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Seasonality of *Ichthyophthirius multifiliis* in the Trout (*Oncorhynchus mykiss*) Farms of the Eastern Black Sea Region of Turkey

Hamdi Ogut[1,*], Abdurrezak Akyol[1], Mehmet Zeki Alkan[1]
[1] Karadeniz Technical University, Faculty of Marine Sciences, Department of Fisheries, 61530 Sürmene, Trabzon, Turkey

* Corresponding Author: Tel.: +90 462 752 2805/128; Fax: +90 462 752 2158;
E-mail: oguth@ktu.edu.tr

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Abstract

Rainbow trout (*Oncorhynchus mykiss*) ranging from fry to marketing size were sampled monthly in 2002 from three farms to determine prevalence, abundance, and intensity of *Ichthyophthirius multifiliis*, the causative agent of ichthyophthiriasis. *I. multifiliis* was present and active in the farms from June to August. However, outbreaks of ichthyophthiriasis occurred only late in summer. Persistent presence of the ciliated parasitic protozoan in the farms suggests that *I. multifiliis* is endemic in the region. Thus, effective measures such as decreasing of host densities and routine prophylactic treatments should be applied to decrease or eliminate the impact of the parasite on growth of the host. This is the first extensive study providing seasonal data on the presence of *I. multifiliis* in the Eastern Black Sea Region of Turkey.

Key Words: Keywords: *Ichthyophthirius multifiliis,* prevalence, intensity, seasonal occurrence, ichthyophthiriasis, rainbow trout

Introduction

The ciliated protozoan, *Ichthyophthirius multifiliis* (Foquet), the causative agent of ichthyophthiriasis or Ich, is one of the most important pathogenic parasites of cultured fish (Schäperclaus, 1991). This protozoan is widely distributed to all parts of the world but Antarctica, and almost all of the freshwater fish are susceptible to infections. Mortality rate of infected fish by ichthyophthiriasis could reach almost 100 % (Meyer, 1974). In some cases, this parasite does not cause mortality, but it still has negative consequences on the population and individual fish (Lom and Dyková, 1992), although clear evidence is not available. Furthermore, no data is available on whether Ich can be a cause of decreased growth rate of fish. However, it should be noted that since an immune reaction is initiated for *I. multifiliis* (Wolf and Markiw, 1982), an energy allocation occurs in immune system probably resulting in decreased growth rates.

Ichthyophthiriasis outbreaks occur during the summer months when temperatures are at the peak in the Northeastern Black Sea Region of Turkey. However, the extent of the natural outbreaks and the factors contributing to the incidence of disease and parasite prevalence are not known. In this study, we monthly examined the incidence of ichthyophthiriasis and the prevalence and intensity of *I. multifiliis* in three fish farms located on a big river system, the Maçka River, Trabzon, Turkey.

Material and Methods

Rainbow trout (*Oncorhynchus mykiss*) ranging from fry (1.2 g) to marketing size (200-300 g) were monthly sampled at three farms in 2002. These farms, separated from each other with less than 1 km, were designated as upper, middle and lower according to their location on the river line. A total of 35 fish was monthly collected from three pools (each 10 or 15 fish) of each farm. This sampling method was used since a large variation was detected in the prevalence and intensity of any parasite among pools in our earlier work (unpublished results). Fish were killed with a blow to the head and the right side body surface above the lateral line was scraped gently to obtain smears. Gills and fins were also removed and examined directly. The smears and other preparations were examined at the farm by a microscope at 40x or 100x magnification. The number of trophonts was counted and recorded. Weight and length of the fish was measured and recorded as well. Temperature was measured, and oxygen concentration (inflow and discharge) was determined by Winkler method at the time of sampling. Moreover, 21 visits were carried out to the farms complaining excess mortality during summer, 2002. At each visit, stocking densities of fish, fish weights and lengths, and water temperature and dissolved oxygen contents were recorded. A minimum of 35 samples was examined with a microscope at the farm as described above to determine whether observed outbreaks were ich-related. Diagnosis of ichthyophthiriasis was carried out as described in Blue Book (Ewing, 1994).

Mean prevalence, intensity, and abundance were calculated using samples collected from three pools at each farm. The terms prevalence, intensity and abundance were used as described in Bush *et al.* (1997). Intensity and abundance at three farms were compared using ANOVA (Rózsa *et al.,* 2000). And Tukey-Kramer test was employed as multiple comparison tests (month to month, pool to pool at each farm) in order to pinpoint statistical differences. In all comparisons, significance level was set at 0.05. All statistical analyses were performed using Statview 5.0 (SAS Institute Inc.).

Results

The trophont of *I. multifiliis* observed in this study is shown in Figure 1. *I. multifiliis* was present in the river system from June through November, 2002 (Figure 2). Statistically significant differences in mean prevalence, mean intensity and mean abundance were observed among the months (ANOVA, P<0.05), peaking in August.

Figure 1. A trophont of *I. multifiliis* and its horseshoe shaped macronucleus (Bar is 20 µm).

The parasite was not detected in the samples from upper and middle farms in September when a serious muddy flooding occurred in the river system. The mean abundance and mean intensities of parasites in samples collected in August, September (only the lower farm), and October were statistically different from the other months (ANOVA, P<0.05).

The mean intensities were significantly correlated with temperature ($F_{1,21}$=15.2, P<0.05, Figure 3). The level of infection was divided into three zones according to temperature and mean intensities. Epizootics and excess mortality were observed at temperatures above 18ºC when host had more than 30 parasites, with prevalence levels above 80%. The mean parasite intensity, however, did not depend on the length (r^2 = 0.23) and condition factor (r^2 = 0.01) of the fish (Figure 4), or water temperature and oxygen contents in the surveyed farms in whole year (Figure 5). The prevalence, intensity and abundance levels of the three farms in August and October were not statistically different (Tukey-Kramer test, P>0.05). Moreover, pool-to-pool variability in the mean prevalence and intensity, when *I. multifiliis* presented, was high (Figure 2). Prevalence at some cases ranged from 0 to 100% among the pools examined.

Twenty-one farms complaining excess mortality were visited or their samples were accepted from late spring to the fall of 2002. Five out of 21 epidemic cases occurred in the region were diagnosed as ichthyophthiriasis. Ichthyophthiriasis - related mortalities ceased after three daily-consecutive formalin treatments (150 ppm for 15 min at 18ºC).

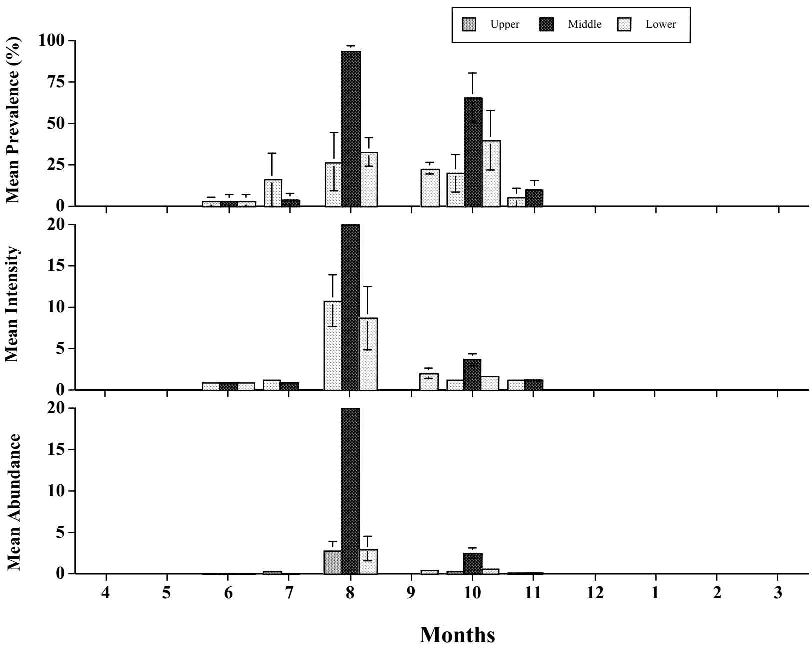

Figure 2. Seasonal variation of the mean abundance, the mean intensity and mean prevalence of *I. multifiliis*. Vertical bars show standard errors (±SE).

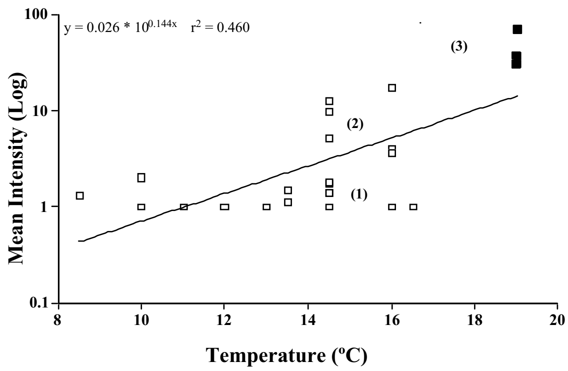

Figure 3. Relationship between water temperature and mean intensity of *I. multifiliis.* Black squares show that excess mortality (epizootic) occurred.

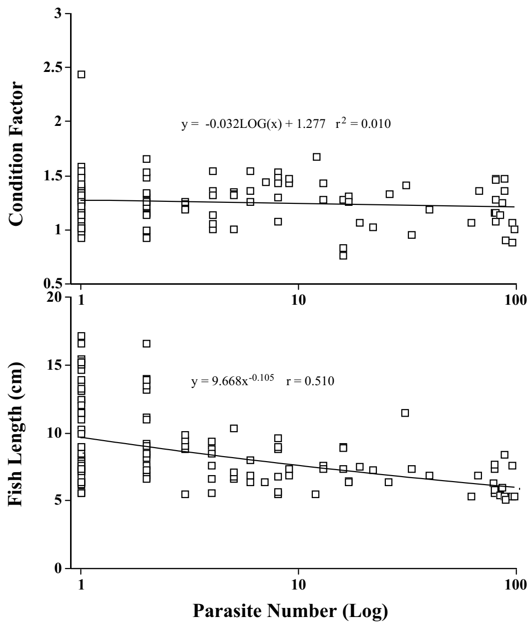

Figure 4. Number of parasites and its relationship to fish length and condition factor. The data include all infected fish from three farms.

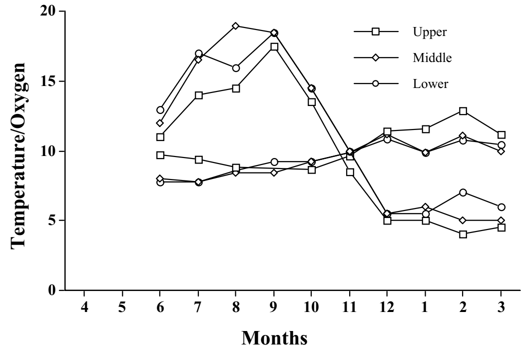

Figure 5. Monthly fluctuation of temperature and oxygen at three farms. Continuous lines represent temperatures and dotted lines oxygen.

Discussion

The ciliated protozoan, *I. multifiliis*, was present and active when temperatures were above 14ºC and ichthyophthiriasis -related mortality was observed when temperatures were above 18ºC. The peak of the mean prevalence, intensity, and abundance of *I. multifiliis* occurred in August. Unexpectedly, the parasites were absent from upper and middle stream farms in the following month. This was probably due to muddy flooding with cold water originated from heavy rain in the mountains. Significantly higher pool-to-pool variations in the mean prevalence, intensity, and abundance of *I. multifiliis* were largely due to the improper water supply and insufficient water circulation.

The life cycle duration of the *I. multifiliis* depends on temperature (Bauer, 1958). The present study indicated a positive logarithmic relationship between magnitude of the mean intensity and temperature. Bodensteiner *et al*. (2000) reported epizootics in channel catfish (*Ictalurus punctatus*) occurring at temperature ranging from 6 to 12ºC, on the contrary to our results, and many others indicated that ichthyophthiriasis epizootics in rainbow trout occur above 18ºC. Moreover, we were unable to detect any trophont of *I. multifiliis* in the winter months in all three farms visited. This difference might be a result of different races of *I. multifiliis* as suggested by some researchers (Nigrelli *et al.,* 1976). Frequent ichthyophthiriasis epizootics at higher water temperature (24– 26ºC according to Bauer 1959) were also reported from the farms of North America (Wood, 1979) and Europe (Bauer, 1959; Valtonen and Keräen, 1981).

Three factors might have contributed to the occurrence of epizootics in farmed trout in late summer. First, the temperature late in the summer was higher, and in parallel to the temperature, water inflow to the farms was low due to shortage of rain. Bodensteiner *et al*. (2000) showed that incoming water is a key factor in determining the levels of ichthyophthiriasis in channel catfish. In the same study, it was suggested that turnover rates of water could be more important than water velocity. A second potential factor is that epizootics (excess mortality) occurred consistently late in the summer. This outcome was probably a result of the small difference in day and night temperatures during the late summer. Early in the summer, daylight temperature of the incoming water was higher than that of the night even though some high temperatures were observed especially at the rivers with low water flows. This suggests that frequent occurrences of the ichthyophthiriasis outbreaks in the late summer are due to low water temperature fluctuation in daylight. Lastly, the race of the *I. multifiliis* could be a determinant of the time when ichthyophthiriasis occurs.

In the spring of 2005, an unusual ichthyophthiriasis epidemic was observed in the rainbow trout brood stock at 12-14 °C (unpublished data). Later on, the parasite spread to the juveniles and mortality of the affected fish reached 96%. The gills were heavily affected by the parasite and formalin treatments were proved to be detrimental to the fish. The parasite was transferred to another farm via transfer of the infected fish. This spread of the parasite in that farm took place in short time (5 days). Mean intensities of the parasite were successfully decreased to ignorable levels with application of salt (6 ppt) every 12 hrs. This case was different in other outbreaks detected in the area for the last five years, in which all occurred at 18°C and above suggesting that the agent caused mortality at lower temperature (12-14°C) is somewhat different from that at higher temperature (over 18°C).

I. multifiliis is endemic in the region. Several factors could be responsible for this endemicity. These might include improper water circulation, insufficient water supply, insufficient tank cleaning and disinfecting regimes, or in some cases, reuse of the water without treatment. One attempted epidemiological study to determine the factors affecting prevalence and incidence of Ich in trout farms in the region was halted due to too many uncontrollable factors (unpublished results).

Farmers in the region generally ignore the parasite since it causes none, or very low levels of mortality only when temperatures are above the seasonal averages. However, inexistence of mortality is not always indicative of a healthy system. Lom and Dyková (1992) suggested that yield could be reduced by half in heavily stocked fish due to non-lethal infections. Moreover, during exceptionally hot years, yield could be reduced both by disease related mortality and/or stress caused by the parasite. Furthermore, even though treatment would not be so costly, treating fish for Ich in hot seasons could impose more risk on host weakened against secondary infections, e.g. yersiniosis caused by *Yersinia ruckeri*.

High pool-to-pool variations in prevalence of *I. multifiliis* were observed. No studies have been conducted in the regional farms to compare the seasonal variations of the protozoan parasite. However, in future epidemiological research designed especially for risk factors enhancing parasite occurrence, pool-to-pool variation should be considered. Especially, farm-to-farm comparisons based on the data collected by point sampling in a farm are not a proper way.

Clayton and Price (1994) reported that in poeciliid fish experimentally infected with *I. multifiliis*, body area affected susceptibility. Although fish size was not associated with the abundance of the protozoan in the present study, high intensities of the parasite were observed in fish ranging 5-10 cm in length. The resistance of bigger fish might be due to acquired immunity from previous infections (Lom and Dyková, 1992). Fish of 5-10 cm in length belong to 0- year class and have never experienced *I. multifiliis* infections. Similarly, no relationship was detected between condition of fish, and infection level when measured as number of trophont per fish.

In short, our results indicate that *I. multifiliis* first appeared in the system in June and caused Ich-related mortality in August and disappeared after December. The parasite (trophonts) were absent in winter months. Mean intensities of the parasite were mainly affected by water temperature. Increased load of grown fish and decreased levels of water due to shortage of rainfalls in August probably enhanced the chance for ichthyophthiriasis outbreaks. An adjustment in loading density of fish in late spring could help decrease the impact of the parasite.

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