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Investigation of high mortalities in eyed eggs and fry of rainbow trout (*Oncorhynchus mykiss* Walbaum) and brook trout (*Salmo trutta*)

Gökkuşağı alabalığı (*Oncorhynchus mykiss* Walbaum) ve kahverengi alabalık (*Salmo trutta*) yumurta ve keseli yavrularında görülen yüksek ölümlerin araştırılması

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Abstract

This work was conducted to investigate high mortalities of eyed-eggs and fry in trout hatcheries during the winter seasons of 1994-1996. Low water temperature (0-6 °C), clays covering eggs, directly sun-light and mechanically made faults were observed as the reasons of mortality in brook trout (*Salmo trutta*) eyed-eggs and fry In one of the hatcheries. Reddening and haemorrhages around eyes of eggs, became hard in yolk-sac of fry. *Vibrio parahaemolyticus* infections were present in rainbow trout eyed-eggs (*Oncorhynchus mykiss* Walbaum) in the other hatcheries. Infections caused reddening and haemorrhages around eyes of eggs. Bacterial isolates were identified in each hatchery, and tested to determine sensitivity against to 12 chemotherapeutants. Also, pathogenicity of two isolates were examined using healthy rainbow trout fry. In a therapeutic experiment baths with chloramine-T and norfloxacin controlled the natural *V. parahaemolyticus* infections.

Key words: fish, disease, trout, *Vibrio parahaemolyticus*, environmental factor

Introduction

Eggs and fry are more sensitive than adult fish to disease as in human and other animals. Therefore, management of hatcheries are important in cultured fish farms.
Bacterial (Barker *et.al.*, 1990; Muroga, 1992), fungal (Hatai *et al.*, 1990) and parasiter infections (Markiw, 1991), genetical (Popp, 1980), nutritional (Roberts and Bullock, 1989) and environmental factors (Popp, 1980; Sarıeyyüpoğlu, 1991) may cause losses in hatcheries. *Vibrio parahaemolyticus* can be found in aquatic environment. Yet, one infection case for fish has been reported (TzongFu *et al.*, 1994) however some information are currently available in the reports regarding its pathogenicity for fish (Muroga, 1990a, b; TzongFu *et al.*, 1994), other animals and human (Popp, 1980; Twiddy, 1995).

The aim of this study was to search for reasons of high mortalities and anomalies appeared in eyed-eggs and fry in three trout farms.

Materials and Methods

Eyed-eggs and yolk-sac fry
Hatcheries 2 and 3 in the basin of Çoruh River (in eastern Turkey) are neighbours. Hatchery 1 is on the other stream of Çoruh River. Rainbow trout fry (*Oncorhynchus mykiss*, Walbaum) have been produced intensively in the three farms. In addition, brook trout (*Salmo trutta*) eggs sent from France had been incubated in Farm 1.

During the investigation period (from December 1994 to February 1995), production water had 3±3 °C temperature, 6.8 pH and 9.5 ppm dissolved oxygen in Hatchery 1. The water analysed in Hatchery 2 had 9±2 °C temperature, 7 pH and 9.8 ppm dissolved oxygen in February 1996.

Microbiological investigations
The gross clinical signs dead eggs and fry were recorded during necropsy. Samples of eggs and fry were homogenised in physiological saline water. For bacterial isolation, inoculums were aseptically obtained from each homogenate and passaged on tryptone soya (TS) agar (Oxoid, UK), deoxycholate hydrogen sulfide lactose (DHL) agar, Sakazaki (Merck, Germany), GSP (*Aeromonas* and *Pseudomonas* selective) agar (Merck, Germany), Bloody agar (Merck), Cytophaga agar (Merck), MacConkey agar (Merck), thiosulfate citrate bile sucrose (TCBS) agar (Merck) and Baird-Parker agar (Merck). After incubation at 25 °C for

48 h, the individual colonies were enriched in 3% saline of tryptone soya (TS) broth (Oxoid) at 25 °C for 48 h, and restreaked on TCBS agar. Individual colonies were used in the identification tests (Plumb and Bowser, 1983; Austin and Austin, 1993). Upon completion of the identification procedures, antibiogram tests were conducted by using agar disc diffusion method on antibiotic medium agar (Merck) (Bauer *et al.*, 1966).

For the fungal isolation, homogenates were passaged on saboraud agar (Merck) and potato dextrose agar (Merck). Additionally, samples of eggs and fry were prepared on the lame, and investigated with light microscopy to diagnose parasiter infection (Plumb and Bowser, 1983).

Chemotherapy
In order to control infection in hatcheries 2 and 3, a single application of chloramine-T (5 mg/l of water, as a bath for 1 h) was used. After the treatment of disinfection, one of the antibiotics (norfloxacin) affecting bacteria in antibiogram test was applied 10 mg/l water/day for 5 days.

Infection experiment
Bacterial cultures isolated from diseased eggs were grown for up to 24 h in TS broth supplemented with 1.5% sodium chloride, and centrifuged, and resuspended in 11 volumes of 1% sodium chloride to approximately 10^7 viable cells/ml (Austin *et al.*, 1997).

Two different 500 l capacity fibre-glass tanks with adequate freshwater circulation were used for the infection and non-infected control of rainbow trout (*Oncorhynchus mykiss* Walbaum) fry. A total of 30 fry were used for the infection experiment. Fry were immersed with 10^9 live cells/l bacterial suspension. The infected fry were maintained up to 2 weeks in the tank. Remaining 10 fry were used as non-infected control fish in other tank. Dead fry were removed and pathogen recovered and identified by standard bacteriological examination (Plumb and Bowser, 1983; Austin and Austin, 1993).

Results and Discussion

Microbiological and parasiter investigations
Bacterial, fungal and parasiter agents could not be isolated from eggs and fry in Hatchery 1. Bacteria were isolated from eyed-eggs in Hatcheries 2 and 3, and according to the regular identification results for the bacteria tabulated in Table 1, all the isolates were identified as *Vibrio parahaemolyticus*. The results given in Table 1 were almost identical with those of isolates from fish (TzongFu *et*

al.,1994), human or other animals (Holt *et al.*, 1994; Twiddy, 1995). It could be existed in aquatic environment (Muroga, 1990a, b; TzongFu *et al.*, 1994), and its pathogenicity for aquarium fish had been known (TzongFu *et al.*, 1994).

Sensitivity of the bacterial isolates to antimicrobial compounds

Sensitivities of the two isolates (isolated from Farm 1 and 2) to antimicrobial compounds are shown in Table 2. NCCLS (1992) was used as reference in the evaluation of antibiogram tests. Gentamycin, cephoperazone and derivatives (enrofloxacin, norfloxacin and ofloxacin) of fluoroquinolone were effect to bacteria. Isolates were moderate sensitive to tetracycline.

Clinical signs of infection and mortalities in hatcheries

Hatchery 1: Reddening and haemorrhages around eyes of eggs and became hard in yolk-sac of fry (Fig. 1). Ataxia was present, a high rate of anomalies as lordosis and scoliosis were also observed in fry.

The hatching started on 21 th January 1995, and continued until 25 th February 1995. However eyed-eggs were spaced on 1 st December 1994. 25 000 fry had alive next yolk-sac period although 100 000 eyed-eggs were incubated (25% survival rate).

It is known which bacterial (Barker *et al.*, 1990; Muroga, 1992), fungal (Hatai *et al.*, 1990) and parasiter (Markiw, 1991) infections, genetical, nutritional and environmental factors (Popp, 1980; Roberts and Bullock, 1989; Sarıeyyüpoğlu, 1991; Atay, 1994; Çelikkale, 1994; Aras *et al.*, 1995) caused losses of eggs and fry in hatcheries. In this study, environmental factors were assumed to have effected the mortalities of eggs and fry in Hatchery 1 considering the fact that infection and nutritional deficiency was not observed. Thus, clays come with water has covered eggs, and cause death.

Eyed period of eggs was along term (about 50 days), and a homogenous hatching had not occurred (it occurred in 30-35 days), and yolk-sac period had also continued a long term. Low water temperature (0-6 °C) could cause high mortality, late hatching and long yolk-sac period. Several workers have reported that hatching could not occur at lower temperature of water than 6 °C (Atay, 1994; Çelikkale, 1994; Aras *et al.*, 1995).

Additionally, it was observed which eggs and fry had not prevented against direct light. Sun-light could cause ataxia in fry as researchers have reported (Popp, 1980; Atay, 1994; Aras *et al.*,1995). In the present

work, it was observed that farmer had attentively executed cleanliness and management of eggs. Parts of dead eggs had also caused death of other eggs by sticking on them.

Hatcheries 2 and 3: Reddening and haemorrhages were present around eye of dead eggs. The infections caused 30% and 48% mortalities in Hatchery 2 and Hatchery 3, respectively. The most of *Vibrio parahaemolyticus* infections caused enteritis in fish (Muroga, 1990a, b; Muroga, 1992; TzongFu *et al.*, 1994), human and other animals (Popp, 1980; Holt *et al.*, 1994; Twiddy, 1995).

Medical Treatments in Hatcheries 2 and 3

Mortalities of eyed-eggs had been remarkably remained where eggs hatched in normal healthy conditions after adoption of chloramine-T and norfloxacin baths.

Figure 1.

Eggs applied antibiotic can slowly grow at the further stage of fry, but antimicrobial compound application is needed to control diseases and may treat without its harm has been considered (Leary, 1990). In the present study, effects on the growth of fry of antimicrobial compound applications at the eyed stage of eggs were outside of our investigation.

Pathogenicity of the *Vibrio parahaemolyticus*: All of the fry infected with *Vibrio parahaemolyticus* died in 15 days following the immersion, and pathogen were isolated from the kidney of dead fry. Some information is currently available in the reports regarding its pathogenicity in freshwater (Muroga, 1990; TzongFu *et al.*, 1994) and marine fishes (Muroga, 1990a, b). Yet, workers described *V. parahaemolyticus* as a pathogenic bacterium for fish however they did not report infection case (Austin and Austin, 1993) except one report (TzongFu *et al.*, 1994). The results of this study showed that *V. parahaemolyticus* could be a significant pathogenic agent for salmonids although further studies were needed under different environmental conditions and fish species. Furthermore, it is a bacterium zoonose due to caused gastro-enteritis in human (Popp, 1980; Twiddy, 1995), and its harm for human must also considered.

Özet

Bu çalışma; alabalık yumurta ve keseli genç yavrularında1994-1996 yılları arasında kışın görülen yüksek orandaki ölümleri araştırmak için yapılmıştır. Kuluçkahanelerden birindeki dere alabalığı (*Salmo trutta*) gözlenmiş yumurta ve genç yavrularının; düşük su sıcaklığı (0-6 °C), kum-silt yapışması, direkt güneş ışığına maruz kalma ve mekanik hatalar yüzünden ölebildiği gözlenmiştir. Ölen gözlenmiş yumurtalarda kızarıklık ve kanama, yavrularda da yumurta kesesinin sertleşmesi ve kanama başlıca klinik belirtiler olarak görülmüştür. Diğer kuluçkahanelerdeki gözlenmiş gökkuşağı alabalığı yumurtalarında (*Oncorhynchus mykiss* Walbaum) ise, yumurtaların göz kısımlarında kızarıklık ve kanamaya sebep olan *Vibrio parahaemolyticus* enfeksiyonu teşhis edilmiştir. Bacteri izolatlarının, 12 antimikrobiyale karşı hassasiyetleri ve sağlıklı gökkuşağı alabalığı genç yavruları (yumurta kesesi çekilmiş) üzerinde patojeniteleri test edilmiştir. Kloramin-T dezenfektanı ve norfloksasin antibiyotiği banyoları, *V. parahaemolyticus* enfeksiyonunu tedavide başarılı şekilde kullanılabilmiştir.

Table 1. Biological and biochemical characteristics of bacteria isolated from naturally infected fry and eggs.

Characteristic	Response		Characteristic	Response	
	Isolate 1	Isolate 2		Isolate 1	Isolate 2
Gram stain	-	-	β-Alanine utilization	-	-
Motility (room temperature)	+	+	Acetate utilization	+	+
Oxidase	+	+	Sodium citrate utilization	+	+
Catalase	+	+	Caprylate utilization	+	+
Lipase	+	+	Citrulline utilization	-	-
Amylase	+	+	Ethanol utilization	+	+
Arginine dihydrolase	-	-	Heptanoate utilization	+	+
Lysine decarboxylase	+	+	Gas production from glucose	-	-
Ornithine decarboxylase	+	+	O/F	F	F
DNA'ase	+	+	Acid production from		
Phenylalanine deanimase	-	-	Cellobiose	-	-
Methyl-Red	+	+ weak	Trehalose	+	+
Voges-Proskauer	-	-	Glucose	+	+
Simmon's citrate	-	-	Mannitol	+	+
Urease	-	-	Lactose	-	-
H_2S production	-	-	Arabitol	-	-
Growth at KCN	-	-	Inositol	-	-
Growth at 8 % NaCl	+	+	Sorbitol	-	-
Indole	+	+	Rhamnose	-	-
Nitrate reduction	+	+	Sucrose	-	-
Esculin hydrolysis	-	-	Melibiose	-	-
Alginase	-	-	Erythritol	-	-
Gelatine degregation	+	+	Dulcitol	-	-
Chitinase	+	+	Maltose	+	+
β-Galactosidase	-	-	Raffinose	-	-
0/129 sensitivity	-	-	Salicin	-	-
Hemolysis	+	+	Arabinose	+	+
L-Arginine utilization	+	+	Glycerol	+	+
L-Leucine utilization	+	+	Adonitol	-	-
L-Tyrosine utilization	+	+	Galactose	+	+
			Xylose	-	-

Table 2. Results of susceptibility test *Vibrio parahaemolyticus* isolates to antibiotics

Antibiotic (disc=µg)	Result (zone diameter=mm)		Reference[1] (zone diameter=mm)		
	Isolate 1	Isolate 2	S[2]	MS[3]	R[4]
Enrofloxacin (5)	S (24)	S (25)	≤14	15-17	≥18
Norfloxacin (10)	S (28)	S (25)	≤12	13-16	≥17
Ofloxacin (5)	S (23)	S (22)	≤12	13-15	≥16
Tetracycline (30)	MS (16)	MS (18)	≤14	15-18	≥19
Gentamycine (10)	S (21)	S (20)	≤12	13-14	≥15
Imipenem (10)	R (0)	R (10)	≤13	14-15	≥16
Sulp./Trim[5] (23.75/1.25)	R (8)	S (19)	≤10	11-15	≥16
Cephoperazone (75)	MS (20)	MS (17)	≤15	16-20	≥21
Cefotaxime (30)	R (0)	R (0)	≤14	15-22	≥23
Erythromycin (15)	R (0)	R (0)	≤13	14-22	≥23
Amp./Sulb.[6] (10/10)	R (11)	R (0)	≤11	12-14	≥15
Penicillin G (10 u)	R (0)	R (0)	≤11	12-21	≥22
Rifampycin (5)	R (12)	R (9)	≤16	17-19	≥20
Kanamycin (30)	R (10)	R (11)	≤13	14-17	≥18

[1] =NCCLS (1992), [2] = Sensitive, [3] = Moderate sensitive, [4] = Resistant
[5] = Sulphamethoxazole/Trimethoprim, [6] = Ampicillin/Sulbactam

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