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PREVALENCE AND INTENSITY OF *HEXAMITA SALMONIS* IN RAINBOW TROUT FARMS IN THE SOUTHEASTERN BLACK SEA AND THEIR RELATIONSHIP TO ENVIRONMENTAL FACTORS

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Key words: *Hexamita salmonis*, intensity, prevalence, rainbow trout, temperature

Abstract

An acute rise of unexplained mortalities (reaching 84,000 fish at one farm in 2002, 32%) was observed at a rainbow trout (*Oncorhynchus mykiss*) farm on the southeastern Black Sea coast from January to June for at least the last four years. Three rainbow trout farms on the same river system were surveyed for eight months to determine the occurrence and spread of the suspected causative agent, *Hexamita salmonis*. We found that this protozoan was endemic to the trout farms from December through late August. The main determinants of the incidence and intensity were water temperature (non-linear regression, $r^2 = 0.77$) and oxygen and nitrate levels (ANOVA, $r^2 = 0.83$). The mean intensity of the parasite gradually decreased while prevalence gradually increased from February to April at the farm with the highest values. There was no weight loss in infected fish compared to uninfected fish ($p = 0.4$). The downstream farm had a higher level of parasitic intensity than the upstream farm during the high risk season. Hexamitiasis was the main cause of the high mortality in May and June when water temperatures were around 6-13ºC. Prophylactic measures should be taken in systems where this parasite is endemic. This is the first report of hexamitiasis from Turkey.

Introduction

Large numbers of unexplained mortalities were recorded in recent years (up to 84,000 fish, 32%, at one farm in 2002) by farmers in the Black Sea region. Unlike mortalities attributed to known disease epizootics, these mortalities were observed at fairly low temperatures (~6ºC). Moreover, except for darkening and lethargy, no indication of systemic infection by viral or bacterial infectious agents was detected. Farmers usually blamed nitrate-based fertilizers applied concomitantly with the mortalities. The causative agent was determined to be the diplomonadid intestinal flagellate, *Hexamita salmonis*. There are no earlier records of hexamitiasis-related mortality in Turkey.

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H. salmonis (Moore 1923) can cause a high level of mortality (Allison, 1963; Bregnballe, 1963). Factors enhancing the occurrence and spread of hexamitiasis and its consequences are unclear. Known and unknown aspects of the disease described by Uzman et al. (1965) still hold true. It has been suggested that inadequate diet, change in diet, low oxygen content in the water, keeping various size fish together (Becker, 1977; Vickerman, 1989) or, more probably, a combination of these factors increase the incidence and intensity of the parasite. In controlled *in vitro* experiments, temperature was found to be a factor in the reproduction rate of *H. salmonis* (Buchmann and Uldal, 1996). However, it is not known whether this holds true for natural epizootics.

The objectives of this study were: (a) to determine the seasonal prevalence, intensity, and potential pathology of infection in three adjacent farms on the Macka River and (b) to discuss the potential impact of the parasite on trout farming.

Materials and Methods

Study site and fish. Rainbow trout, aged one year and up (14-181 g), from three commercial trout farms close to each other on the same river, were sampled monthly from December to July 2004 to determine the prevalence and intensity of infection. This time period was selected since water temperatures were around 6ºC, the temperature at which *H. salmonis* reproduces most efficiently (Buchmann and Uldal, 1996). It was assumed that if the parasite were present, it would have the highest impact at this time. Temperature nitrate, nitrite, phosphate, and oxygen concentrations were measured by the Winkler method at each site visit.

Determination of infection intensity. Procedures described by Tojo and Santamarina (1998) were followed with minor modifications. Fish were killed by an overdose of anesthesia. Fecal samples were obtained through the anus by squeezing the abdomen. All intestinal contents were emptied and immediately examined under a microscope without a slide cover. Since the feces were undisturbed (*H. salmonis* was found between the feces and the lumen of the intestine), no water was added to the slide, in contrast to the procedures of Tojo and Santamarina (1998). The terms 'prevalence' and 'intensity' were used as described in Bush et al. (1997).

Statistics. Monthly differences in mean intensity and condition factor (CF = 100 x weight/length^3) were tested with a conservative test, Sheffe's test. One way ANOVA was used to determine statistical differences in mean intensity. Regression analysis was carried out to determine the association between temperature and mean intensity. Multivariate analysis was used to determine whether a combination of factors was responsible for the prevalence and intensity of the protozoan.

Results

The infected fish were lethargic and swam close to the surface of the water. Their color had darkened and they had swollen abdomens containing serosanguinous ascites. The color of the intestines and intestinal contents of infected fish were mostly indistinguishable from those of uninfected fish. However, when there was an epizootic onset, most of the infected fish had no food in the intestine and the intestinal contents were yellowish. There were no differences in weight, length, or condition factor between infected and uninfected fish (p = 0.40).

There were statistical differences among mean intensities of *H. salmonis* in the three farms (ANOVA, $p<0.05$). High levels were observed at the downstream farm, that decreased gradually from January to April (Fig. 1). Mean intensity at the upstream farm did not significantly differ between months, while mean intensity at the midstream farm was statistically higher in January than in other months ($p<0.05$). On the other hand, the lowest intensity at the downstream farm was detected in May-June (Sheffe's test, $p<0.01$). Unexpectedly, the midstream farm had an erratic level of mean intensity. Prevalence at the downstream farm gradually increased until April while, unexpectedly, intensity decreased. Thus, prevalence was generally the highest in April, while mean intensity was the highest in February.

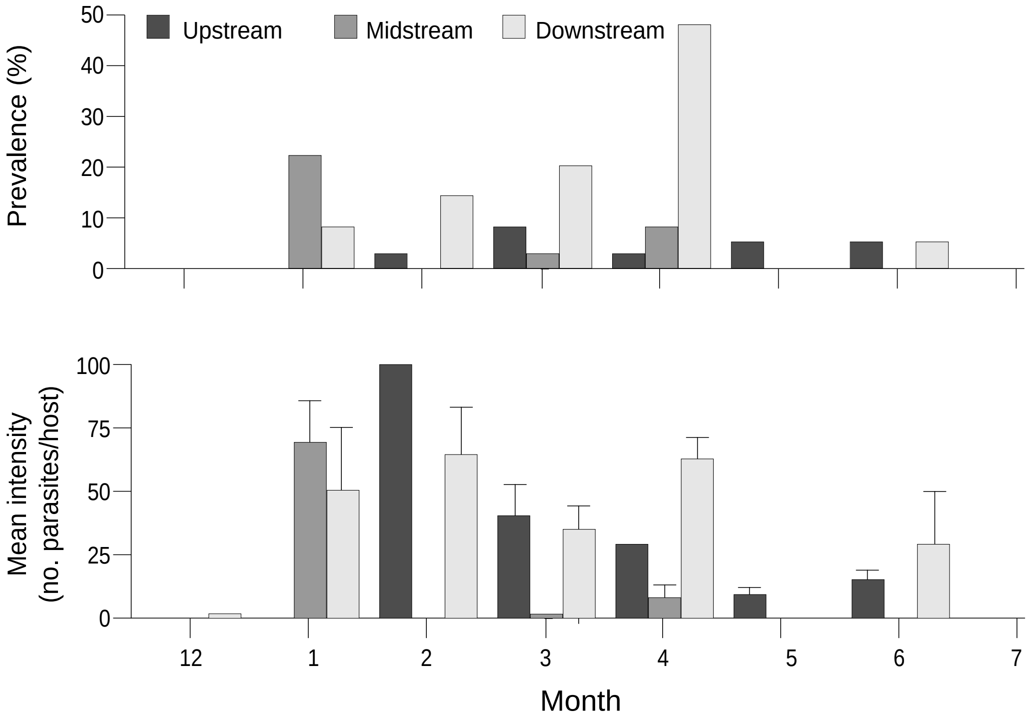

Fig. 1. Monthly mean prevalence and intensity of *H. salmonis* in three trout farms on the Black Sea coast of Turkey.

Prevalence and intensity were closely related to water temperature (Fig. 2). Prevalence sharply increased with the increase in temperature from 4 to 6ºC, then gradually decreased to negligible with further increases in temperature. Seventy-seven percent of the variation in mean intensity was associated with the variation of water temperature (regression analysis). Multivariate analysis of water quality parameters (Table 1) suggests that oxygen and nitrate were responsible for 83% of the variation in mean intensity. The best model was mean intensity = (17.28 x oxygen) + (2.0 x nitrate) - 186.37 ($F = 14.67$, $p = 0.0049$). Temperature was related to intensity ($p<0.033$, $r^2 = 0.77$), but replacement of oxygen in the model with temperature decreased the predictive capability of the model ($p = 0.097$). Only addition of nitrate to the model improved its predictive capability (from $p<0.0198$ to $p<0.0049$).

In a case study of a fourth farm located further upstream than the surveyed farms, large numbers of *H. salmonis* related mortality occurred during May. Daily mortality generally increased with slight increases in temperature (Fig. 3). Mean intensity was so high that it was uncountable and prevalence was 96% in May. On June 10, the parasite was undetectable in treated fish but was present in one group of untreated fish at a lower prevalence at the same farm (mean intensity = 6.3±1.3, prevalence = 8%).

Discussion

The gradual decrease in mean intensity of *H. salmonis* from February to July can be explained by the increase in temperature. Similar seasonal variations in intensity of this parasite were reported by Moore (1923), Davis (1956), and Buchmann et al. (1995). An

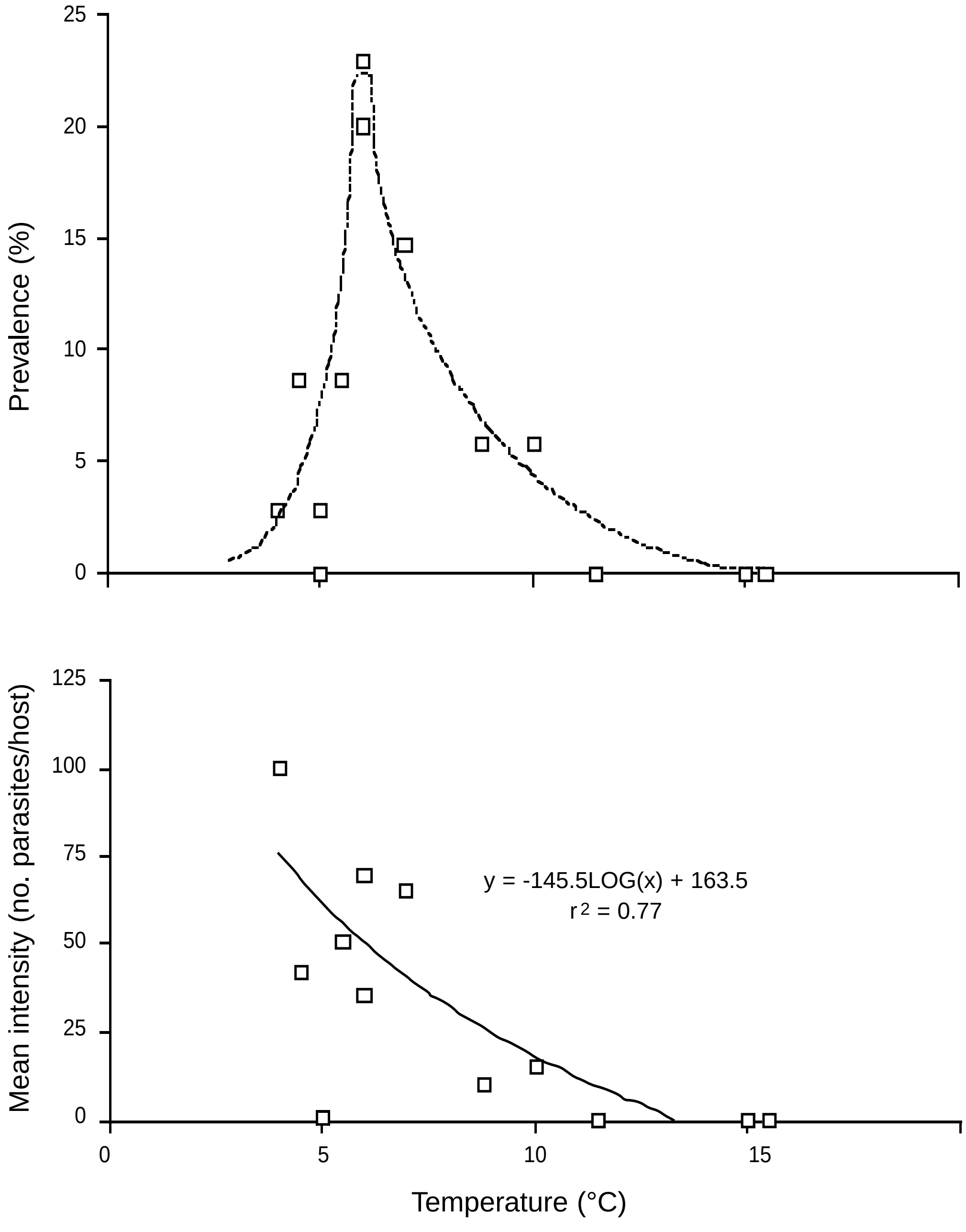

Fig. 2. The relationships between (a) water temperature and prevalence and (b) water temperature and mean intensity.

Table 1. Water quality parameters (±standard error of means from three farms) in three rainbow trout fish farms on the southeastern coast of the Black Sea.

Month	*Temperature (°C)*	*Oxygen (mg/l)*	*Nitrate (μa/l)*	*Nitrite (μa/l)*	*Phosphate (μa/l)*
December	5.33±0.17	11.15±0.16	23.27±0.37	0.04±0.0	1.13±0.17
January	5.5±0.29	10.43±0.57	47.16±6.80	0.05±0.02	0.09±0.05
February	5.33±0.88	11.57±0.65	24.54±0.32	0.05±0.01	0.17±0.02
March	5.17±0.44	10.54±0.35	21.8±2.03	0.04±0.01	0.29±0.07
April	ND	ND	34.37±16.11	0.14±0.03	0.39±0.05
May	ND	ND	24.79±2.11	0.22±0.08	0.99±0.07
June	12±0.58	8.52±0.62	25.72±5.47	0.08±0.01	1.78±0.27
July	15.83±0.93	8.32±0.52	27.87±1.67	0.03±0.02	0.67±0.39

ND = no data

interesting phenomenon occurred at the downstream farm – the mean intensity of the parasite gradually decreased whereas the prevalence increased from February to April, suggesting that the parasite reproduces during February at low temperatures but spreads later on at higher temperatures. This new finding deserves further study.

Multivariate analysis unexpectedly suggested that oxygen and nitrate were the two main factors determining parasite intensity. Since our sample size was small (18 samples), it is possible that the observed association occurred by chance. On the other hand, it is possible that environmental oxygen rather than temperature was responsible for effective protozoan reproduction since they are interrelated; as temperature increases, the oxygen carrying capacity of the water decreases. Moreover, the high levels of environmental nitrate could have been caused by high loading densities (high nitrate) and agricultural fertilization. More research is needed to determine whether oxygen or temperature is more important in the reproduction of this protozoan. Since temperature and oxygen are related and temperature is more practical, temperature was used in the presentations in this study.

Both larval fish (0.25 g) and one-year-old fish (50-181 g) were infected at varying levels (Ogut, unpubl. results), similar to the findings of Uldal and Buchmann (1996). The protozoan was not detected in fish larger than 181 g. Size dependent occurrence was reported by Poynton (1986) and Uldal and Buchmann (1996). The latter speculated that an inadequately developed immune system or other physiological factors might be responsible. We observed *H. salmonis*-related mortality in one-year-old fish. Therefore, immature immunity cannot be a contributing cause. Temperature, on the other hand, was positively correlated to the variation (77%) in parasite intensity. Fish are small during the winter and spring when water temperatures are below 10ºC. Larvae are also kept at temperatures lower than 10ºC. The protozoan may be more prevalent in smaller fish at this time of year, likely as a result of the high fish density in the small, poorly circulated tanks where they are kept. It was frequently observed that one-year-old fish in farms at higher altitudes were affected and died when temperatures were appropriate whereas no impact was observed in smaller fish at lower farms on the same river (Ogut, unpubl. results). *H. salmo-*

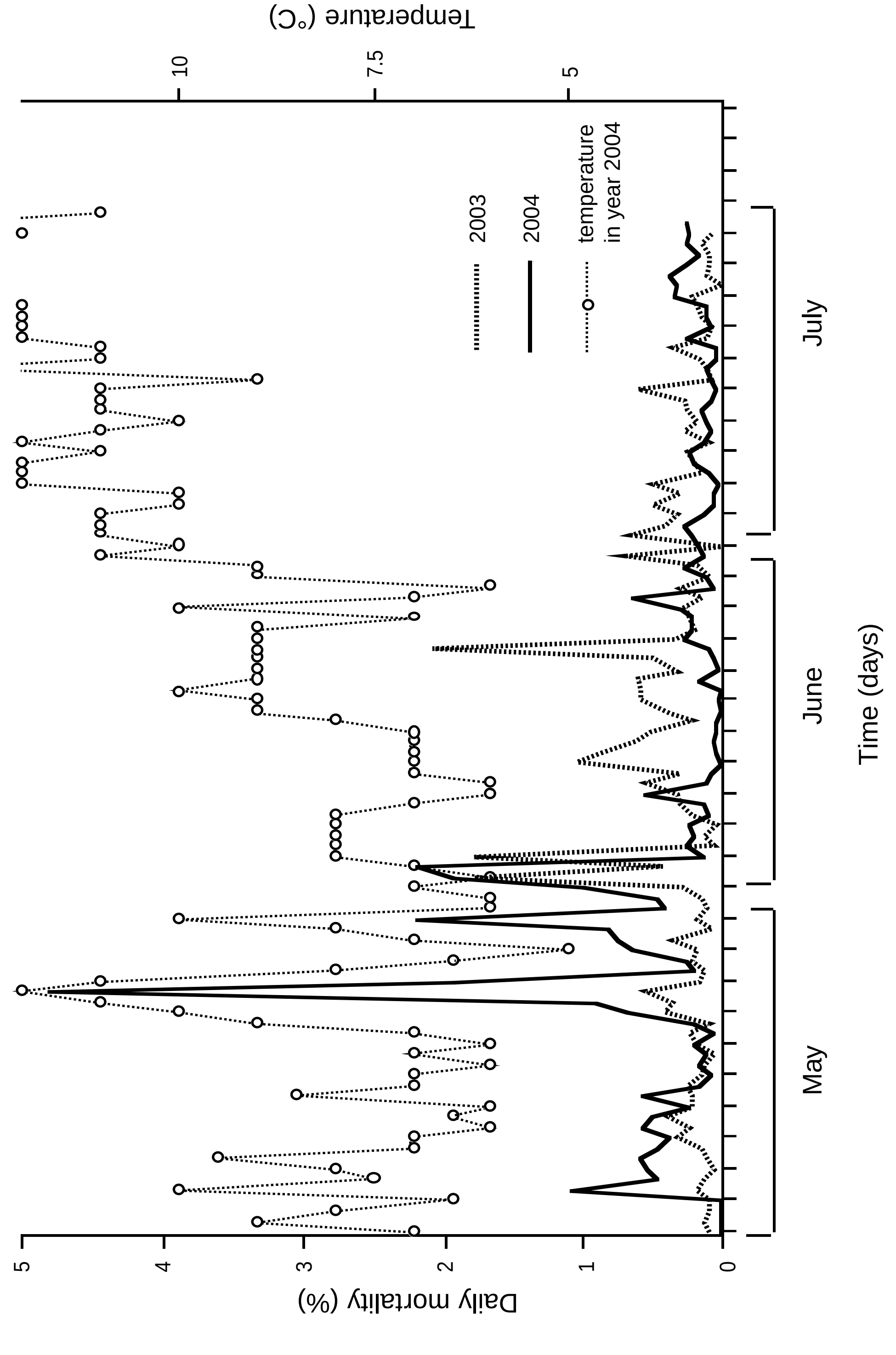

Fig. 3. Daily mortality during May-July. Initial stocking was 88,000 fish in 2003 and 50,000 in 2004.

nis caused mortality in August at a farm located 1800 m above sea level where the water temperature was 13ºC during the day (Ogut, unpubl. data). Diet, as suggested by Becker (1977) and Vickerman (1989), may have been a contributing factor but there are no studies on this aspect; further study is necessary. Greater emphasis should be given to the influence of temperature since there is firm evidence that temperature has a strong effect on the reproduction rate of *H. salmonis* (Buchmann and Uldal, 1996). Thus, temperature rather than fish size or other suspected factors should be targeted first to explain high intensities and incidences of this protozoan.

Hexamitiasis and its impact on trout culture are somewhat confusing. Allison (1963) and Bregnballe (1963) reported hexamitiasis-related mortality in rainbow trout. On the other hand, Noga (1995) reported that an *H. salmonis* intensity of less than 35 is safe and no treatment is required. This parasite caused no mortality on the farms studied during the survey even though the protozoan was present at varying levels. However, in two other farms on the same river system, chronic mortality (~1-3%/week) was observed at 6±1ºC. Prevalence levels in these farms were above 90% and the mean intensity of the parasite was considerably high (uncountable). Mortality ceased with application of medicated feed (magnesium sulfate at 3% of the feed).

In contrast to our findings, Uldal and Buchmann (1996) found that infected fish weighed less and were shorter than uninfected fish. Considering the suggestion that no treatment is needed for fish with less than 15 protozoan, one can assume that infection of a fish does not necessarily mean that it will be seriously affected. Thus, it is possible that above certain intensities (i.e., epizootics), weight loss could be anticipated. Moreover, as suggested by Uldal and Buchmann (1996), fish with other problems may be more susceptible to *H. salmonis*.

Maintenance of proper water quality and good husbandry are essential for controlling this parasite. In controlled experiments, Uzman et al. (1965) found that morbidity was higher in fish raised with poor husbandry practices. As suggested by Uldal and Buchmann (1996), fish with a reduced appetite and growth rate due to other reasons were probably more susceptible to infection. We observed that infected fish were very fragile to stresses generated from other sources such as turbidity in the spring. When cleaning or bath treatments (e.g., formalin or chloramin-T) were carried out on infected fish, large amounts of mortality were anticipated. Thus, prior to the high risk season, fish should be screened for *H. salmonis*. If the parasite is detected, fish should be treated immediately since heavily infected fish avoid medicated feed.

Unexpectedly, during the one-year survey, no outbreaks of yersiniosiz caused by *Yersinia ruckeri* were observed. Fish in the three farms were checked monthly and received appropriate prophylactic treatment against ectoparasites. Thus, efficient control of ectoparasites may have prevented secondary infections such as yersiniosiz. On the other hand, carriers of *Y. ruckeri* discharge bacteria every 45 days from colonies that settle in the posterior intestine (Bush and Lingg, 1975). It is possible that bacteria discharged in the intestine were consumed by *H. salmonis*, preventing the spread of *Y. ruckeri* to susceptible fish. The use of *H. salmonis* as a prophylactic method to control yersiniosiz needs further exploration.

The fourth farm had heavy mortalities from May to July for at least the last four years in spite of an appropriate temperature of 6ºC during those months. In 2004, the mortality was attributed to the protozoan *H. salmonis*. It is unlikely that the mortalities in previous years were caused by bacterial disease since the seasonal temperatures are below 10ºC with an average of approximately 6ºC. Sometimes, especially during May, mortality increased with a sudden increase of temperature, perhaps due to added stress from lowered water quality. Fish density was also a factor in the large number of daily mortalities. In 2002, 263,000 fish were stocked at the beginning of May. Almost half the stock died within three months. In 2003 and 2004, stocking density was much lower: 88,000 and 50,000, respectively. Thus, the impact of the parasite was probably the weakest in 2004. In early June 2004, a treatment of magnesium sulfate (3% of feed for three days) lowered the parasite load to almost undetectable levels. A respected brand of magnesium sulfate should be used for treatment; otherwise, results may vary.

In conclusion, accumulated evidence suggests that *H. salmonis*, previously not well known in the area, probably spread and became endemic in trout farms in the Black Sea region of Turkey due to uncontrolled and erratic fish transfers. Temperature is a key factor in occurrence, incidence, and spread of hexamitiasis epizootics. The effects of oxygen and nitrate on the reproduction of *H. salmonis* need further exploration. In locations where the protozoan occurs frequently, fish should be checked at 4-6ºC and treated if necessary to minimize the impact of the parasite on the fish.

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