Chilodonella piscicola*, *Trichodina claviformis*, *Ichthyophthirius multifiliis*, and *Apiosoma piscicolumn*. The distribution and fluctuation of the ectoparasites were seasonal. Among the most problematic, *I. necator* and *C. piscicola* occurred in cold temperatures. *Ichthyophthirius multifiliis* occurred in summer and caused problems in late summer and early fall.

Our results indicate that in Turkey, as in North America and Europe (Wood, 1979; Robertson, 1985), *I. necator* is one of the most detrimental parasites in cultured rainbow trout. In the past, it was reported that this parasite occurs mainly in poor water quality (Bauer, 1959). Recently, however, the disease has been reported in optimal aquaculture conditions (Urawa, 1992). In our region, new incidences of the parasite caused disease epizootics from April and May as reported by Wood (1979) and persisted through mid-summer if not treated. At the midstream farm, the protozoan was eliminated from the system by application of formalin as suggested by Wood (1979), Robertson (1985), and Woo and Poynton (1995). At the downstream farm, where infected fish were not treated, the prevalence of the parasite gradually dropped from May to August although intensity did not, indicating that transfer of the parasite from fish to fish did not occur despite the initial high prevalence and intensity.

Mortality of fish infected by *I. necator* in April-May may have been caused by costiasis or other synergically added stressors such as low water quality since, during this period, the number of fish per pond is at its highest within the 10-14 month rearing period. After this period, farmers begin to lower the number of fish per pond. The condition factor of the fish sig-

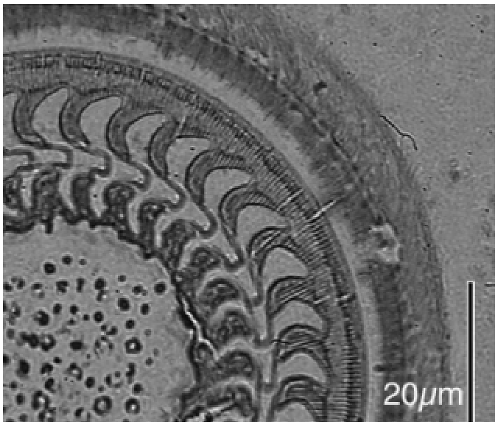

Fig. 4. *Trichodina claviformis*, silver stained.

nificantly rose (Tukey-Kramer Test, $p<0.05$) during the months that costiasis was observed at the midstream and upstream farms. In the following month, the condition factor dropped to levels before costiasis was observed. The fluctuation in condition factor did not occur at the upstream farm where costiasis was not observed, suggesting water intake due to tissue damage and recovery in the following month. The condition factor at the midstream farm was significantly higher than at the upstream or downstream farms during January-April. Possibly, the effective control of the ectoparasites resulted in better fitness of larvae and postlarvae during this period.

Gyrodactylus sp. was present in the system throughout the year but at a higher intensity during the winter. The monogenean became a problem when the O_2 level in the water rose to 8 mg/l or above. In April and May, for example, the parasite was present only in the upstream farm where the O_2 level was 9.75 mg/l. At the same time, the O_2 level was below 8 mg/l at the other two farms. The reason for this should be further explored.

This parasite rarely causes problems in rainbow trout. During the last five years, only two cases of fish heavily infected by *Gyrodactylus* sp. were submitted to the Fish Diseases Laboratory at the Faculty of Marine Sciences. However, even though *Gyrodactylus* sp. itself does not seem to significantly impact growth and survival of trout in the region, if this parasite coexists with other ectoparasites or stressors, serious damage may result. Barker et al. (2002) reported that mixed infections of *G. pleuronecti* and *Trichodina murmanica* synergically caused significant growth loss. In our study, there were few cases of co-infection, preventing us from comparing growth rates of individuals infected by one ectoparasite with those of individuals infected by more than one.

Chilodonella piscicola is an important ectoparasite affecting the region's marketable trout. Unfavorable factors such as high population density, low dissolved oxygen, and high fluctuations in water temperature are factors for the occurrence and severity of *C. piscicola* outbreaks (Snieszko, 1974; Wedemeyer, 1976; Wood, 1979; Roberts and Sheperd, 1986). However, Urawa (1996) experimentally demonstrated that the disease can occur in optimal environmental conditions. The parasite was in the system mainly during the cold season (late fall to early spring), similar to

Table 3. Total number of infected fish and number of fish infected by more than one ectoparasite.

	Total	*Chilodonella piscicola*	*Trichodina claviformis*	*Ichthyobodo necator*	*Gyrodactylus sp.*
I. multifiliis	128	0	8	0	4
C. piscicola	35		8	0	2
T. claviformis	122			0	7
I. necator	63				0

reports by Bauer (1959) that its maximum reproduction rate occurs at 5-10ºC. The single case of *C. piscicola* in August in our study can be explained by heavy snow melts which lowered the water temperature to levels favorable for *C. piscicola*. However, persistent infection at 12-16ºC in late spring to early summer at other farms in the region cannot be explained by temperature.

The parasite appeared only on the skin and was harmless at low intensities, in many cases requiring no treatment. However, sometimes the protozoan causes persistent infections due to unknown reasons (Noga, 1995) and may be found on both the gills and skin, always forming cysts if found on the gills (Bauer and Nikolskaya, 1957). In this case, fish need to be treated multiple times with formalin for at least one hour (Hoffman and Meyer, 1974; Noga, 1995). If, however, the epidemic occurs after May, the suggested one-hour formalin treatment is almost impossible due to high water temperatures. Losses as high as 38% of the marketable trout can be anticipated if multiple formalin treatments are not applied in 3-30 min treatments each day at intervals of four hours until complete removal of the parasite from the gills. Even a one-hour formalin treatment per day would not effectively remove the parasite (Urawa, 1996).

Trichodina claviformis was also present in the system mainly in the colder months. It was persistent in the upstream farm during the winter and early spring, possibly due to the increased organic pollution (nitrate and phosphate) of the river during this period (Ogut and Palm, 2005). Although data did not indicate any mortality related to this species, controlling *T. claviformis* could prevent secondary infection. *Trichodina claviformis* was frequently detected on fish submitted for diagnosis to the Fish Diseases Laboratory at the Faculty of Marine Sciences. Intensity of the protozoan was frequently high at ponds with significantly less water or ponds experiencing water cutoffs for some time (pers. comm.). This species was the only ectoparasite that coexisted with other ectoparasites. There is no data about this species regarding its pathogenity to salmonids. Comparing its effects on trout with that of other trichodinid species could be misleading. Therefore, more study is needed to fully determine its impact on trout culture.

In conclusion, our survey showed that *I. necator, Gyrodactylus* sp., *C. piscicola, T. claviformis,* and *I. multifiliis* economically affect trout culture. These species, known to cause extensive damage to skin and gills, could be important factors during grow-out in the area since they can significantly limit the ability of fish to osmoregulate after transfer to sea water and can lead to secondary bacterial and viral infections.

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