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Article *in* Bulletin- European Association of Fish Pathologists · January 2019

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Seasonal distribution of protozoan parasite infections in rainbow trout (*Oncorhynchus mykiss*) farms in the Eastern Black Sea of Turkey

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Abstract
In the present study a total of 8850 rainbow trout (*Oncorhynchus mykiss*) fry (0.2-10 g) and juveniles (30-80 g) were sampled monthly from 15 farms in the Eastern Black Sea region of Turkey during a one-year period. The parasites *Trichodina* sp., *Chilodonella* sp., *Ichthyobodo* sp., *Ichthyophthirius multifiliis* and *Spironucleus salmonicida* were recorded from all trout farms. Infections with these protozoa are responsible for the high mortality (30% to 50%) rates found in rainbow trout farms in this region. Infested trout displayed exophthalmia, skin discoloration and abdominal distension. The seasonal prevalence of the infections varied between 5.77±1.46 and 37.72±4.41% for *Trichodina* sp., 5.63±1.36 and 38.52±12.27% for *Chilodonella* sp., 5.77±1.46 and 37.72±4.41% for *Ichthyobodo* sp., 5.34±1.37 and 49.96±7.91% for *I. multifiliis* and 8.02±4.30 and 38.52±12.27% for *S. salmonicida*, respectively. This is the first study to provide seasonal data on the presence and intensity of five protozoan parasites in cultured rainbow trout in Turkey.

Introduction

The availability of suitable fresh water resources in Turkey has permitted the growth of commercial rainbow trout aquaculture with an annual production of 101 761 tonnes in 2017 (TUIK, 2018). Protozoan parasites represent one of the most important groups of pathogens which negatively affect the health of cultured and feral fish in Turkey and decrease their market value. In the last decade, there has been a tremendous increase in protozoan infestation restricting rainbow trout production. It has been determined that protozoan parasitic infestations affected sustainable production leading to 70% mortality and major economic losses, if not diagnosed and/or treated at the early stage. The most important protozoan infections in rainbow trout (*Oncorhynchus mykiss*) farms are *Chilodonella* sp., *Ichthyobodo* sp., *Spironucleus* sp., *Ichthyophthirius multifiliis* and *Trichodina* spp. It was reported that parasitic ciliate infections may cause significant production and economic losses in fresh water fish farms (Lom and Dykova, 1992; Scholz, 1999; Bastos Gomes et al., 2017). *Ichthyobodo necator* is a flagellated protozoan causing infections of the skin which lead to significant loss in salmonid fish culture (Durborow, 2003). *Spironucleus* sp. is a flagellated parasite of the intestine which has previously been described as *Hexamita* or *Octomitus*

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(Poynton and Sterud, 2002). *Spironucleus salmonicida* (previously *Hexamita salmonis*) has been reported in the gastrointestinal tracts of juvenile salmonids cultivated in fresh water (Buchmann and Uldal, 1996; Poynton and Sterud, 2002), marine reared Atlantic salmon (Mo et al., 1990; Poppe et al., 1992; Jørgensen and Sterud, 2006) and in ornamental fish (Klinger and Floyd, 2009; Paull and Matthews, 2001). *Chilodonella* is a flat and oval shaped ciliate that occurs on fish gills and skin. Infections with *Chilodonella* spp. cause epithelial hyperplasia, gill lamellae fusion, inflammatory infiltrate, hemorrhages, oedema and necrosis, and these can be dangerous in cultured fish (Durborow, 2003; Klinger and Floyd, 2009). *Trichodina* are pathogenic ciliated protozoa usually found on the gills and various parts of the body. Trichodinosis may affect most fresh water fish causing mortality (Durborow, 2003). *Ichthyophthirius multifiliis* (Ich) is an obligatory parasite which resides as a trophozoite below the epithelium of the skin of freshwater fish. Ichthyophthiriasis (white spot disease) can cause high mortality in cultivated fish stocks within a short time due to damage to the epithelium (Durborow et al., 1998; Davis et al., 2002; Klinger and Floyd, 2009).

There are no comprehensive studies focusing on seasonal prevalence and intensity of these parasitic protozoans in farmed rainbow trout in Turkey. Earlier reports from Turkey document the prevalence and the intensity of *H. salmonis* in rainbow trout (Ogut and Akyol, 2005), the seasonality of *I. multifiliis* in trout farms (Ogut at al., 2005), external protozoan parasites in three trout species of the Eastern Black Sea Region (Balta at al., 2008) and a histopathological study of hexamitiasis in rainbow trout (Timur et al., 2009).

Given the severity of the current situation, the aim of the present study was to describe seasonal prevalence and intensity of five different protozoan parasites in cultured rainbow trout in Turkey.

Material and Methods

Trout collection and sampling area

A total of 60 rainbow trout (0.2-10 g fry to 30-80 g juveniles) were randomly sampled from six polls

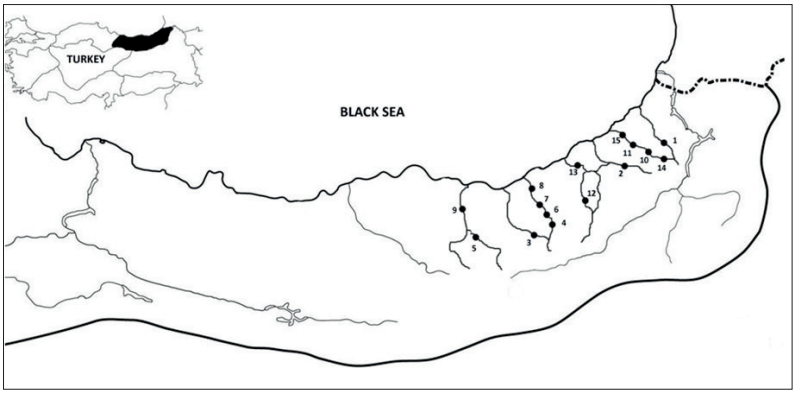

Figure 1. Sampling area, the numbers represent the fish farms, (The map modified from URL-1/http://cografyaharita.com/turkiye-dilsiz-haritalari.html).

per month from each of 15 farms (Figure 1) over a year. All farms used untreated surface water while some farms used second-pass untreated water. The farms ranged in elevation from 10 to 2100 m. In the winter months, sampling from some farms could not be carried out due to heavy snow. Annual production among farms ranged from 50 to 250 tonnes.

Parasitological examinations

Rainbow trout were examined on location in a field laboratory. Standard investigation for fish parasites was conducted according to Buchmann (2007). Fresh mucus smears were separately collected from gills, skin and fins to identify protozoan parasites by microscopic examination (Lom and Dykova, 1992). Morphological identification of the protozoan parasites were performed directly on wet mounts of gills, skin and fins mucus under a microscope at 10 to 40x magnification. The intestine of each trout was longitudinally opened and microscopic examination was carried out on a scrape preparation of the posterior part of intestine at 40x magnification. While *Trichodina* positive slides were air-dried and stained by using silver methods, other protozoan positive slides were stained using Giemsa solution (Lom and Dykova, 1992). The stained slides were examined and photographed under a light microscope at 100x magnification (Leica DM4000 B) in the laboratory. All protozoan parasites on each independent slide were counted under a light microscope and recorded for each species. Mean prevalence and intensity were calculated as described by Bush et al. (1997). Parasite counts from gills, skin and fins scrapings and intestinal content were assigned a rank number differentiating between; no infection, low infection (1-10 parasites), intermediate infection (11-25 parasites) and high infection (25-80 parasites). Water temperature, hardness, dissolved oxygen and pH were monitored on all farms.

Statistical analyses were performed using SPSS (Ver. 23.00). One-way analysis of variance (ANOVA) was used to determine the statistical significance of monthly differences in mean water temperature, mean intensity and mean prevalence and mortality rate. Multiple comparisons were performed with Duncan multiple range test.

Results

During the study, seasonal mean water temperature ranged from 2.71±0.19°C to 21.44±0.16°C. The mean water temperature (°C), total hardness (mg/l as CaCO3), oxygen (mg/l) and pH

Table 1. Distribution of protozoan species according to location on the rainbow trout and definition of relative infection levels

Parasite species	Skin surface	Gills	Fins	Intestinal content
Chilodonella sp.	+++	++	+	-
Ichthyobodo necator	+++	++	+	-
Ichtyophthirius multifilis	+++	+++	++	-
Trichodina sp.	+++	+++	++	-
Spironucleus salmonicida	-	-	-	+++

-: No parasite, +: Parasite between 1-10 (low), ++: Parasite between 11-25 (intermediate), +++: Parasite between 26-80 (high)

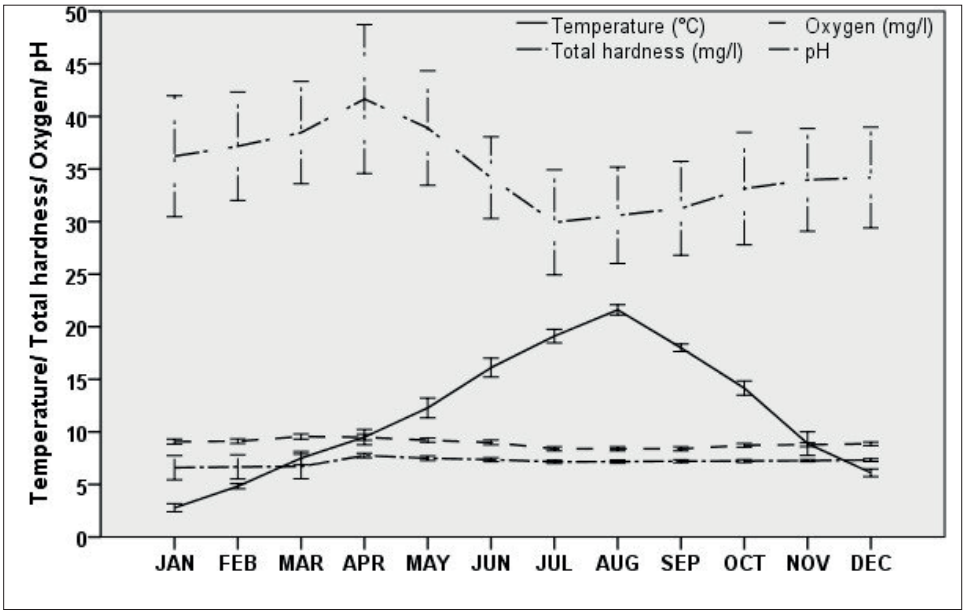

Figure 2. Annual monthly mean water temperature (°C), total hardness (mg/l as $CaCO_3$), oxygen (mg/l) and pH from fish farms.

measured in fish farms are shown in Figure 2. The protozoan parasites *Chilodonella* sp., *I. necator*, *I. multifiliis* and *Trichodina* sp.were found on the gills, skin and fins of rainbow trout examined from all farms. Moreover, *S. salmonicida* was found especially in the intestine, but rarely in the kidney. The locations of the identified protozoans and relative infection levels are shown in Table 1. Infected rainbow trout swam near the water surface and showed severe signs of lethargy, emaciation and anorexia. The seasonal prevalence of the infections varied between 5.77±1.46 and 37.72±4.41% for *Trichodina* sp., 5.63±1.36 and 38.52±12.27% for *Chilodonella* sp., 5.77±1.46 and 37.72±4.41% for *Ichthyobodo* sp., 5.34±1.37 and 49.96±7.91% for *I. multifiliis* and 8.02±4.30 and 38.52±12.27% for *S. salmonicida*, respectively. Many cases of protozoan parasites in rainbow trout fry occur as mixed infestation (e.g., *Chilodonella* sp., *Trichodina* sp. and Ich; *Ichthyobodo* and *Spironucleus* or Ich and *Spironucleus*). Moreover, the prevalence of mixed infestation reached 35.5%. Between 10 and 80 protozoan parasites were observed per microscopic field in a typical scrape preparation of the gills or skin. Fry infected with *S. salmonicida* displayed a slow spiral swimming behaviour. Thin brown faeces extending from the anus of infected trout and faecal examination revealed an average of 10-100 *S. salmonicida* per microscope field. Furthermore, mortality associated with the advanced stages of protozoan infestations reached seasonally 100% when the affected trout were anorexic. Mortality rates from March through to June when the water temperature ranged from 5 - 14°C were 35 to 40% whereas from June through to September, when the water temperature exceeded 16°C, mortality rates were 40 to 50%.

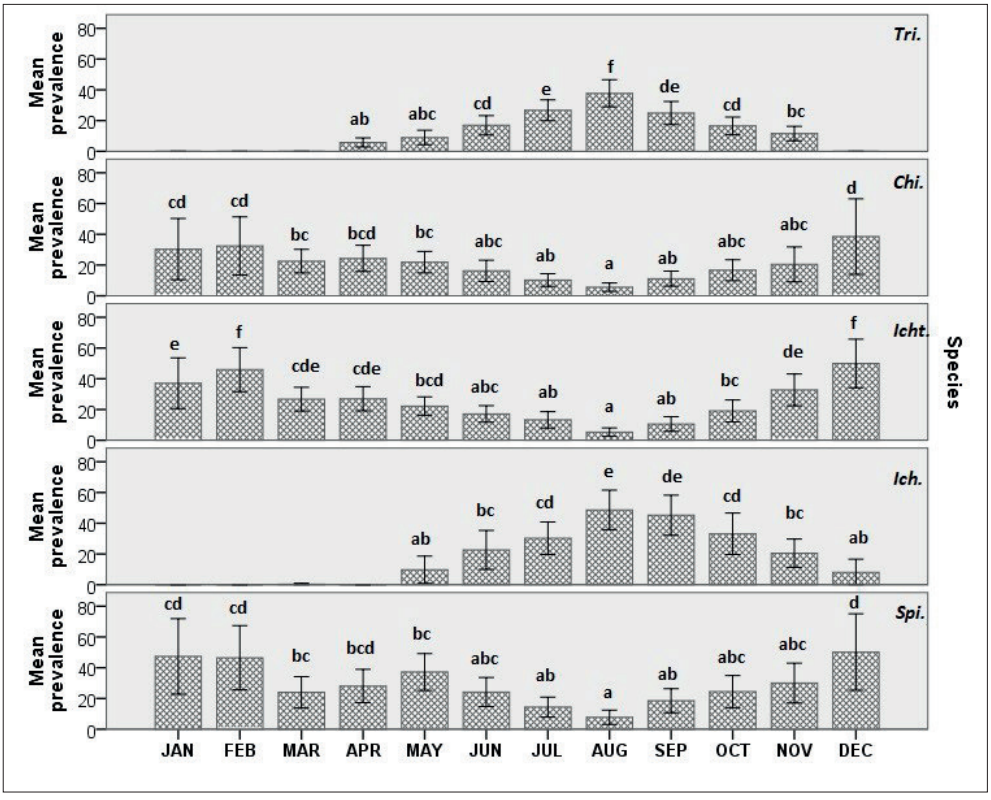

Figure 3. Seasonal mean prevalence of five protozoan parasites from examined fish farms. *Trichodina* sp. *(Tri.), Chilodonella* sp. *(Chi), I. necator (Icht), I. multifiliis (Ich.), S. salmonicida (Spi.).* Different letters (a,b,c,d,e) above error bars indicate significant differences ($p<0.05$). Data were expressed as mean ±SE.

While three protozoan species (*I. necator, S. salmonicida* and *Chilodonella* sp.) were detected in fish examined throughout the year, Ich and *Trichodina* sp. were detected from April to November (Figure 3). All parasites were observed at all farms in different months depending on the water temperature and the elevation. The mean prevalence ranged from 9.78±4.44 to 48.67±6.40 for Ich and 5.77±1.46 to 37.72±4.41 for *Trichodina* sp. (Figure 3). The highest prevalence was observed in August for both Ich and *Trichodina* sp. Infestations with *Chilodonella* sp., *I. necator* and *S. salmonicida* were observed all year with the highest prevalence observed in December: 38.52±12.27 for *Chilodonella* sp., 49.96±7.91 for *I. necator* and 50.16±12.45 for *S. salmonicida* (Figure 3).

The highest mean intensity of *Trichodina* sp. and Ich was 59.77±2.18 and 70.02±2.59, respectively. The intensity of these two protozoan species was highest in August and lowest in April (Figure 4). The highest mean intensity was 67.15 ±1.97 for *I. necator* in May, 66.43±2.41 for *S. salmonicida* in May and 32.95±1.69 for *Chilodonella* sp. in June (Figure 4).

Discussion

The widespread occurrence of parasite infesta-

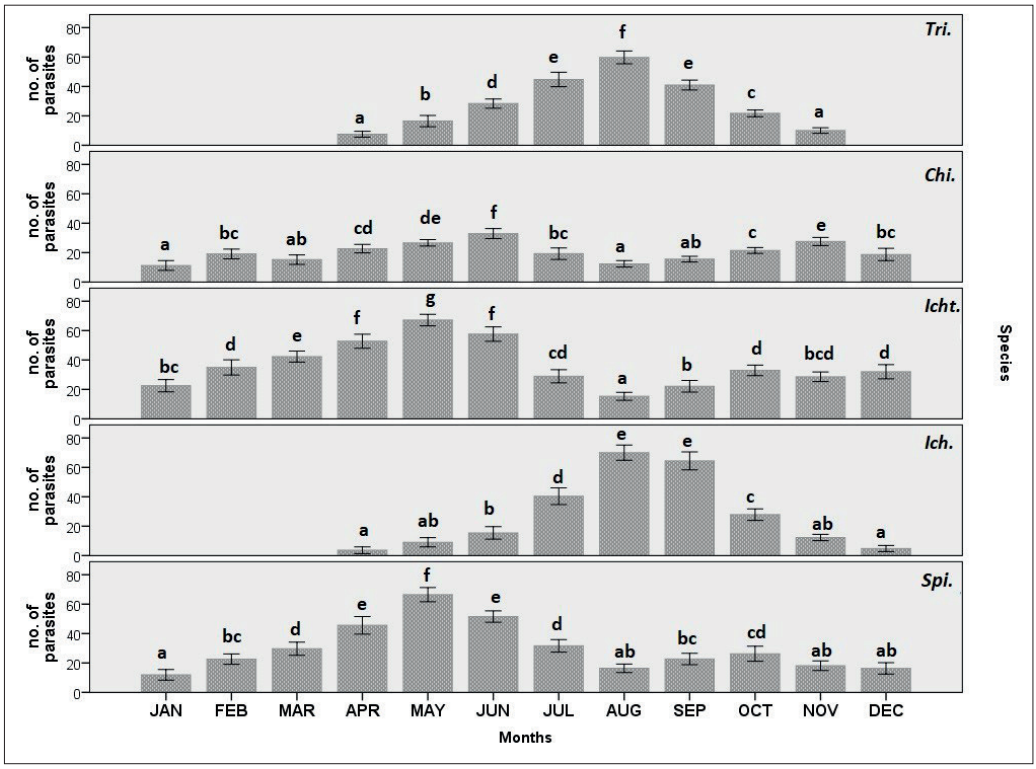

Figure 4. Seasonal mean intensity of five protozoan parasites from examined fish farms. *Trichodina* sp. *(Tri.), Chilodonella* sp. *(Chi), I. necator (Icht), I. multifiliis (Ich.), S. salmonicida (Spi.).* Different letters (a,b,c,d,e) above error bars indicate significant differences ($p<0.05$). Data were expressed as mean ±SE.

tion among trout populations in this study may be related to farms sharing untreated water on common river systems as well as the practice of transporting fish between farms within the region. In addition, reservoirs of infection may occur in natural fish populations. In the present study, four external parasitic species (*Chilodonella* sp., *I. necator, I. multifiliis* and *Trichodina* sp.) were identified compared to three species (*Chilodonella* sp., *I. necator* and *I. multifiliis*) identified in a previous study on rainbow trout from the Eastern Black Sea region (Balta et al., 2008). We observed that the mean prevalence and intensity of the protozoans were monthly differentiated depending on the water temperature (Figure 3, 4). A positive relationship between *Trichodina* sp. and *I. multifiliis* infestations and water temperature were reported by some researchers (Buchmann and Bresciani, 1997; Buchmann et al., 2001; Ogut and Akyol, 2005; Ogut et al., 2005; Jørgensen et al., 2009).

S. salmonicida is the most common internal fish parasite encountered in salmonid hatcheries for which trout fry are especially affected in the South Eastern Black Sea region of Turkey (Kayis et al., 2009). Moreover, we found *S. salmonicida* in cultured rainbow trout in the in the

Eastern Black Sea of Turkey similar to Kayis et al. (2009). Moreover, there are some reports associated with mortalities in fry corresponding to previous observations on *Hexamita salmonis* (Davis, 1926; Moore, 1923; Ferguson, 1979). In addition, evidence of more prevalent *H. salmonis* infections in the cold season was observed by Poynton (1986). Similarly, we observed mortality associated with *S. salmonicida* infestation in March through to June when the temperature of the water was cold in the present study.

Chilodonella infections may result in rapid epizootic events on fish farms with mortalities within two or three days of infection and losses of 50–95% of fish stocks (Paperna and Van As, 1983; Karvonen et al., 2010). Rintamäki-Kinnunen and Valtonen (1997) reported that *I. multifiliis* and *I. necator* infestations in salmon usually occurred at temperatures between 10 and 20°C. On the other hand, *I. necator* infestation occurred in a wide temperature range from 3.5 to 38°C (Robertson, 1979). Trichodinids are important parasites of both freshwater and marine fish. The heavy infestations caused significant pathological changes to the skin and gills of affected fish (Lom and Dykova, 1992). Heavy infestations of *I. necator* and *Trichodina* causing skin hyperplasia and destruction of the normal gill structure were associated with mortality in rainbow trout (McArdle, 1984). Moreover, *I. multifiliis* was associated with high fish mortality in some rainbow trout farms (Ogut et al., 2005; Jørgensen et al. 2009).

In the present study, *S. salmonicida*, *I. necator* and *Chilodonella* sp. infestations were associated with mortality (35 to 40%) in March through to June, when water temperatures ranged from 5-14°C. Whereas, Ich and *Trichodina* sp. were associated with mortality (40 to 50%) in June through to September when the water temperature exceeded 16°C. These data indicate that routine examination and treatment are necessary to minimise the impacts of protozoan infections in farmed rainbow trout.

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