Acta Veterinaria Hungarica

69 (2021) 4, 338–346

DOI:
10.1556/004.2021.00041

RESEARCH ARTICLE

Low-level pathogen transmission from wild to farmed salmonids in a flow-through fish farm

MARKÉTA PRAVDOVÁ[1,2,3]*, MARKÉTA ONDRAČKOVÁ[1]*, MIROSLAVA PALÍKOVÁ[4,5], IVANA PAPEŽÍKOVÁ[4,5], YURIY KVACH[1,6], PAVEL JURAJDA[1], VERONIKA BARTÁKOVÁ[1], VERONIKA SEIDLOVÁ[4,5], MONIKA NĚMCOVÁ[4,5] and JAN MAREŠ[5]

[1] Institute of Vertebrate Biology, Czech Academy of Sciences, Květná 8, 603 65 Brno, Czech Republic

[2] Faculty of Agronomy, Mendel University, Brno, Czech Republic

[3] Faculty of Science, Masaryk University Brno, Brno, Czech Republic

[4] Department of Ecology and Diseases of Zoo Animals, Game, Fish and Bees, Faculty of Veterinary Hygiene and Ecology, University of Veterinary Sciences Brno, Brno, Czech Republic

[5] Department of Zoology, Fisheries, Hydrobiology and Apiculture, Faculty of Agronomy, Mendel University in Brno, Brno, Czech Republic

[6] Institute of Marine Biology of the National Academy of Sciences of Ukraine, Odessa, Ukraine

Received: 28 June 2021 • Accepted: 21 September 2021

ABSTRACT

While the potential effects of pathogens spread from farmed fish to wild populations have frequently been studied, evidence for the transmission of parasites from wild to farmed fish is scarce. In the present study, we evaluated natural bacterial and parasitic infections in brown trout (*Salmo trutta* m. *fario*) collected from the Černá Opava river (Czech Republic) as a potential source of infections for rainbow trout (*Oncorhynchus mykiss*) reared in a flow-through farm system fed by the same river. The prevalence of bacterial and protozoan infections in farmed fish was comparable, or higher, than for riverine fish. Despite this, none of the infected farmed fish showed any signs of severe diseases. Substantial differences in metazoan parasite infections were observed between wild and farmed fish regarding monogeneans, adult trematodes, nematodes, the myxozoan *Tetracapsuloides bryosalmonae* found in riverine fish only, and larval eye-fluke trematodes sporadically found in farmed fish. The different distribution of metazoan parasites between brown and rainbow trout most probably reflects the availability of infected intermediate hosts in the two habitats. Despite the river being the main water source for the farm, there was no significant threat of parasite infection to the farmed fish from naturally infected riverine fish.

KEYWORDS

rainbow trout, brown trout, parasites, bacteria, freshwater fish farm

INTRODUCTION

Freshwater aquaculture is an important and integral part of the agricultural sector in many countries, and particularly in Central Europe. In the Czech Republic, there is a strong emphasis on the farming of common carp (*Cyprinus carpio*) in ponds, using traditional forms of semi-intensive aquaculture based on knowledge gained over hundreds of years (Všetičková et al., 2012). While the common carp is by far the dominant species farmed in the Czech Republic (88% of the total biomass), aquaculture-reared salmonids (Salmonidae), such as brown trout (*Salmo trutta* m. *fario*), rainbow trout (*Oncorhynchus mykiss*), and brook trout

*Corresponding authors. Tel.: +420 543 422 515.
E-mail: pravdova8@email.cz; ondrackova@ivb.cz

(*Salvelinus fontinalis*) are gaining an increasingly important share of the market. Although salmonids occur naturally in cold waters, mainly at high latitudes in the Holarctic region, their value as both a food source and a recreational fishing species has led to extensive stocking outside the native range (Fausch, 1988; Halverson, 2008).

The rainbow trout, native to North America and East Asia (Kamchatka), is commercially farmed in many countries throughout the world, and it is frequently stocked into rivers and lakes to attract recreational anglers (Frimodt, 1995). The species naturally inhabits clear, cold headwaters, creeks, small to large rivers, lakes and intertidal stretches (Page and Burr, 2011). Today, rainbow trout is the most widely introduced salmonid worldwide, and one of the most widely introduced fish species in general (Crawford and Muir, 2008). Its global success can be ascribed to a combination of factors, including its importance as a game-fish species in western culture, its rapid growth and overall suitability for hatchery cultivation and, thanks to this latter one, its economic importance in food production (Crawford and Muir, 2008).

The brown trout is native to European waters and, similarly to the rainbow trout, the species has been widely introduced into suitable environments around the world, although its environmental tolerance limits are lower than those of rainbow trout. Brown trout prefer cold, well-oxygenated streams, rivers and lakes and ponds in upland/mountainous areas, with adequate cover in the form of submerged rocks, undercut banks and overhanging vegetation (Scott and Scott, 1988). In recent decades, the density of brown trout populations in parts of Europe has declined due to a number of factors such as habitat loss, climate change, overfishing, and diseases (Borsuk et al., 2006; Borgwardt et al., 2020). Hence, stocking programs, in some cases, have been put in place to support natural populations (Borgwardt et al., 2020). In the Czech Republic, natural populations are rare (Kohout et al., 2012; Jurajda et al., 2020), with the majority of populations being a mixture of several stocked populations originating from different river basins. Farm-reared brown trout released into the wild may differ from native wild populations in many ways, whether it be genetically, behaviourally and/or phenotypically (Ferguson, 2007). The stocking may reduce fitness and disrupt local adaptation to the wild population, and may enhance their susceptibility to diseases (Einum and Fleming, 2001). Moreover, the translocation of farmed brown trout from multiple areas may lead to the introduction of diseases and parasites into the wild populations (Pinter et al., 2019).

Infectious diseases caused by viral, bacterial or parasitic agents are common in farmed salmonids, including brown trout and rainbow trout. *Flavobacterium psychrophilum*, for example, is an important cause of mortality in both fresh and brackish waters (Nilsen et al., 2011). The myxozoan parasite *Tetracapsuloides bryosalmonae*, which causes proliferative kidney disease (PKD), is also an economically important salmonid disease agent both in aquaculture (Palíková et al., 2017) and under natural conditions (Pojezdal et al., 2020). Moreover, whirling disease (WD) is an ecologically and economically debilitating disease of rainbow trout and other salmonids, caused by the myxozoan parasite *Myxobolus cerebralis* (Sarker et al., 2015). Monogenean parasites of the genus *Gyrodactylus* can seriously injure fish and may cause considerable mortality, especially among small fingerlings (Stoltze and Buchmann, 2001). The majority of problems with protozoan and metazoan parasites in aquaculture are associated with their ability to spread without an intermediate host. However, parasites with complex life cycles, i.e. that require intermediate hosts, may also cause serious fish diseases should they enter the aquacultural system (Palíková et al., 2014). In many instances, disease outbreaks in fish farms are the result of increased stress in the host animal and/or decreased water quality and, in such cases, environmental manipulation may help to mitigate both the level of infection and any associated mortality (Paladini et al., 2017). The potential effects of pathogen spread from farmed fish to the wild population have frequently been studied. Freshwater aquaculture creates conditions (e.g. high stocking levels) conducive to pathogen transmission and disease; hence pathogens can overspill back, resulting in a relevant challenge to wild populations (Peeler and Murray, 2004). The evidence for the transmission of parasites from wild to farmed fish is scarce. Thus, the aim of this study was to evaluate whether wild brown trout stocks inhabiting a river that serves as the source of water for a commercial flow-through fish farm have any epidemiological impact on farmed rainbow trout by disease transmission.

MATERIALS AND METHODS

Study area

The brown trout, as the potential source of trout parasites/diseases, were sampled directly from the River Černá Opava (50°1′35.848″N, 17°3′78.396″E; 17.9 km long, av. depth 30 cm, av. width 10 m, flow rate 0.3 m^3s), a tributary of the River Opava (Odra River basin, Baltic Sea drainage), which serves as water source for the fish farm, approx. 200 m far from the sampling point in the river (see below; No. 1, Fig. 1). Although the brown trout population is self-reproducing, stocking of fingerlings is regularly carried out by the local angling club from the downstream fish farm.

The rainbow trout were obtained from a small-scale trout farm (Pstruhařství Jaroslav Žalák) located at Vrbno pod Pradědem in the north-east of the Czech Republic (50°1′37.762″N, 17°3′79.324″E). The fish farm's ponds are fed with water from the adjacent River Černá Opava, the water first passing through a 10 × 10 mm mesh screen. The water is of high quality and requires no further treatment. The fish farm uses a 'flow-through system', whereby water from the river flows through separate channels into external breeding ponds (min. flow rate 50 l/s; capacity 8,600 m^3) and indoor tanks (max. flow rate 10 l/s; capacity 85 m^3), from where it then flows into a drainage channel and back to the river (Fig. 1, Table 1). The fish are fed with 4.5-mm Aller Gold

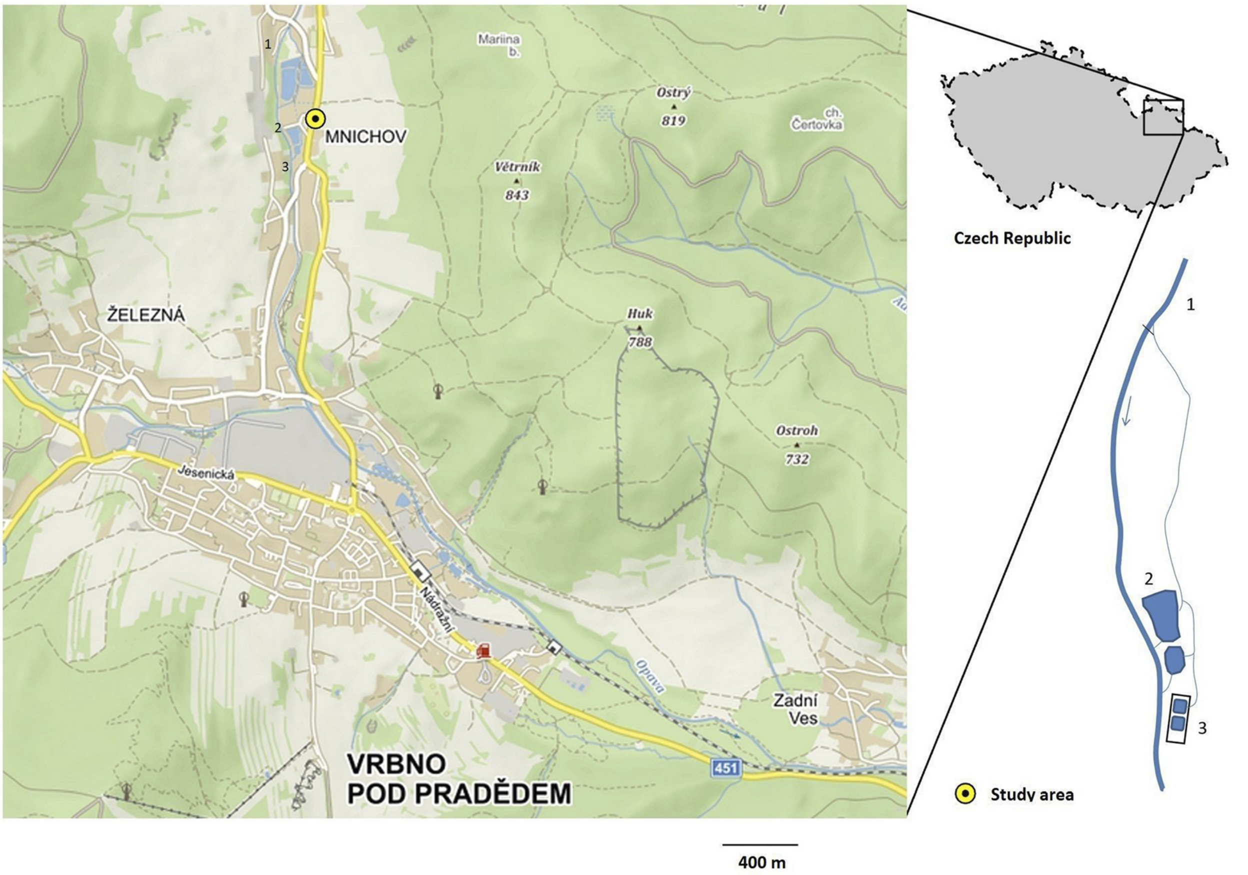

Fig. 1. The map of the sampling site and schematic representation of the Žalák fish farm, showing the Černá Opava River (1) flowing into the external ponds (2) and indoor tanks (3). The thinner lines represent the drainage channels from the river

pellets (Aller-Aqua Group, Denmark) from an automatic fish feeder and by invertebrates naturally established in the pond. Only early life stages are treated against ectoparasites, using formaldehyde and salt baths, in a special tank. For this study, fish were obtained from both the external ponds (No. 2, Fig. 1) and the indoor tanks (No. 3, Fig. 1).

Fish and parasite sampling

The examined brown trout specimens were obtained from the Černá Opava in the autumn (September) of 2019 using a backpack electroshocker (Lena, Bednář, Czech Republic; pulsed DC maximum output 225–300 V, maximum output current 1.5 A, pulse frequency 50–120 Hz) fitted with a 3-m anode pole with an anode ring 25 cm in diameter (for numbers of fish examined, see Table 1).

The rainbow trout sampling took place in spring (April), summer (July/August) and autumn (September/October) of 2019. Fish collected in spring and autumn were obtained from the external ponds and were screened for bacterial, protozoan and metazoan parasite infection. In summer, fish were collected from both the indoor tanks and the external ponds, and they were examined for bacterial and protozoan infections. Swab samples from gills and from spleen tissue were collected from all fish and put into Amies transport medium (Oxoid, UK) to preserve them during transport to the laboratory. In the laboratory, the swabs were inoculated in duplicate onto blood agar and TYES agar (Oxoid, UK) and cultured aerobically at 17 °C. The grown bacteria were identified according to colony morphology, followed by the use of matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) system (Microflex LT; Bruker Daltonics, Bremen, Germany). In all cases, rainbow trout were collected randomly using a dip net.

Table 1. Number (n) of farmed rainbow trout (*Oncorhynchus mykiss,* OM) and riverine brown trout (*Salmo trutta,* ST) collected for pathogen evaluation, showing the number of fish examined (n), the water temperature (t, °C), the range of body length (SL, mm) and the total weight (Wt, g)

		n	*t*	SL (range)	Wt (range)
OM external pond	spring	25	5.5	212–280	141–334
	summer	10	14.5–17	341–380	457–650
	autumn	21	7.5	280–400	380–393
OM indoor tanks	summer	10	13.5–14	289–333	403–610
	autumn	10	7.5	313–358	399–935
ST river	autumn	14	7.0	140–215	42–126

All fish were transported to the laboratory alive in tanks with aerated river water, where they were maintained individually until dissection. In order to ensure minimal loss of parasites, fish from each locality were dissected alternately within two days of capture (see Kvach et al., 2016). The fish were measured for standard length (SL, nearest 1 mm) and total body weight (Wt, nearest 1 g). The fish were then sacrificed humanely, and tissue samples were examined under a binocular microscope for the presence of parasites using standard protocols (Ergens and Lom, 1970).

The microparasites collected from the skin and gills were identified directly to genus level, while the macroparasites were preserved in ammonium picrate–glycerine mixture (Monogenea), 4% formaldehyde or 70% ethanol (Trematodes, Nematodes) and identified under an Olympus BX51 light microscope equipped with a QuickPhoto 3.2 digital image analyser (Olympus Optical Co., Japan). The ciliates from the gills and skin were examined in wet-mounts under a microscope (Reavill and Roberts, 2007). The microbial cultures from the gills and spleen were used to determine the type of bacteria and their abundance by culturing on appropriate media (see Roberts, 2005). The monogenean parasites were identified according to the shape of their haptoral hard parts and copulatory organs using the key by Gusev et al. (1985). The keys of Bykhovskaya-Pavlovskaya and Kulakova (1985) and Moravec (2013) were used for the identification of trematode and nematode species, respectively, while both morphological and molecular methods (28S rDNA) were used to identify the recently described trematode *Crepidostomum pseudofarionis* (Faltýnková et al., 2020). Total genomic DNA was extracted using the Invisorb® Spin Forensic Kit (STRATEC Molecular, Germany), following the manufacturer's guidelines. A partial 28S rDNA was used for molecular characterisation, using the primers LSU5 (TAGGTCGACCCGCTGAAYTTAAGCA) (Littlewood, 1994) and ECD2 (CCTTGGTCCGTGTTTCAAGACGGG) (Littlewood et al., 1997). The reaction mix contained 4 µL of extracted DNA, 0.3 µM of each primer, 1 × buffer A, 0.2 µL dNTPs (10 mM), 0.2 µL $MgCl_2$ (25 mM), 0.5 U Taq polymerase and ddH_2O up to a total volume 10 µL. For PCR analysis, we used the KAPA2G Robust HotStart PCR Kit (Kapabiosystems, USA). Amplification was taken in a Mastercycler ep gradient S thermocycler (Eppendorf, Germany), and the temperature profile of the PCR reaction consisted of an initial denaturation step at 95 °C for 2 min followed by 33 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 60 s, followed by a final extension step at 72 °C for 5 min. All PCR products were purified using an ExoSAP-IT kit (Affymetrix Inc., Santa Clara, USA), according to the manufacturer's protocol. PCR products were sequenced commercially by Eurofins Genomics Germany GmbH (Ebersberg). The sequences were checked and aligned using Geneious v. 9.0.5. software (http://www.geneious.com), and the newly generated sequences were compared with the NCBI database using BLASTn to assess sequence similarity.

The caudal kidney samples (ca. 20 mg) from six rainbow trout and eight brown trout collected in autumn were fixed in 70% ethanol for molecular detection of *T. bryosalmonae* presence. DNA was extracted using the NucleoSpin®DNA Tissue kit (Macherey-Nagel GmbH & Co., Germany), according to the manufacturer's instructions. For molecular detection, a real-time PCR assay of 18S rDNA was performed as described previously by Bettge et al. (2009) with forward and reverse primers PKDtaqf1: 5′-GCG AGA TTT GTT GCA TTT AAA AAG-3′ and PKDtaqr1 5′-GCA CAT GCA GTG TCC AAT CG-3′. The internal probe (probePKD: 5′-CAA AAT TGT GGA ACC GTC CGA CTA CGA-3′) was labelled at the 5′ end with the reporter dye 6-carboxyfluorescein (FAM) and at the 3′ end with the quencher dye 6-carboxytetramethyl-rhodamine (TAMRA). The quantitative PCR (qPCR) amplification was performed using a reaction volume of 20 µl containing 3.76 µl of water, 10 µl LightCycler® 480 Probe Master (Roche), 0.6 µl forward primer (300 nM), 0.6 µl reverse primer (300 nM), 0.4 µl probe (200 nM) and 2 µl of extracted DNA. Real-time PCR results were scored as positive on $Ct < 32$ (Seidlova et al., 2021).

Data analysis

The parasitic infections were characterised by calculating the prevalence, the abundance, and the intensity of infections, for all examined parasite taxa. The prevalence was calculated as the proportion of parasitised fish among all sampled fish, the mean abundance as the mean number of parasites in all hosts in the sample, and the intensity of infection as the mean number of parasite individuals found in infected hosts (Bush et al., 1997). Overall parasite abundance was measured as the total abundance of all parasite taxa per host individual.

RESULTS

The microbiological examination of the gills showed the regular presence of flavobacterial infection in rainbow trout, along with the occasional occurrence (10%) of *Chromobacterium violaceum* and *Pseudomonas fluorescens* in spring samples. In summer, *Pseudomonas putida*, *P. koreensis* and *Hafnia alvei* were confirmed in pond fish (all at 20% prevalence), while *Bacillus cereus* and *Proteus* sp. were found in fish from the indoor tanks (both at 10% prevalence). In autumn, no bacterial infections were detected in rainbow trout from the external pond; however, 20% of fish from the indoor tanks were positive for *Flavobacterium saccharophilum* and 70% for *Aeromonas* sp., both infecting the gills. Natural river populations of brown trout showed the presence of *Aeromonas bestiarum* (20%) and *H. alvei* (10%). Bacterial infection of the spleen was very rare and only confirmed in rainbow trout from the external pond in summer (*Aeromonas sobria*, 20%) and riverine brown trout in autumn (*H. alvei*, 20%; Table 2).

Examination of the protozoan parasites confirmed the presence of *Apiosoma* spp., *Trichodina* spp. and *Ichthyophthirius multifiliis* in both farmed rainbow trout and riverine

Table 2. Prevalence of microparasites (i.e. protozoan parasites) and bacterial infections (%) and, in parentheses, the (minimum to maximum) number of parasite individuals detected in rainbow trout (*Oncorhynchus mykiss,* OM) and brown trout (*Salmo trutta,* ST)

	OM (pond)		OM (indoor tank)			ST (river)
	Spring	Summer	Autumn	Summer	Autumn	Autumn
Protozoa						
Apiosoma sp. skin	40 (0–1)	10 (0–2)	10 (0–1)	–	–	50 (0–8)
Epistylis sp. skin	–	–	–	–	–	40 (0–2)
Ichthyophthirius multifiliis skin	–	40 (0–1)	20 (0–1)	30 (0–1)	15 (0–1)	10 (0–1)
Ichthyophthirius multifiliis gills	–	10 (0–1)	–	10 (0–1)	5 (0–1)	10 (0–1)
Trichodina sp. skin	–	60 (0–3)	10 (0–1)	–	5 (0–1)	–
Trichodina sp. gills	–	10 (5–10)		–	–	10 (5–10)
Bacteria						
Aeromonas bestiarum gills	–	–	–	–	–	20
Aeromonas sobria spleen	–	20	–	–	–	–
Aeromonas sp. gills	–	–	–	–	70	–
Bacillus cereus gills	–	–	–	10	–	–
Flavobacterium saccharophilum gills	100	–	–	–	20	–
Hafnia alvei gills	–	20	–	–	–	10
Hafnia alvei spleen	–	–	–	–	–	20
Chromobacterium violaceum gills	10	–	–	–	–	–
Proteus sp. gills	–	–	–	10	–	–
Pseudomonas fluorescens gills	10	–	–	–	–	–
Pseudomonas koreensis gills	–	20	–	–	–	–
Pseudomonas putida gills	–	20	–	–	–	–

brown trout, and *Epistylis* sp. in brown trout only (Table 2). *Apiosoma* spp. were observed on the skin of rainbow trout from the external ponds (but not from the indoor tanks) over all three seasons with a prevalence between 10 and 40%. These ciliates also occurred on brown trout with 50% prevalence. Trichodinid ciliates were observed on the skin and/or gills of rainbow trout from the external ponds (prevalence 10–60%) and the indoor tanks (5%), and in riverine brown trout (10%). The presence of *I. multifiliis* was confirmed in both the skin and gills of all rainbow trout samples (ponds and indoor tanks; prevalence 10–40%) and in riverine brown trout (10%), during summer and autumn (Table 2).

Metazoan parasite infection of farmed rainbow trout was only recorded occasionally and consisted of two eye-fluke trematodes, the metacercariae of *Tylodelphys clavata* and *Diplostomum* sp. Despite the relatively high prevalence (spring 60%, autumn 45%), the abundance of *T. clavata* was low, 1.7 in spring and 0.9 in autumn. The presence of *Diplostomum* sp. was confirmed in a single rainbow trout in autumn (Table 3). In contrast, brown trout specimens were infected with a wide range of macroparasite species at both high prevalence and abundance (Table 3), the parasite community consisting mainly of the ectoparasitic monogeneans *Gyrodactylus derjavinoides* and *G. teuchis* infecting the gills, fins and body surface, and endoparasites, such as the nematode *Salmonema ephemeridarum* and the trematodes *Crepidostomum metoecus* and *C. pseudofarionis*, located in the intestine. In addition, trematodes were recorded in the pyloric caeca and gall bladder (*C. metoecus, C. pseudofarionis*), while nematodes (*S. ephemeridarum*) were found in the stomach. Real-time PCR indicated that

Table 3. The prevalence (P) in %, the mean abundance (A ± standard deviation [SD]) and the intensity of infection (I; mean number and the minimum to maximum range of parasite individuals) in rainbow trout (*Oncorhynchus mykiss*) from the fish farm pond, and in brown trout (*Salmo trutta*) collected from the river

	Oncorhynchus mykiss (pond)						*Salmo trutta* (river)		
	Spring (n = 15)			Autumn (n = 11)			Autumn (n = 14)		
Parasite species	P	A ± SD	I (range)	P	A ± SD	I (range)	P	A ± SD	I (range)
Gyrodactylus teuchis	–	–	–	–	–	–	64.29	3.43 ± 4.68	5.33 (1–17)
Gyrodactylus derjavinoides	–	–	–	–	–	–	57.14	4 ± 5.79	4 (2–20)
Crepidostomum metoecus	–	–	–	–	–	–	92.86	5.21 ± 5.28	9.13 (1–20)
C. pseudofarionis	–	–	–	–	–	–	85.7	2 ± 1.37	2.33 (1–5)
Salmonema ephemeridarum	–	–	–	–	–	–	92.86	27.1 ± 17.69	29.15 (7–72)
Tylodelphys clavata	66	1.73 ± 1.81	2.6 (1–6)	45.45	0.9 ± 1.31	2 (1–4)	–	–	–
Diplostomum sp.	–	–	–	9.1	0.09 ± 0.29	1 (1)	–	–	–

Tetracapsuloides bryosalmonae, the agent of PKD, was present in brown trout only, in the river (3 of 8 were positive, i.e. 37.5% prevalence). The examined rainbow trout specimens were not affected by *T. bryosalmonae* infection.

DISCUSSION

In the study, we evaluated bacterial as well as protozoan and metazoan parasitic infections in rainbow trout reared in a fish farm and in brown trout inhabiting the Černá Opava, the main source of water for the farm's flow-through pond system and a potential source of natural infections. Rainbow trout from both the external ponds and indoor tank were infected with bacteria and protozoan parasites at slightly higher or similar prevalence than riverine brown trout. Although brown trout were sampled in autumn only, these findings suggest that the spread of the detected microparasites from wild brown trout populations to farmed rainbow trout is negligible. While there were slight differences in the prevalence of bacterial and microparasite infections in rainbow trout from ponds and indoor tanks, none of the fish examined showed signs of infection or behavioural abnormalities, indicating no relevant correlation between breeding conditions and the risk of fish disease. Metazoan parasites in farmed fish were very rare and comprised just two digenean species. In contrast, the prevalence and the abundance of macroparasites were higher in riverine fish. Overall, parasites with complex life cycles were predominant in wild fish, whereas those with simple life cycles and bacteria were predominant in farmed fish.

It is known that high stocking density and associated stress, low water quality, oxygen deficiency, other diseases (viral, eukaryotic agents), inappropriate aquacultural techniques and/or reproductive stage of aquacultural fish are often associated with bacterial diseases (Austin and Austin, 2016). One of the most frequently detected bacteria in the pond system were *Flavobacterium* spp., which were recorded at 100% prevalence in rainbow trout in the external ponds (spring) and 20% prevalence in the indoor tank (autumn). In comparison, these bacteria were not found in the autumn sample of riverine brown trout. However, this might be caused by the uneven sampling regime for rainbow and brown trout (3 samplings at two sites vs. 1 sampling at 1 site).

Flavobacteria infecting gills included *F. sacharophilum* and a number of other benign species that could not be determined to species level. Species with pathogenic potential, such as *Flavobacterium branchiophilum*, *F. psychrophilum* or *Flavobacterium columnare*, were not detected. Both the presence and the abundance of bacteria such as *Pseudomonas* sp. and *Aeromonas* sp. are usually associated with the physicochemical parameters of the holding water, which may also be affected by the type and quantity of food provided, and the fish stocking density. Consequently, these bacteria are more often found in farmed fish than in wild populations (Gołaś et al., 2019). These findings were confirmed in our study, with no pseudomonads and very few aeromonads recorded in brown trout from the river.

Protozoan parasites are the most common parasite type encountered in fish hatcheries (Pillay, 1995), with *I. multifiliis* tending to dominate in freshwater fish (Colorni, 2008). It is usually considered a generalist species (e.g. Hines and Spira, 1974), and young fish tend to be more vulnerable than adults. In our study, however, the prevalence of this parasite was generally similar in both farmed rainbow trout and riverine brown trout. On the other hand, Hoffman (1999) noted that the risk of infection in aquacultural facilities increases with water temperature. This was confirmed in our study; the highest prevalence was found in summer samples, although no massive infections were detected in either the external ponds or the indoor tanks. Overall, the farmed rainbow trout were in good somatic condition, which limited any negative effects of this parasite on host health. And most likely, the antiparasitic in-bath treatment applied as prevention effectively controlled the amount of protozoan parasites. Other ciliates, such as trichodinids and *Apiosoma* spp., were found on the gills and body surface, mainly in the external ponds, although trichodinid parasites were also rarely observed in fish from the indoor tanks. These parasites, along with *Epistylis* sp., which was found solely in riverine wild brown trout, commonly occur in salmonids throughout the year, usually with moderate infection rates and without any serious negative effect on fish survival (Buchmann and Bresciani, 1997). Despite the relatively high fish density in the pond system, infection with these ciliates was not unusually high, with the observed prevalence being up to 60%, and no negative effects on the health of the fish host are expected.

At least 22 macroparasite species have been reported for rainbow trout, and 33 for brown trout, in the Czech Republic (Moravec, 2001), of which 18 are common to both species. Of these, gyrodactylids (Monogenea) are frequent ectoparasites of salmonids, and their direct life cycle often contributes to fish host mortality, depending on host susceptibility (e.g. Busch et al., 2003). Fish usually get infected via contact with affected fish. Owing to their ability to survive outside the fish host for some time (Peeler and Thrush, 2004), gyrodactylids may potentially be spread from rivers into fish farms that use the river water. However, none of the macroparasites detected in brown trout were found in farmed rainbow trout. This might be due to the high host specificity of most of these parasite species, especially monogeneans. While *G. derjavinoides* is a specialist for brown trout (Malmberg et al., 2007), *G. teuchis* has been found on several salmonid species, including both brown and rainbow trout (Cunningham et al., 2001). Furthermore, the relatively long channel distance between the river and the pond (approx. 500 m) appears to have represented a sufficient barrier for preventing transmission of gyrodactylids to farmed fish.

Myxosporean infection of *T. bryosalmonae* was confirmed in riverine brown trout only. While the presence of *T. bryosalmonae* has previously been reported in some Czech rivers (see Pojezdal et al., 2020), our study reports the presence of the species at a new locality, the Černá Opava. The absence of *T. bryosalmonae* in farmed rainbow trout, despite its presence in the adjacent river, may be related to a range of factors, such as resistance of the rainbow trout strain stocked (e.g. Grabner and El-Matbouli, 2009), the effectiveness of treatment against bryozoans, the lack of the parasite's intermediate host or, possibly, the distance of the feed channel acting as a barrier. However, the transmission of *T. bryosalmonae* between natural waters and fish farms may require further investigation.

Five macroparasite species with complex life cycles, i.e. those requiring multiple hosts to complete their life cycle, were detected in brown and rainbow trout, with no overlap in species composition. Two eye-fluke species, i.e. metacercariae of *T. clavata* and *Diplostomum* sp., were found to infect rainbow trout in the external ponds, though both were found at low abundance (presence of macroparasites was not investigated in the indoor tanks). No eye-flukes were detected in the brown trout, presumably as the environmental conditions in the river were unsuitable for completion of the parasite's life cycle. Aquatic snails, which serve as the first intermediate host for diplostomid trematodes, prefer lentic waters and usually do not inhabit running rocky streams or high mountain streams (Bailey et al., 2005). On the other hand, brown trout were heavily infected with the trematodes *C. metoecus* and *C. pseudofarionis* and the nematode *S. ephemeridarum*, with prevalence levels reaching 86–93%. Both *Crepidostomum* and *Salmonema* require ingestion by an infected intermediate host (e.g. invertebrates) for completion of their life cycles (for details see Pravdová et al., 2021). The high prevalence and abundance of these parasites in the brown trout is an indicator of their frequent occurrence in intermediate hosts. As the presence of these infected invertebrates is very likely in the external ponds at least, the absence of such foodborne macroparasites (adult trematodes or nematodes) in the rainbow trout may reflect their altered feeding behaviour under farmed conditions. The high prevalence of *Crepidostomum* spp. in brown trout might then be explained by the ontogeny and seasonal changes in parasitic infection (Prati et al., 2020a). *Crepidostomum* infection tends to be higher in autumn and winter as trout tend to shift diet from surface/terrestrial insects toward amphipods and insect larvae (Prati et al., 2020a). The size range of brown trout used in this study also corresponds to size classes of trout that tend to feed mainly on these preys. In contrast, larger brown trout tend to be piscivorous; thus, *Crepidostomum* infections may decrease due to ontogenetic switches in the diet (Prati et al., 2020b).

In freshwater fish farming, *Aeromonas* sp., *I. multifiliis*, monogenean and trichodinid infections are of serious concern worldwide (Valladão et al., 2016). Aside from monogeneans, all of these pathogens were detected in rainbow trout from the external ponds and indoor tanks; however, none had any significant impact on the heath of the fish host. While there is widespread concern about the potential effects of pathogens spreading from farmed fish to wild populations, there is little evidence for the transmission of parasites from wild fish to farmed fish (Johansen et al., 2011). Our results support this statement. We found little evidence of any significant transfer of macroparasites, microparasites or bacteria to farmed rainbow trout, despite the fact that river water inhabited by naturally infected wild brown trout was the main water source for the flow-through pond system.

ACKNOWLEDGEMENTS

We would like to thank Jaroslav Žalák for providing fish from his fish farm and for creating ideal conditions for our survey. We would also like to thank Ing. Michal Urban (Mendel University) for providing important information about the farm and Dr. Zdenka Jurajdová (Institute of Vertebrate Biology, Czech Academy of Sciences) who helped with fish dissection. Thanks are also due to all the students from Mendel University who helped us with fish processing for microbiological investigation. Finally, we would like to thank Dr. Kevin Roche (Institute of Vertebrate Biology, Czech Academy of Sciences) for his help with proofreading of the English text. This work was supported by the project PROFISH CZ.02.1.01/0.0/0.0/16_019/0000869.

REFERENCES

Austin, B. and Austin, D. A. (2016): Bacterial Fish Pathogens: Disease of Farmed and Wild Fish. 6th edition. Springer International Publishing, UK.

Bailey R. C., Benke, A. C. and Cushing, C. E. (2005): Rivers of North America, Yukon River Basin. Elsevier Academic, Amsterdam. pp. 774–802.

Bettge, K., Wahli, T., Segner, H. and Schmidt-Posthaus, H. (2009): Proliferative kidney disease in rainbow trout: Time- and temperature-related renal pathology and parasite distribution. Dis. Aquat. Organ. **83**, 67–76.

Borgwardt, F., Unfer, G., Auer, S., Waldner, K., El-Matbouli, M. and Bechter, T. (2020): Direct and indirect climate change impacts on brown trout in central Europe: how thermal regimes reinforce physiological stress and support the emergence of diseases. Front. Environ. Sci. **8**, 59.

Borsuk, M. E., Reichert, P., Peter, A., Schager, E. and Burkhardt-Holm, P. (2006): Assessing the decline of brown trout (*Salmo trutta*) in Swiss rivers using a Bayesian probability network. Ecol. Model. **192**, 224–244.

Buchmann, K. and Bresciani, J. (1997): Parasitic infections in pond-reared rainbow trout *Oncorhynchus mykiss* in Denmark. Dis. Aquat. Organ. **28**, 125–138.

Busch, S., Dalsgaard, I. and Buchmann, K. (2003): Concomitant exposure of rainbow trout fry to *Gyrodactylus derjavini* and *Flavobacterium psychrophilum*: effects on infection and mortality of host. Vet. Parasitol. **117**, 117–122.

Bush, A. O., Lafferty, K. D., Lotz, J. M. and Shostak, A. W. (1997): Parasitology meets ecology on its own terms: Margolis et al. J. Parasitol. **83**, 575.

Bykhovskaya-Pavlovskava, I. E. and Kulakova, A. P. (1985): Class Trematoda. In: Bauer, O. N. (ed.) Key to the Parasites of the Freshwater Fish Fauna of the USSR. Nauka, Moscow, Russia. pp. 77–198.

Colorni, A. (2008): Diseases caused by Ciliophora. In: Eiras, J. C., Segner, H., Wahli, T. and Kapoor, B. G. (eds) Fish Diseases. Vol. **1**. Science Publishers, Enfield, New Hampshire, USA. pp. 569–612.

Crawford, S. S. and Muir, A. M. (2008): Global introductions of salmon and trout in the genus *Oncorhynchus*: 1870–2007. Rev. Fish Biol. **18**, 313–344.

Cunningham, C. O., Mo, T. A., Collins, C. M., Buchmann, K., Thiery, R., Blanc, G. and Lautraite, A. (2001): Redescription of *Gyrodactylus teuchis* Lautraite, Blanc, Thiery, Daniel & Vigneulle, 1999 (Monogenea: Gyrodactylidae), a species identified by ribosomal RNA sequence. Syst. Parasitol. **48**, 141–150.

Einum, S. and Fleming, I. A. (2001): Implications of stocking: ecological interactions between wild and released salmonids. Nord. J. Freshw. Res. **75**, 56–70.

Ergens, R. and Lom, J. (1970): Agents of Fish Diseases. Academia, Prague. 384 pp.

Faltýnková, A., Pantoja C., Skírnisson, K. and Kudlai, O. (2020): Unexpected diversity in northern Europe: trematodes from salmonid fishes in Iceland with two new species of *Crepidostomum* Braun, 1900. Parasitol. Res. **119**, 2439–2462.

Fausch, K. D. (1988): Tests of competition between native and introduced salmonids in streams: What have we learned? Can. J. Fish. Aquat. Sci. **45**, 2238–2246.

Ferguson, A. (2007): Genetic Impacts of Stocking on Indigenous Brown Trout Populations. Science Report: SC040071/SROV. Environmental Agency, Bristol, UK. 81 pp.

Frimodt, C. (1995): Multilingual Illustrated Guide to the World's Commercial Coldwater Fish. Fishing News Books, Osney Mead, Oxford, England. 215 pp.

Gołaś, I., Szmyt, M., Potorski, J., Łopata, M., Gotkowska-Płachta, A. and Glińska-Lewczuk, K. (2019): Distribution of *Pseudomonas fluorescens* and *Aeromonas hydrophila* bacteria in a recirculating aquaculture system during farming of European grayling (*Thymallus thymallus* L.) brood stock. Water **11**, 376.

Grabner, D. S. and El-Matbouli, M. (2009): Comparison of the susceptibility of brown trout (*Salmo trutta*) and four rainbow trout (*Oncorhynchus mykiss*) strains to the myxozoan *Tetracapsuloides bryosalmonae*, the causative agent of proliferative kidney disease (PKD). Vet. Parasitol. **165**, 200–206.

Gusev, A. V., Dubinina, M. N., Raikova, E. V., Khotenkovskiy, I. A., Pugachev, O. N. and Ergens, R. (1985): Monogenea. In: Bauer, O. N. (ed.). Key to the Parasites of the Freshwater Fish Fauna of the USSR. Vol. **2**. Nauka, Leningrad. pp. 1–425.

Halverson, M. A. (2008): Stocking trends. A quantitative review of governmental fish stocking in the United States, 1931 to 2004. Fisheries **33**, 69–75.

Hines, R. S. and Spira, D. T. (1974): Ichthyophthiriasis in the mirror carp, *Cyprinus carpio* (L.). V. Acquired immunity. J. Fish. Biol. **6**, 373.

Hoffman, G. (1999): Parasites of North American Fishes. Comstock Publishing Associates, Ithaca, New York. 539 pp.

Johansen, L.-H., Jensen, I., Mikkelsen, H., Bjørn, P.-A., Jansen, P. A., Bergh, Ø. (2011): Disease interaction and pathogens exchange between wild and farmed fish populations with special reference to Norway. Aquaculture **315**, 167–186.

Jurajda, P., Bednařík, A., Bartáková, V., Mendel, J., Jurajdová, Z. and Mikl, L. (2020): Genetic structure of brown trout (*Salmo trutta*) populations in selected localities of the Krkonoše National Park [in Czech]. Opera Corcon. **57**, 95–106.

Kohout, J., Jašková, I., Papoušek, I., Šedivá, A. and Šlechta, V. (2012): Effects of stocking on the genetic structure of brown trout, *Salmo trutta*, in Central Europe inferred from mitochondrial and nuclear DNA markers. Fish. Manag. Ecol. **19**, 252–263.

Kvach, Y., Ondračková, M., Janáč, M. and Jurajda, P. (2016): Methodological issues affecting the study of fish parasites. I. Duration of live fish storage prior to dissection. Dis. Aquat. Organ. **119**, 107–115.

Littlewood, D. T. J. (1994): Molecular phylogenetics of cupped oysters based on partial 28S rRNA gene sequences. Mol. Phylogenet. Evol. **3**, 221–229.

Littlewood, D. T. J., Rohde, K. and Clough, K. A. (1997): Parasite speciation within or between host species? – Phylogenetic evidence from site-specific polystome monogeneans. Int. J. Parasitol. **27**, 1289–1297.

Malmberg, G., Collins, C. M., Cunningham, C. O. and Jalali, B. (2007): *Gyrodactylus derjavinoides* sp. nov. (Monogenea, Platyhelminthes) on *Salmo trutta trutta* L. and *G. derjavini* Mikailov, 1975 on *S. t. caspius* Kessler, two different species of *Gyrodactylus* – combined morphological and molecular investigations. Acta Parasitol. **52**, 89–103.

Moravec, F. (2001): Checklist of the Metazoan Parasites of Fishes of the Czech Republic and the Slovak Republic (1873–2000). Academia, Prague. 168 pp.

Moravec F. (2013): Parasitic Nematodes of Freshwater Fishes of Europe. Revised second edition. Academia, Prague. 601 pp.

Nilsen, H., Olsen, A. B., Vaagnes, O., Hellberg, H., Bottolfsen, K., Skjelstad, H. and Colquhoun, D. J. (2011): Systemic *Flavobacterium psychrophilum* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum), farmed in fresh and brackish water in Norway. J. Fish. Dis. **34**, 403–408.

Page, L. M. and Burr, B. M. (2011): A Field Guide to Freshwater Fishes of North America North of Mexico. Houghton Mifflin Harcourt, Boston. 663 pp.

Paladini, G., Longshaw, M., Gustinelli, A. and Shinn, A. P. (2017): Parasitic diseases in aquaculture: their biology, diagnosis and control. In: Austin, B. and Newaj-Fyzul, A. (eds) Diagnosis and Control of Diseases of Fish and Shellfish. John Wiley & Sons, Ltd., Chichester. pp. 37–107.

Palíková, M., Navrátil, S., Čížek, A., Soukupová, Z., Lang, Š., Kopp, R. and Mareš, J. (2014): Seasonal occurrence of diseases in salmonid recirculation system in the Czech Republic. Acta Vet. Brno **83**, 201–207.

Palíková, M., Papežíková, I., Marková, Z., Navrátil, S., Mareš, J., Mareš, L., Vojtek, L., Hyršl, P., Jelínková, E. and Schmidt-Posthaus, H. (2017): Proliferative kidney disease in rainbow trout (*Oncorhynchus mykiss*) under intensive breeding conditions: Pathogenesis and haematological and immune parameters. Vet. Parasitol. **238**, 5–16.

Peeler, E. J. and Murray, A. G. (2004): Disease interaction between farmed and wild fish populations. J. Fish. Biol. **65**, 321–322.

Peeler, E. and Thrush, M. (2004): Qualitative analysis of the risk of introducing *Gyrodactylus salaris* into the United Kingdom. Dis. Aquat. Organ. **62**, 103–113.

Pillay, T. V. R. (1995): Aquaculture Principles and Practices. Wiley-Blackwell, Oxford. 640 pp.

Pinter, K., Epifanio, J. and Unfer, G. (2019): Release of hatchery-reared brown trout (*Salmo trutta*) as a threat to wild populations? A case study from Austria. Fish. Res. **219**, 105296.

Pojezdal, L., Adámek, M., Syrová, E., Steinhagen, D., Minářová, H., Papežíková, I., Seidlová, V., Reschová, S. and Palíková, M. (2020): Health surveillance of wild brown trout (*Salmo trutta fario*) in the Czech Republic revealed a coexistence of proliferative kidney disease and piscine orthoreovirus-3 infection. Pathogens **9**, 604.

Prati, S., Henriksen, E. H., Knudsen, R. and Amundsen, P. A. (2020a): Seasonal dietary shifts enhance parasite transmission to lake salmonids during ice cover. Ecol. Evol. **10**, 4031–4043.

Prati, S., Henriksen, E. H., Knudsen, R. and Amundsen, P. A. (2020b): Impacts of ontogenetic dietary shifts on the food-transmitted intestinal parasite communities of two lake salmonids. Int. J. Parasitol. Parasites Wildl. **12**, 155–164.

Pravdová, M., Kolářová, J., Grabicová, K., Mikl, L., Bláha, M., Randák, T., Kvach, Y., Jurajda, P. and Ondračková, M. (2021): Associations between pharmaceutical contaminants, parasite load and health status in brown trout exposed to sewage effluent in a small stream. Ecohydrol. Hydrobiol. **21**, 233–243.

Reavill, D. and Roberts, H. (2007): Diagnostic cytology of fish. Vet. Clin. North Am. Exot. Anim. Pract. **10**, 207–234.

Roberts, R. J. (2005): Bacteria from fish and other aquatic animals: a practical identification manual. J. Fish. Dis. **28**, 627.

Sarker, S., Kallert, D., Hedrick, R. and El-Matbouli, M. (2015): Whirling disease revisited: Pathogenesis, parasite biology and disease intervention. Dis. Aquat. Organ. **2**, 155–175.

Scott, W. B. and Scott, M. G. (1988): Atlantic Fishes of Canada. University of Toronto Press, Scholarly Publishing Division. ISBN-13: 978-0802057129. 730 pp.

Seidlová, V., Syrová, E., Minarova, H., Zukal, J., Baláž, V., Němcová, M., Papežíková, I., Pikula, J., Schmidt-Posthaus, H., Mareš, J. and Palíková, M. (2021): Comparison of diagnostic methods for *Tetracapsuloides bryosalmonae* detection in salmonid fish. J. Fish. Dis. (in press). https://doi.org/10.1111/jfd.13375.

Stoltze, K. and Buchmann, K. (2001): Effect of *Gyrodactylus derjavini* infections on cortisol production in rainbow trout fry. J. Helminthol. **75**, 291–294.

Valladão, G. M. R., Gallani, S. U. and Pilarski, F. (2016): South American fish for continental aquaculture. Rev. Aquac. **10**, 1–19.

Všetičková, L., Adámek, Z., Rozkošný, M. and Sedláček, P. (2012): Effects of semi-intensive carp pond farming on discharged water quality. AIeP **42**, 223–231.