

Bacterial and Parasitic Co-Infection in *Carassius gibelio* Bloch, 1782 Caught in the Onaç Reservoir, Türkiye

P. Yıldırım¹, Ş. Kan², S.S. Güçlü³, Ö. Özmen⁴, A. Özdamar⁵, E. Bahrioğlu^{6*},
A. Kubilay⁷

¹Isparta University of Applied Sciences, Eğirdir Fisheries Faculty, 32260, Isparta-Türkiye

²Isparta University of Applied Sciences, Eğirdir Fisheries Faculty, 32260, Isparta-Türkiye/

³Isparta University of Applied Sciences, Eğirdir Fisheries Faculty, 32260, Isparta-Türkiye

⁴Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Burdur, Türkiye

⁵Isparta University of Applied Sciences, Eğirdir Fisheries Faculty, 32260, Isparta-Türkiye

⁶Isparta University of Applied Sciences, Eğirdir Fisheries Faculty, 32260, Isparta-Türkiye

^{7*}Isparta University of Applied Sciences, Eğirdir Fisheries Faculty, 32260, Isparta-Türkiye

ABSTRACT: This study investigates the bacterial and parasitic co-infection of Prussian carp (*Carassius gibelio* Bloch, 1782). *C. gibelio* belongs to the Cyprinidae family, an alien species in the freshwater systems of Türkiye. Alien species can cause the emergence of new diseases in native species that constitute the ichthyofauna. *C. gibelio* specimens were examined from Onaç Reservoir in Burdur, Türkiye. Symptoms were skin lesions, exophthalmos, darkening in color, rupture, and hemorrhage in the radius of the caudal fin, and loss of scale of the fish. *Gyrodactylus sprostonae* and *Trichodina* sp. were also observed. Additionally, *Shewanella putrefaciens* and *Aeromonas sobria* were isolated from the fish specimens. Erosive and ulcerative lesions were frequently seen during the histopathological investigation. Both isolates were resistant to penicillin, oxacillin, lincomycin, and ampicillin. In conclusion, the presence of various bacterial strains, a ciliate parasite, and a monogenic parasite was determined in *C. gibelio*. We report that the aforementioned fish pathogens created a co-infection in the region. In the future, more study is needed to a clearer awareness of the *C. gibelio* dealing with co-infection and the details of the interaction between bacterial and parasitic diseases. This will be beneficial for fish disease management.

Keywords: *Carassius gibelio*; *Gyrodactylus sprostonae*; *Trichodina* sp.; *Shewanella putrefaciens*; *Aeromonas sobria*

Corresponding Author:
Aysegül Kubilay, Isparta University of Applied Sciences, Eğirdir Fisheries Faculty,
32260, Isparta-Türkiye
E-mail address: aysegulkubilay@isparta.edu.tr

Date of initial submission: 10-04-2023
Date of acceptance: 03-10-2023

INTRODUCTION

Türkiye has 25 alien fish species (İnnal and Erk'akan, 2006). One of these exotic species is *C. gibelio*, reported from 46 different freshwater systems in Türkiye. The first record of *C. gibelio* was reported from Lake Gala in the Thrace Region, Turkey. *C. gibelio* is a dangerous fish species for native fish communities (Baran and Ongan, 1988; Arslan and Emiroğlu, 2011). As any alien species can transport alien organisms such as parasites, they cause new diseases in the seriously threatened habitats of native species (Uğurlu and Polat, 2006).

Generally, seasonal diseases recur in the natural environment due to regular seasonal environmental changes. Seasonal diseases in natural habitats were often parasitic or bacterial infections. Bacterial infections generally develop secondary to parasitic infections (Plumb, 1999). One of the most important problems encountered in aquatic products is parasitic diseases. Parasites have a lethal effect on fish, whereas their growth, behavior, and resistance to stress factors were affected. At the same time, it causes the fish to be deprived of vitamins and minerals by exploiting the nutrient from the fish (Timur and Timur, 2003; Austin and Austin, 2016). In addition, parasites form wounds, incisions, and similar damage in muscles and other internal organs by adhering to the adhesion organs in their hosts (Demir, 2014).

Parasites of the genus *Gyrodactylus* can be seen in freshwater and marine fish. Approximately 409 *Gyrodactylus* species have been identified so far, and it is predicted that 20,000 species can be identified (Özer et al., 2011). There are no eyes or eye spots. The parasite is generally located in the skin, fins, and nostrils. Fish-to-fish transmission occurs through direct contact (Stoskopf, 2015).

There is a great number of Trichodinid ectoparasites species reported in nature, and they are a group of widely distributed ectoparasitic ciliates causing serious diseases (Wang et al. 2018). Trichodinid ciliates are one of the most common ectoparasites in wild fish (Basson and As, 2006; Martins and Ghiraldelli, 2008; Martins et al., 2012). With the rapid increase in the number of trichodinids in aquatic environments with high stocking density, the circular movements of the parasite can cause abrasive lesions on the host's body surface (Wang et al., 2022). Secondary bacterial infections that develop from lesions on the body surface can increase fish losses (Iqbal and Haroon, 2014; Valladao et al., 2014).

The damage caused by parasites creates potential port antre for bacterial pathogens (Cusack and Cone, 1985; Cusack and Cone, 1986). There withal, the development of bacteria causes a reduction in the immune system of the fish, which makes the host body suitable for parasitic infestation. Co-infection has often been seen in natural habitats (Gorgoglione et al., 2020). Co-infections can frequently occur in fish. The potential impact of co-infections is much more significant than developing a single disease.

Shewanella putrefaciens (formerly *Pseudomonas putrefaciens*, *Alteromonas putrefaciens*) is a Gram-negative facultatively anaerobic bacteria in Vibrionaceae (MacDonell and Colwel, 1985; Kozińska and Pêkala, 2004; Varalakshmi et al., 2022). In recent years, the new agent of the disease called Shewanellosis in freshwater fish has been identified as *S. putrefaciens*. This infection has been seen in aquaculture conditions and in many marine and freshwater fish found in nature (Kozińska and Pêkala, 2004; Qin et al., 2012a; Rusev et al., 2016; Pêkala et al., 2015; Mohammed and Peatman, 2018)

Aeromonas species are motile or non-motile and generally give positive results in glucose tests, catalase, and oxidase (Martinez-Murcia et al., 2005). The motile Aeromonas-caused infections (*Aeromonas hydrophila*, *A. sobria*, and *A. caviae*) are mostly known as the reason for hemorrhagic septicemia, mostly associated with fish diseases (Austin and Austin, 2016). Diseases are progressing with high mortality and significant economic losses in freshwater fish, crustaceans, and sometimes marine fish.

In this study, disease-related symptoms were seen in the natural environment on *C. gibelio*. Therefore, we have conducted an investigation on Prussian carp, *C. gibelio* for bacterial and parasitic agents in the Onaç Reservoir, Burdur, Türkiye.

MATERIAL AND METHODS

Study area and fish sampling

Onaç Reservoir was built on the Onaç Stream (37°30'22.17" N; 30°34'31.71" E) near Bucak (Burdur) in 2006 for irrigation purposes (Figure 1). The water level rises considerably, especially in spring, due to the runoff waters. In addition to the *C. gibelio* species, alien species *Pseudorasbora parva*, *Gambusia holbrooki*, and the endemic species *Pseudophoxinus ninae* are distributed at the subject lake.



Figure 1. The study area (Onaç Reservoir) and the sampling point (asterisk).

C. gibelio individuals (n=20) were examined in April 2022 from Onaç Reservoir in Burdur, Türkiye. Fish samples were taken with an electroshocking device. Diseased fish were transported to the laboratory alive (n=20) with ice and oxygen assistance for isolation, diagnosis, and pathogen identification. Firstly, the length-weight measurements and sexuality of the moribund fish were determined. Moribund Prussian carp was 10.96 cm in mean length and 25.48 gr in live weight. The water temperature at the sampling date was 16.9°C, and dissolved oxygen was measured as 4.44 mg/l.

Bacterial Isolation and Identification

The examination was performed in 20 individuals. Samplings were gathered from the head kidney, spleen, and liver and streaked onto tryptic soy agar (TSA) and TSA with 2% NaCl added (TTSA).

Incubation of the plates was done at 24°C for 48 hours. Colonies were identified based on morphological characteristics, physiological and biochemical. All procedures were performed in accordance with guidelines for diagnosing fish diseases and international and national animal welfare guidelines (OIE, 2003; Buller, 2004; Austin and Austin, 2016).

Biochemical, physiological, and morphological characteristics of the strains were determined with the following tests: cytochrome oxidase test kit (Merck), catalase-peroxidase (EC 1.11.1.21) test, motility observed under light microscopy (Olympus CX21),

oxidative and fermentative degradation of glucose with O/F basal medium (Merck) supplemented with 1% glucose, resistance to vibriostatic agent O/129 (Buller, 2004; Austin and Austin, 2016).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed to determine the antimicrobial resistance profile of the isolated bacterial strains. For this purpose, the specification of the antimicrobial susceptibility of colonies was done according to the Kirby-Bauer disc diffusion method (Oxoid) (Biemer, 1973). Results were assessed by the instructions of the Clinical and Laboratory Standards Institute (CLSI, 2014).

Molecular Identification

Bacterial colonies were taken individually from the first isolates, and then serial streaking to an agar plate was done for purification. The differentiated two colonies from biochemical, morphologic, and physiological tests were identified by sequencing the 16S rRNA (16s ribosomal Ribonucleic Acid) gene. According to the manufacturer's protocol, the genomic DNA from selected isolates was extracted using the GeneMatrix Bacterial and Yeast Genomic Purification Kit (EURx, Gdansk, Poland). The 16S rRNA gene was amplified by the following primers:

27F (5'AGAGTTTGATCMTGGCTCAG-3')

1492R (5' TACGGYTACCTTGTTACGACTT-3').

PCR reactions were prepared with the following ingredients: 1X PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTP mix, 0.3 µM forward primer, 0.3 µM reverse primer, 2U of Taq DNA polymerase from 5U/µl stock, and 3 µl of DNA template. The reaction mixture was prepared to be 35 µl for each reaction. Thermal cycles were set to 95°C 5 min. - initial denaturation, 30 cycles of “95°C for 45 s - denaturation, 57°C for 45 s - annealing, 72°C for 60 s - extension”, and 72°C for 5 min for a final extension. PCR products were controlled with the Gel electrophoresis (1x TAE buffer, %1.5 Agarose, 100 volts, 90 min., EtBr stained). PCR products were purified with MAGBIO “HighPrep™ PCR clean-up System” following the manufacturer’s protocols. ABI 3730XL Sanger sequencing instrument (Applied Biosystems, Foster City, CA) and Big-Dye Terminator v3.1 Cycle Sequencing Kit were used for Sanger Sequencing samples (Applied Biosystems, Foster City, CA). Reads obtained with primers 27F and 1492R were contiguous to form a consensus se-

quence. CAP contig assembly algorithm was used in BioEdit software to perform this process. Obtained final sequences were submitted to NCBI nucleotide search for identification.

Parasitological Examination

Each fish’s external and internal organs were examined for parasites at the Faculty of Eğirdir Fisheries in Isparta, Türkiye. Parasite species were identified using an Olympus light microscope (Olympus CX21). Diagnosis of the present parasites was made morphologically using various references (Basson and As, 2006; Buchmann and Bresciani, 2006; Schäperclaus, 1984; Stoskopf, 1993).

Histopathological Analysis

Lesioned skin and visceral organ samples were taken during the necropsy and fixed in a 10% buffered formalin solution for histopathological examination. An automatic tissue processor (Leica ASP300S, Wet-



Figure 2. External lesions in *Carassius gibelio* individuals from Onaç Reservoir (a- 6.6 cm SL, b- 8 cm SL, c-12.7 cm SL, d-19.4 cm SL, e- 10.8 cm SL, f- 113.3 cm SL, g- 1016.6 cm SL)

zlar, Germany) was used to process the tissue samples, then embedded in paraffin wax. A fully automatic rotary microtome (Leica RM2155, Wetzlar, Germany) was used to cut paraffin blocks with a thickness of 5 µm. Then sections were stained with Hematoxylin-eosin (H&E) staining and analyzed under a light microscope (Luna, 1968). The Database Manual Cell Sens Life Science Imaging Software System created microphotographs and evaluated morphometric data (Olympus Corporation, Tokyo, Japan).

RESULTS

Gross findings

Common symptoms seen in infected fish (Figure 2) at the gross examination were ulcerative skin lesions (n=20), exophthalmos (n=15), darkening in color (n=15), rupture and hemorrhage in the radius of the caudal fin (n=20), loss of scale (n=18), hemorrhage (n=18), and hyperemia in the tail and mouth (n=18), excessive amount of mucus (n=18), hyperemia of the gills (n=20), and swelling of the gill filaments (n=18), local hemorrhages (n=18), and prolapse of the anus (n=18).

Parasitic Findings

In the parasite examinations, *Gyrodactylus sprostonae* from the monogenic group and *Trichodina* sp. from protozoan ciliate parasites were detected (Figure 3). Morphological features were used to define the *G. sprostonae* species. *Gyrodactylus* are small, spindle-shaped, ectoparasitic trematodes. The body length of the parasites we caught varied between 0.3-0.6 mm (The body of a normal *Gyrodactylus* varies between 0.2-1.0 mm). The front end of the body had 2 contractile suckers and it didn't have the eye. The attachment organ had 18 peripheral hooks and two connecting bars. It had anchor-shaped hooks.

The presence of both parasites was observed intensively in all fishes. During the study period, 20 Prussian carp were examined, and parasites were found in all (100%).

Bacteriologic Findings

In bacteriologic examinations, inoculations made from the head kidney, spleen, and liver have resulted in morphologically different two colonies only on the TSA plate for all samples. Analysis of pure colonies revealed that stain 1 (n=18) was Gram-negative, motile, catalase and cytochrome oxidase positive, and fermentative. Stain 2 (n= 20) was Gram-negative,

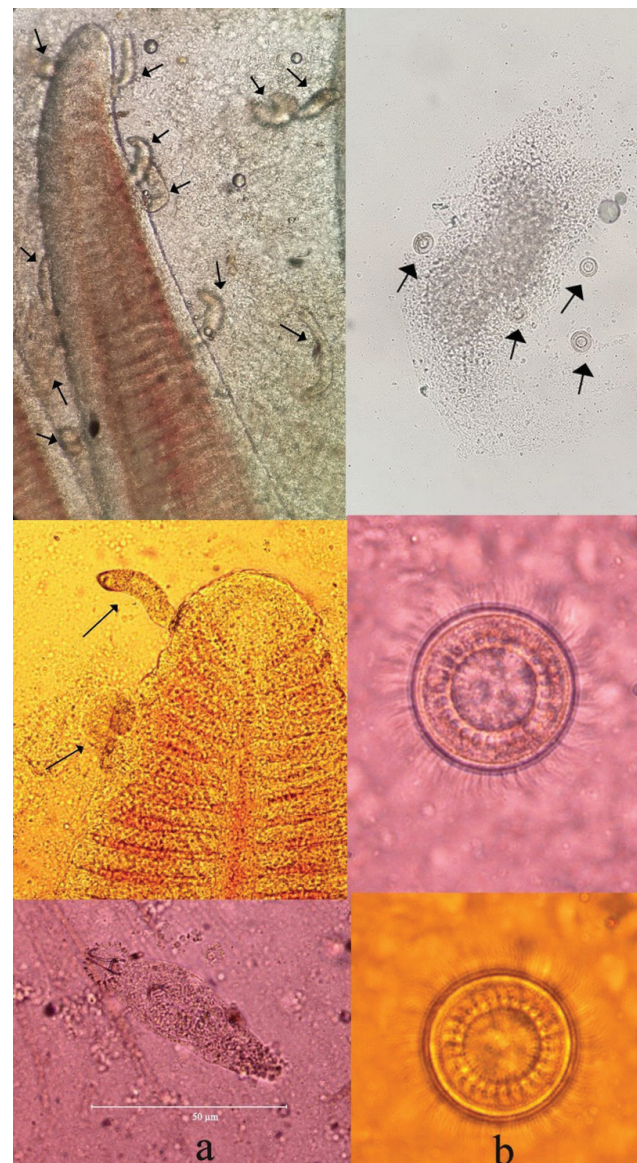


Figure 3 *G. sprostonae* (a) and *Trichodina* sp. (b) on gill filaments in *C. gibelio* individuals from Onaç Reservoir.

catalase and cytochrome oxidase-positive, non-fermentative, and O/129 sensitive.

The partial 16S rRNA sequence (ca. 1.4 kb) of the *S. putrefaciens* isolate was submitted to the GenBank database with accession no: OR468003. Similarities between its 16S rRNA sequence and those of *S. putrefaciens* strains in the GenBank database were to 100%.

The partial 16S rRNA sequence (ca. 1.4 kb) of the *Aeromonas sobria* isolate was submitted to the GenBank database with accession no: OR468004. Similarities between its 16S rRNA sequence and those of *A. sobria* strains in the GenBank database was to 100%.

The antibiogram test was performed in 3 parallels. The diameters of the inhibition zones averages are recorded in Table 1. According to the antibiogram test results, both isolates were susceptible to Oxolinic Acid, Florfenicol, Enrofloxacin, Norfloxacin, Tetracycline, Doxycycline, Kanamycin, Spectinomycin, Chloramphenicol, Flumequine, Nitrofurantoin, and Erythromycin. However, both isolates were resistant to Penicillin, Oxacillin, Lincomycin, and Ampicillin.

Histopathological Findings

In the affected skin areas, erosive and ulcerative lesions were frequently seen during the histopathological investigation. These areas had completely necrotic skin layers, and some muscle tissues were significantly affected. In locations with lesions, inflammatory cells infiltration was moderate to noticeable. Additionally, hemorrhages and edema were also noted. Although melanomacrophages were also seen, heterophile leukocytes and lymphocytes make up the majority of the inflammatory cells (Figure 4).

In addition to skin lesions, numerous parasites were seen in the gills and visceral organs. Pathologi-

cal findings, including inflammatory cell infiltrations or gill fusions, were brought on by parasites. Subepithelial necrotic cells, subepithelial leukocyte-based inflammation, and adaptive hyperplastic alterations involving mucus and epithelial cells were common changes in gills. Around the parasites, the other locations had a slight inflammatory reaction (Figure 5)

DISCUSSION

It is known that the resistance of fish against diseases decreases at the end of the winter period and at the beginning of spring in fish in natural aquatic environments. When it was reduced resistance to diseases, bacterial and parasitic diseases commonly relapse, and co-infection occur (Schade et al., 2016; Akaylı et al., 2019).

Parasites of the genus *Gyrodactylus* have the capability to rapidly widen their population in a relatively short period due to high breeding rates and direct transmission between hosts (Rawson and Rogers, 1973). Thus, the parasites reproduce rapidly in a short time, take their place in the hosts' skin, gills, and fins, and cause significant damage to the fish. Wounds

Table 1 The diameters of the inhibition zones (mm) of *S. putrefaciens* and *A. sobria* isolates

Antibiotic Disc	Isolate, <i>S. putrefaciens</i>	Isolate, <i>A. sobria</i>
Gentamicin	16 (S)	15 (I)
Oxolinic acid	26 (S)	31 (S)
Penicillin	6 (R)	0 (R)
Oxacillin	6 (R)	0 (R)
Florfenicol	35 (S)	34 (S)
Apramycin	16 (S)	10 (R)
Enrofloxacin	29 (S)	40 (S)
Streptomycin	9 (R)	15 (I)
Norfloxacin	23 (S)	38 (S)
Tylosin	19 (S)	0 (R)
Tetracycline	21 (S)	30 (S)
Doxycycline	22 (S)	27 (S)
Kanamycin	19 (S)	17 (S)
Spectinomycin	18 (S)	20 (S)
Pristinamycin	24 (S)	10 (R)
Lincomycin	0 (R)	0 (R)
Chloramphenicol	31 (S)	34 (S)
Ampicillin	0 (R)	0 (R)
Clindamycin	19 (S)	0 (R)
Flumequine	29 (S)	41 (S)
Nitrofurantoin	20 (S)	23 (S)
Vancomycin	21 (S)	11 (I)
Erythromycin	30 (S)	20 (S)

“S” = Sensitive ($d > 15$ mm); “I” = Intermediate ($10 \text{ mm} < d \leq 15$ mm); “R” = Resistant ($d \leq 10$ mm)

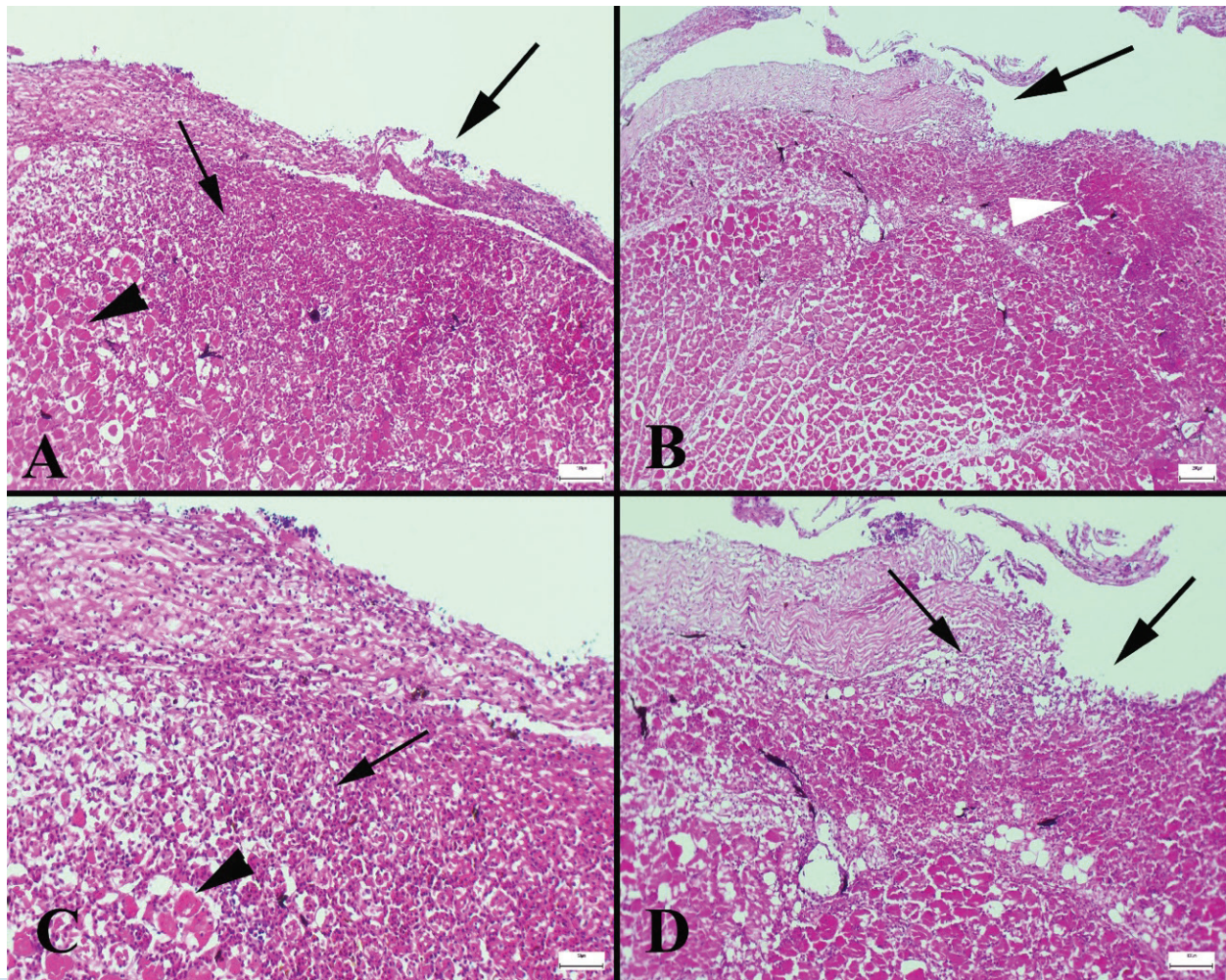


Figure 4 Histopathological appearance of the lesioned skin areas. (a) Totally ulcerated skin (thick arrow) and the inflamed muscle area, inflammatory cell infiltrations (thin arrow), and degenerated muscle cells (arrowhead), H&E, Scale bar=100µm. (b) Totally ulcerated skin and hypodermis (thick arrow) and a big necrotic area in the muscle (white arrowhead), H&E, Scale bar=200µm. (c) Higher magnification of the inflammatory reaction (thin arrow) and degenerated muscle cells (arrowhead), H&E, Scale bar=50µm. (d) Higher magnification of the lesioned area in another fish inflammatory reaction (thin arrow) and skin ulcer (thick arrow), H&E, Scale bar=100µm

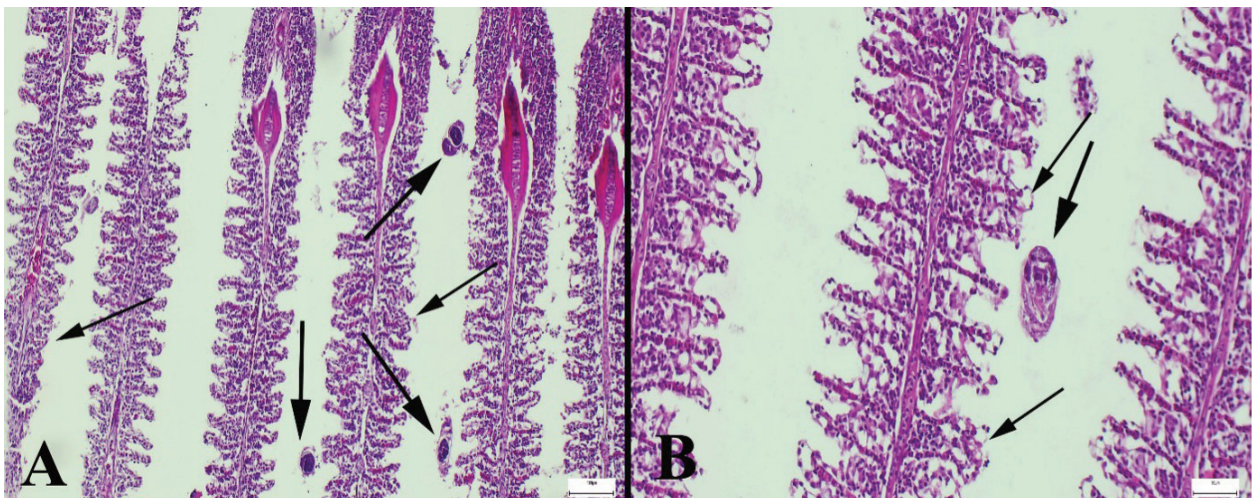


Figure 5 The microscopical appearance of the parasites. (a) Numerous parasites (thick arrows) in the gills of a fish and fusions in gill lamellas (thin arrows), H&E, Scale bar=100µm. (b) Another gill parasite (thick arrow) and lamellar fusions (thin arrow) in a fish, H&E, Scale bar=50µm.

inflicted by parasites create a port antre for bacteria (Cusack and Cone, 1985; Cusack and Cone, 1986).

Kayış et al. (2018) detected parasitic and bacterial pathogens in naturally distributed fish species in Deriner Dam Lake. Similar to our study, it was observed that both bacterial agents, along with the *Trichodina* and *Gyrodactylus* parasites, caused co-infection in different fish species. Despite co-infections, the presence of different types of parasites was determined. Akaylı et al. (2019), *Gyrodactylus* and *Trichodina* parasites were reported in Seven khramulya (*Capoeta capoeta*). Bacterial infections *S. capitis*, *Vibrio fluvialis*, *Staphylococcus warneri*, and *A. hydrophila* were also observed, which caused co-infection in the host.

Studies have shown that parasites of the genus *Gyrodactylus* are generally located on the fish skin and fins (Schäperclaus, 1984; Stoskopf, 1993). However, in the examinations we have made in Prussian carp, it has been seen that *G. sprostanæ* was dominantly located in the gill tissue.

Trichodina has been studied for almost 200 years. Species identification of this genus and methods based on morphological features for identification remain challenging due to their high-level interspecific similarity, variation, and low host specificity (Tang et al., 2017; Wang et al., 2019; Wang et al., 2022). Therefore, the parasite was identified as *Trichodina* sp in the present study.

In bacteriologic examinations, two different pure colonies were found, and molecular studies showed that there were *S. putrefaciens* and *A. sobria* species. The bacterial fish pathogen *S. putrefaciens* poses a serious problem because it is facultative and can cause disease under unfavorable environmental conditions (Paździor, 2016). This pathogen was isolated from *Carassius* spp by Qin et al. (2012b). However, for *C. gibelio*, it is the first isolation in Türkiye. There are plenty of reports declare that *S. putrefaciens* was isolated together with other species, e.g., *Pseudomonas* sp. and *Aeromonas* sp. (Pêkala et al., 2015), *Listonella* sp. (Qin et al., 2012a; b), and *S. warneri* (Rusev et al., 2016). Our study is similar to other researchers'; the pathogen was isolated with *A. sobria*. The disease usually occurs in spring when the water temperature is 7-10 °C. (Pêkala et al., 2015). Furthermore, other studies have shown that *S. putrefaciens* grows in pond water with temperatures between 5 and 15 °C. In this study, diseased fish were found in spring at 16.9 °C. This shows a correlation between fish infection and

the spring season. Symptoms of Shewanellosis infection included ulcers and lethargy on the dorsal part of the body, hemorrhage in the kidney and spleen, and petechiae in the swim bladder. The observed symptoms were similar to other research reports (Jiang et al., 2022). *S. putrefaciens* was first observed in *Siganus rivulatus* reared in the Red Sea in 1985 (Saeed et al., 1987). *S. putrefaciens* was also isolated in 2004 from *Cyprinus carpio* and *Oncorhynchus mykiss* farms in Poland (Koziańska and Pêkala, 2004). First isolation from marine finfish was reported in *Dicentrarchus labrax* in the Aegean region of Türkiye (Korun et al., 2009), from the Black Sea region of Türkiye by Kayış et al. (2009), and *Carassius auratus auratus* by Altun et al. (2014). *S. putrefaciens* infection is also seen in humans (Bulut et al., 2004).

Antibiotic resistance of *S. putrefaciens* varies by source. Nowadays, studies have been conducted on the susceptibility of not only humans but also freshwater fish and marine fish to various chemotherapeutic agents. *Shewanella* spp are generally resistant to penicillins, also found in the present study. Most isolates showed sensitivity to tetracycline as in this study (Heritier et al., 2004) (Table 1).

Bacterial infections caused by motile members of the genus *Aeromonas* are known as motile *Aeromonas* septicemia (Haemorrhagic septicemia). It is among the common diseases in fish farms and natural populations. The *Aeromonas* bacteria that cause these infections are called aeromonads. Motile *Aeromonas* were responsible for significant financial losses each year, whether they moved alone or co-infections with other organisms. All members of the *Aeromonas* genus are small, motile, Gram-negative, and rod-shaped bacteria (Camus et al., 1998; Austin et al., 2007). *A. sobria* was isolated from wild egg-laying *Dorosoma cepedianum* in Maryland (Toranzo et al., 1989) and cultured *Perca fluviatilis* in Switzerland (Wahli et al., 2005; Austin et al., 2007). In Türkiye, the Elazığ region by Muz et al. (1995). Hemorrhagic septicemia is characterized by small surface lesions (lead to scale shedding), local hemorrhages in the gills and vent, ulcers, abscesses, exophthalmia, and abdominal distension (Austin et al., 2007). Our study, found that sepsis infection was concentrated in the abdominal cavity. Moribund fish showed loss of scales and occasional skin bleeding on the abdomen and fin bases. Additionally, there was some evidence of abdominal distension. It has been reported in many studies that *S. putrefaciens* bacteria cause co-infection with *Aer-*

omonas species in fish (Yi et al., 2012; Kotob et al., 2017).

CONCLUSIONS

This may contribute to the development of infection prevention methods. In recent years, *S. putrefaciens* infection has been common in freshwater fish. Therefore, there is a need to characterize this bacterium for the future protection of fish health, the environment, and humans. Co-infections are very common in natural aquatic environments. Co-infection can have serious consequences, altering the course and severity of diseases in different fish. This study determined the presence of various bacterial strains in Prussian carp, a ciliate parasite, and a monogenic parasite. Co-infection of fish has been shown to cause severe disease

and tissue damage. Accordingly, the synergistic effects of pathogens cause more damage to fish. In this study, it was observed that *S. putrefaciens* co-infected with *A. sobria* for the first time. Investigation of the pathogenesis and epidemiology of such co-infections in fish is essential in taking necessary precautions.

ACKNOWLEDGMENTS

One of the authors, Pınar Yıldırım, acknowledges the Council of Higher Education for its support with the 100/2000 PhD scholarship program.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

- Akaylı T, Ürku Ç, Bozkurt, ER (2019) Determination of Co-infection in Diseased Seven khamulya (*Capoeta capoeta*). KSU J Agric Nat 226:965-971.
- Altun S, Büyükekiz AG, Duman M, Özyiğit MÖ, Karataş S, Turgay E (2014) Isolation of *Shewanella putrefaciens* from goldfish (*Carassius auratus auratus*). Israeli Journal of Aquaculture-Bamidgeh, 66.
- Arslan N, Emiroğlu Ö (2011) First Record of Parasitic Annelida-Hirudinea (*Piscicola geometra* Linnaeus, 1761) on *Carassius gibelio* (Bloch, 1782) in Lake Ulubat (Turkey). Kafkas Univ Vet Fak Derg 17:131-133.
- Austin B, Austin, DA, Munn, CB (2007) Bacterial fish pathogens: disease of farmed and wild fish. Springer, Chichester.
- Austin B, Austin A (2016) Bacterial fish pathogens: Disease in farmed and wild fish, 6th edn. Springer-Praxis, Godalming.
- Baran I, Ongan T (1988) Gala Gölü'nün limnolojik özellikleri, balıkçılık sorunları ve öneriler. Gala Gölü ve Sorunları Sempozyumu, Doğal Hayatı Koruma Derneği Bilimsel Yayınlar Serisi 46-54.
- Basson L, As JV (2006) Trichodinidae and Other Ciliophorans (Phylum Ciliophora). In: Woo PTK (ed) Fish Diseases and Disorders Volume 1: Protozoan and Metazoan Infections, 2nd edn. CABI, London, pp 154-182.
- Biemer JJ (1973) Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. Ann Clin Lab Sci 3:135-140.
- Buchmann K, Bresciani J (2006) Monogenea (Phylum Platyhelminthes). In: Woo PTK (ed) Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections, 2nd edn. CABI, London, 297-344.
- Buller N (2004) Bacteria from fish and other aquatic animals: A practical identification manual. CABI, London.
- Bulut C, Ertem GT, Gökçek C et al (2004) A rare cause of wound infection: *Shewanella putrefaciens*. Scand J Infect Dis. <https://doi.org/10.1080/00365540410022620>
- Camus AC, Durborow RM, Hemstreet WG et al (1998) Aeromonas bacterial infections-motile aeromonad septicemia (Vol. 478). Southern Regional Aquaculture Center, Mississippi.
- Cusack R, Cone DK (1985) A report of bacterial microcolonies on the surface of *Gyrodactylus* (Monogenea). J Fish Dis 8:125-127.
- Cusack R, Cone DK (1986) A review of parasites as vectors of viral and bacterial diseases of fish. J Fish Dis 9:169-171.
- Demir S (2014) Marmara Gölü'nde (Manisa) Yaşayan Sazan (*Cyprinus carpio* L., 1758) ve Havuz Balığı (*Carassius gibelio* Bloch, 1782)'nin Parazit Faunasının Araştırılması. Dissertation, Ege University.
- Gorgoglione B, Bailey C, Fast MD et al (2020) Co-infections and multiple stressors in fish: EAFF 19th International Conference on Diseases of Fish and Shellfish special edition workshop report. Bull Eur Assoc Fish Pathol 40:4-19.
- Heritier C, Poirel L, Nordmann P (2004) Genetic and biochemical characterization of a chromosome-encoded carbapenem-hydrolyzing amblar class D beta-lactamase from *Shewanella algae*. Antimicrob Agents Chemother 48:1670-1675.
- Iqbal Z, Haroon F (2014) Parasitic infections of some freshwater ornamental fishes imported in Pakistan. Pakistan J Zool 46(3):651-656.
- İnnal D, Erk'akan F (2006) Effects of exotic and translocated fish species in the inland waters of Turkey. Rev Fish Biol Fisheries 16:39-50.
- Jiang X, Wang X, Li L et al (2022) Identification of *Shewanella putrefaciens* as a novel pathogen of the largemouth bass (*Micropterus salmoides*) and histopathological analysis of diseased fish. Front Cell Infect Microbiol 1569.
- Kayış S, Capkin E, Altınok I (2009) Bacteria in Rainbow Trout (*Oncorhynchus mykiss*) in the Southern Black Sea Region of Turkey - A Survey. Isr J Aquacult-Bamid 61(4):339-344.
- Kayış Ş, Düzgün A, Er A (2018) Bacterial and parasitic pathogens isolated from some wild cyprinid fishes. El-Cezeri 5(3):763-772.
- Korun J, Akgün-Dar K, Yazıcı M (2009) Isolation of *Shewanella putrefaciens* from cultured European sea bass, (*Dicentrarchus labrax*) in Turkey. Rev Med Vet, 160, 532-536.
- Kotob MH, Menanteau-Ledouble S, Kumar G, et al (2017) The impact of co-infections on fish: a review. Vet Res 47:1-12.
- Kozińska A, Pékala A (2004) First isolation of *Shewanella putrefaciens* from freshwater fish-a potential new pathogen of fish. Bull Eur Assoc Fish Pathol 24(4):189-193.
- MacDonell MT, Colwell RR (1985) Phylogeny of the Vibrionaceae, and recommendation for two new genera, *Listonella* and *Shewanella*. Syst Appl Microbiol 6:171-182.
- Martínez-Murcia AJ, Soler L, Saavedra MJ, Chacón MR, Guarro J, Stackebrandt E, Figueras MJ (2005) Phenotypic, genotypic, and phylogenetic discrepancies to differentiate *Aeromonas salmonicida* from *Aeromonas bestiarum*. Int Microbiol 8(4):259-269.
- Martins ML, Ghirdelli L (2008) *Trichodina magna* van As and Basson, 1989 (Ciliophora: Peritrichia) from cultured Nile tilapia in the state of Santa Catarina, Brazil. Braz J Biol 68:169-172.
- Martins ML, Marchiori, N, Roumbekakis K et al (2012) *Trichodina nobilis* Chen, 1963 and *Trichodina reticulata* Hirschmann et Partsch, 1955 from ornamental freshwater fishes in Brazil. Braz J Biol 72:281-286.
- Mohammed HH, Peatman E (2018) Winter kill in intensively stocked channel catfish (*Ictalurus punctatus*): Coinfection with *Aeromonas veronii*, *Streptococcus parauberis* and *Shewanella putrefaciens*. J

- Fish Dis 41:1339-1347.
- Muz A, Sarıyüpoğlu, M, Ertaş HB, Simsek A (1995) Keban Baraj Gölü'nden yakalanan bazı balıkların serobik ve mikroaerofilik bakteriler yönünden incelenmesi. FU Sağlık Bil Derg 9:212-219.
- OIE (2003) Manual of diagnostic tests for aquatic animals, 4th edn. Office International des Epizooties, Paris.
- Özer A, Öztürk T, Çam A, et al (2011) Kapımızdaki Potansiyel Risk: *Gyrodactylus*. Türk Bil Derle Derg 1:11-18.
- Paździor, E. (2016) *Shewanella putrefaciens* a new opportunistic pathogen of freshwater fish. J Vet Res 60(4):429-434.
- Pékala A, Kozinińska A, Paździor E et al (2015) Phenotypical and genotypical characterization of *Shewanella putrefaciens* strains isolated from diseased freshwater fish. J Fish Dis 38:283-293.
- Plumb JA (1999) Overview of Warmwater Fish Diseases, Journal of Applied Aquaculture, 9:2, 1-10. : https://doi.org/10.1300/J028v09n02_01
- Rawson MV, Rogers WA (1973) Seasonal abundance of *Gyrodactylus macrochiri* Hoffman and Putz, 1964 on bluegill and largemouth bass. Journal of Wildlife Diseases, 9(2):174-177. <https://doi.org/10.7589/0090-3558-9.2.174>
- Rusev V, Rusenova N, Simeonov R, et al (2016) *Staphylococcus warneri* and *Shewanella putrefaciens* Co-infection in Siberian Sturgeon (*Acipenser baerii*) and Hybrid Sturgeon (*Huso huso* x *Acipenser baerii*). J Microbiol Exp 3:00078.
- Qin L, Zhu M, Xu L (2012a) First report of *Shewanella* sp. and *Listonella* sp. infection in freshwater cultured loach, *Misgurnus anguillicaudatus*. Aquacult Res 45:602-608.
- Qin L, Zhang X, Bi K (2012b) A new pathogen of gibel carp *Carassius auratus gibelio*-*Shewanella putrefaciens*. Acta Microbiol Sin 52:558-565.
- Saeed M, Alamoudi M, Al-Harbi A (1987) A Pseudomonad associated with disease in cultured rabbitfish *Siganus rivulatus* in the Red Sea. Dis Aquatic Org 3:177-180.
- Schade FM, Raupach MJ, Wegner KM (2016) Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual temperature fluctuations. J Sea Res 113:73-84.
- Schäperclaus W (1984) Fish Disease. Berlin-Friedrichshagen.
- Stoskopf MK (1993) Fish medicine. Philadelphia, WB Saunders Co.
- Stoskopf MK (2015) Biology and management of laboratory fishes. In: Fox J (ed) Laboratory Animal Medicine, 3rd edn. Academic Press 1063-1086.
- Tang F, Zhang Y, Zhao Y (2017) Morphological and molecular identification of the new species, *Trichodina pseudoheterodontata* sp. n. (Ciliophora, Mobilida, Trichodinidae) from the channel catfish, *Ictalurus punctatus*, in Chongqing China. J Eukaryo Microbiol 64:45-55.
- Timur G, Timur M (2003) Balık hastalıkları. İstanbul Üniversitesi Su Ürünleri Fakültesi yayımları.
- Toranzo AE, Baya AM, Romalde JJ et al (1989) Association of *Aeromonas sobria* with mortalities of adult gizzard shad, *Dorosoma cepedianum* Lesueur. Journal of Fish Diseases 12:439-448.
- Uğurlu S, Polat N (2006) Exotic fish species inhabiting in freshwater sources within the province of Samsun. J Fish Sci 1:139-151.
- Valladao GMR, Gallani SU, De Padua SB, Laterca M, Pilarski F (2014) *Trichodina heterodontata* (Ciliophora) infestation on *Prochilodus lineatus* larvae: a host-parasite relationship study. Parasitology 141(5):662-669.
- Varalakshmi B, Shanmugapriya A, Karpagam T, Suganya V, Firdous J, Arumugam VA, SrideviR, Abinaya M, Saradhasri V (2022) Bacterial Fish Diseases and Treatment. In Aquaculture Science and Engineering (pp. 517-572). Singapore: Springer Nature Singapore.
- Wahli T, Burr SE, Pugovkin D et al (2005) *Aeromonas sobria*, a causative agent of disease in farmed perch, *Perca fluviatilis* L. J Fish Dis 28:141-150.
- Wang Z, Deng Q, Zhou T et al. (2018) First record of two ectoparasitic ciliates of the genus *Trichodina* (Ciliophora: Trichodinidae) parasitizing gills of an invasive freshwater fish, *Micropercops swinhonis*, in Tibet. Parasitol Res 117:2233-2242.
- Wang Z, Zhou T, Yang H et al (2019) First diagnosis of ectoparasitic ciliates (*Trichodina* and *Chilodonella*) on farmed juvenile yellow catfish, *Tachysurus fulvidraco* in China. Aquac Res 50:3275-3285.
- Wang Z, Liu M, Ma H et al (2022) Redescription and molecular characterization of two *Trichodina* species (Ciliophora, Peritrichia, Mobilida) from freshwater fish in China. Parasitol Int 86:102470.
- Yi SW, You MJ, Lee HB et al (2012) A case of *Aeromonas veronii* infection in Israeli carp (*Cyprinus carpio*): Phylogenetic analysis and antimicrobial resistance. Korean J Vet 35:239-243.