Vol. 155: 103–108, 2023
https://doi.org/10.3354/dao03744

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published August 31

NOTE

Application of UV-C irradiation prevented a severe outbreak of proliferative kidney disease in rainbow trout aquaculture

Christopher Naas[1,*], Alexander Kappe[2], Heike Schmidt-Posthaus[3], Markus Lichtenecker[4]

[1]Potsdam Institute of Inland Fisheries, Im Königswald 2, 14469 Potsdam, Germany
[2]The Thuringian State Office for Consumer Protection, Department 5, Tennstedter Straße 8/9, 99947 Bad Langensalza, Germany
[3]Institute for Fish and Wildlife Health, Department for Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Länggassstrasse 122, 3012 Bern, Switzerland
[4]Themar Fischzuchtanlagen GmbH, Steinlache 4, 98660 Themar, Germany

ABSTRACT: There is an urgent need to establish protocols on how to protect salmonids in aquaculture from outbreaks of proliferative kidney disease (PKD). For this purpose, systems for a continuous application of peracetic acid (PAA, 0.1 mg l^{-1}) and of ultraviolet C light (UV-C, 323.5–158.6 mW s cm^{-2}) were installed in the inlet of raceway-channels within a sub-unit of a commercial rainbow trout *Oncorhynchus mykiss* farm. After 127 d of rearing, a fish health examination was conducted. Fish in the control and PAA treatment groups showed signs of PKD. In contrast, fish in the UV-C treatment group showed almost no signs of disease based on clinical examinations and necropsy. This observation indicates that UV-C irradiation could be a promising tool to protect fish from PKD in the future.

KEY WORDS: Proliferative kidney disease · PKD · Rainbow trout · *Oncorhynchus mykiss* · UV-C · Peracetic acid · PAA · Aquaculture

1. INTRODUCTION

Trout aquaculture in flow-through systems using surface waters is facing many different challenges. One particular issue are outbreaks of proliferative kidney disease (PKD). This can result in impaired fish health and high mortalities (Hedrick et al. 1984, Ghittino et al. 2003), which subsequently reduces fish welfare and aquaculture output.

Around 100 yr ago, PKD was described for the first time (Plehn 1924). The cause of the disease is the myxozoan parasite *Tetracapsuloides bryosalmonae*, which requires 2 different hosts in its life cycle: a vertebrate host (salmonid fish) and an invertebrate host (freshwater bryozoans). The spores are released by infected bryozoans in surface waters and enter the aquaculture facility together with the inflowing water. Here, parasite spores penetrate the gill epithelium of the host and disperses with the blood stream in order to reach the main target organ, the kidney (Grabner & El-Matbouli 2010). Infected fish show signs of anemia (Ghittino et al. 2003), such as pale gills and livers

*Corresponding author: christopher.naas@ifb-potsdam.de

Publisher: Inter-Research · www.int-res.com

(Hedrick et al. 1984, 1986, 1993), and kidney enlargement due to a proliferative and necrotizing interstitial nephritis with intralesional parasites (Hedrick et al. 1986, Bettge et al. 2009a). Clinical PKD outbreaks, including fish mortality, occur from May to September when water temperatures reach 15°C and higher (Ghittino et al. 2003). However, infections can also take place at lower temperatures (Gay et al. 2001).

There is no available treatment for PKD-infected fish (Ghittino et al. 2003, Ros et al. 2022). Thus, aquaculturists have to implement preventive measures to protect their fish stocks. One option to inactivate fish pathogens is to continuously treat the inflowing water of the fish farms. Peracetic acid (PAA) is considered an environmentally friendly product that has been used for parasite control in salmonid flow-through aquaculture (Pedersen & Henriksen 2017). Whether or not continuous PAA application could protect rainbow trout from PKD remains currently unknown. Another option is to treat the inflowing water with ultraviolet light of the C-band (UV-C). Even though UV light is recommended as a preventive measure against PKD (Hedrick et al. 1986), there are no studies available indicating efficiency, UV-C intensity or dosage necessary for the inactivation of *T. bryosalmonae*.

This experiment investigated the continuous use of PAA and UV-C light as treatment methods for flow-through aquaculture rearing rainbow trout. The investigations were conducted under field conditions in a fully operating fish farm.

2. MATERIALS AND METHODS

In spring 2022, juvenile rainbow trout *Oncorhynchus mykiss* originating from a well-water hatchery were stocked in equal shares (110 kg biomass, 27.6 g body weight, 3985 ind.) in 3 identical raceways within a 21-channel subunit of a fully operating commercial trout farm. In the well-water hatchery, fish were regularly checked for pathogen presence; thus, no further diagnostics were performed on introduced fish. Each raceway had an effective production volume of 4 m^3 and was supplied with approx. 10 l s^{-1} surface water. Fish were hand-fed twice a day with aqua feed based on an operational protocol. Throughout the summer, the overall water supply of the sub-unit decreased from 190–207 to 67 l s^{-1} (FlowMate Model 2000, Marsh-McBIRNEY). At times of water shortage in mid June, a recirculating pump was activated. This pump was able to convey water at a rate of 120 l s^{-1} from the central outlet of the subunit back to the central inlet. Before entering the raceways, the mixed water (surface and pumped water) had to pass the corresponding treatment units. Basic parameters of the inflowing surface water were determined from grab samples once a month (n = 5). The pH-value ranged from 7.9 to 8.5, alkalinity from 0.7 to 1.4 mmol l^{-1}, turbidity from 2.4 to 9.3 formazin nephelometric units (FNU), conductivity from 231 to 427 µS cm^{-1}, ammonia from 0.022 to 0.035 mg l^{-1}, nitrite from below the detection limit (0.05 mg l^{-1}) up to 0.083 mg l^{-1} and nitrate between 6.9 to 9.4 mg l^{-1}. The chemical oxygen demand peaked once at 5.2 mg l^{-1}, and the spectral absorption coefficient (at 254 nm) of the filtered water samples (0.45 µm) varied between 3.8 and 4.9 m^{-1}. The minimum and maximum water temperatures determined every day at about 09:00 and 17:00 h in the inflow were 3.2 and 20.0°C, respectively. Recorded water temperature exceeded 15°C for the first time in May and in total for 91 out of 127 d. The lowest and highest oxygen concentrations measured were 8.1 and 14.3 mg l^{-1}, respectively.

To inactivate fish pathogens, the inflowing water of 1 raceway was treated continuously with PAA, 1 raceway was treated with UV-C-irradiation and 1 raceway was kept as a control without a continuous treatment. For the treatment with PAA, a stock solution with 24 l of the regular inflow water and 0.25 l of PAA (40% [w/v]-PAA product, Wofasteril classic, KESLA) was prepared every morning and was continuously administered by a peristaltic pump (DOSAFlex 2.0 l h^{-1}, DOSATRONIC GmbH) during the following 24 h. Thus, a nominal PAA concentration of 0.1 mg l^{-1} was applied, which is tolerated by rainbow trout (Liu et al. 2017, Pedersen & Henriksen 2017). To ensure a defined contact time and mixing of the inflowing water with the PAA solution, a pipe-based inlet structure was constructed. The structure with 30 l of volume allowed a contact time of about 3 s, between the PAA solution and inflowing water, before entering the raceway. PAA was controlled regularly by measuring the redox-potential (Multi 3510 IDS, SensoLyt®ORP 900-P, Xylem Analytics) of the water (Table 1).

For the treatment of the water with UV-C-light, an open channel unit (UMEX GmbH) was installed directly into the inlet of the raceway. The system was mounted on a polypropylene frame and included seven 50 W UV-C-lamps covered by quartz-glass tubes. To increase contact time between the water and the UV-C-irradiation, a baffle plate was installed at the outflow site of the UV-system. The water entered the unit in the upper quarter, and the baffle

Table 1. Redox potential (mV) of the water before and after peracetic acid (PAA) dosage and at the end of the raceway when applying a nominal PAA concentration of 0.1 mg l^{-1} at given water temperatures. N: number of redox potential measurements per date. Data in bold indicate time period during which a circulating pump was operated at a rate of approx. 120 l s^{-1}

Date (mo.yr)	Redox potential (mV) (mean ± SD) Inflow	After PAA	Outflow	Temp. (°C)	n
04.2022	283 ± 1	351 ± 1	359 ± 1	9.1–9.4	3
05.2022	248 ± 0.1	350 ± 0.5	354 ± 0.05	11.4–12.0	3
07.2022	**262 ± 3**	**328 ± 35**	**336 ± 7**	**15.5–17.4**	**9**
08.2022	**277 ± 3**	**363 ± 6**	**368 ± 7**	**16.6–17.7**	**6**

plate directed it down to the bottom. At the same time, the baffle plate provided an effective protection of the fish from UV-C light (Fig. 1). With an inflow of approx. 10 l s^{-1} and a UV-system's volume of 75 l, a hydraulic retention time of 7.5 s was realized in theory. The UV-C intensity (UV meter SXL 55, sglux GmbH; spectral range: 230–280 nm) and calculated UV-C dosage are displayed in Table 2 for the start and end of this field trial.

The water in the control group did not receive a continuous treatment. However, the fish farmer used 10 ml of PAA (40% [w/v]-PAA product, Wofasteril classic, KESLA) that was pre-diluted with water every 3 d for 3 consecutive days with the aim to reduce the pathogen burden and vitalize the fish.

At the end of the summer and after 127 d of rearing, an examination of the fish health was conducted by local authorities (The Thuringian State Office for Consumer Protection). For this purpose, 30 individuals from each of the 3 raceways were randomly selected and sacrificed for analyses by a blow to the head. Directly on site, 20 fish were macroscopically examined and a semi-quantitative classification of gill, liver, and kidney alterations ranging from 0 (no alterations) to 3 (severe diffuse alterations) was conducted independently by 2 persons. The remaining 10 fish were examined in a state laboratory (Thuringian State Office for Consumer Protection) by one person that worked at the fish farm. In addition to the above mentioned semi-quantitative classification of the macroscopic (mc) organ lesions, a histological examination (he) was performed, including the whole carcass (mc), heart (mc, he), spleen (mc), swim bladder (mc), intestine (mc), pyloric caeca (he), liver (mc, he), skin (mc), gills (mc, he), and trunk kidneys (mc, he). All organ samples were fixed in 10% buffered formalin, embedded in paraffin, stained with hematoxylin/eosin (H/E) and cut to 3 or 5 µm thickness. Qualitative assessment was performed at 100 and 400× magnification. For quantitative assessment of *Tetracapsuloides bryosalmonae* infestation of kidneys, 10 separate visual fields per individual fish were randomly chosen, and parasites were counted at 400× magnification.

Macroscopic data collected directly on the farm (n = 20) and in the laboratory (n = 10) were combined for presentation of results. For histological examinations, an n of 10 applies.

For the detection of *T. bryosalmone* DNA in the inflowing water, one water sample was taken on the evening before and a second sample on the day of fish health investigation. After homogenization, 500 ml of each sample were filtered through a series of 4 filters (Sartorius Microsart® @filter 100, 0.45 µm) and stored at −80°C until further investigation. The filters were transported overnight at frozen conditions to the

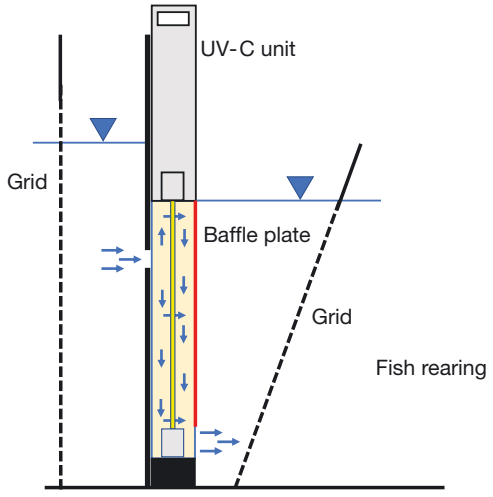

Fig. 1. Schematic drawing of the ultraviolet C light (UV-C) unit in the inlet of the raceway. Blue triangles and arrows indicate water level and flow, respectively. Baffle plate drawn in red

Table 2. Operating hours, ultraviolet C light (UV-C) intensity (mean ± SD, n = 7–8; measured directly in air at the midpoint of the quartz-glass tube of the second lamp on the left) and calculated UV-C dose of the unit (mean ± SD, based on inflow rate of 10 l s^{-1} and total volume of 75 l)

	Operation hours (h)	UV-C intensity (mW cm^{-2})	UV-C dose (mW s cm^{-2})
Start: 03.2022	0	43.3 ± 0.9	323.5 ± 6.7
End: 08.2022	3632	21.2 ± 0.3	158.6 ± 2.2

Institute of Fish and Wildlife Health (FIWI), University of Bern, Switzerland. At FIWI, DNA was extracted from the filters using DNeasy® Blood & Tissue Kit (QIAGEN) according to manufacturers' instructions with minor modifications (M. Stelzer et al. unpubl. data). DNA concentration and quality were checked with NanoDrop™ One UV-Vis Spectrophotometer (Thermo Fisher Scientific). All samples were examined by quantitative PCR (qPCR) according to Bettge et al. (2009b).

Applying established methods for pathogen reduction under site-specific conditions, the responsible authorities were informed about the project procedure (Thuringian State Office for Consumer Protection, File number: 2684-04-15-IFB-20-101). For diagnostic purposes, fish health was examined during a routine check of the fish farm by the authorities. Fish were raised and reared by the fish farmer following best practice.

3. RESULTS AND COMMENTS

Tetracapsuloides bryosalmone DNA was detected in the filter samples analyzed. This finding confirms the presence of the pathogen in surface water which supplies the aquaculture facility.

The macroscopic examination of the carcasses, as well as of heart, spleen, swim bladder and intestine revealed no abnormalities. Also, results of the histopathology of pyloric caeca did not indicate any changes (data not shown). In contrast, gills, livers and kidneys showed pathology.

Gill alterations, e.g. pale gills, were present in control animals and fish exposed to PAA treatment (Fig. 2A). Pale gills are a sign of anemia, a typical observation in PKD affected trout (Sudhagar et al. 2019). In contrast, gills of fish in the UV-C treatment group were macroscopically inconspicuous. Histologically, low to moderate epithelial proliferation with moderate diffuse necrosis of epithelial cells and mild infiltration with lymphocytes and macrophages, mainly in the basal parts of the lamella, were noted in all fish irrespective of the treatment.

Macroscopic liver alterations, consisting of anemia and yellowish discoloration were present in control and PAA treated fish but not in the UV-C group (Fig. 2B). Histologically, moderate periportal infiltrations with lymphocytes and macrophages were observed in control animals while only scattered inflammatory cells were present in animals of the PAA treatment group. In contrast, livers of fish originating from the UV-C treatment group were macroscopically and histologically inconspicuous.

In the control group, 87% of examined fish and 73% of fish in the PAA treatment group showed mild to severe kidney proliferation with multifocal nodular appearance (Fig. 3). Histopathology revealed mild to severe interstitial proliferation and infiltration with mainly macrophages and fewer lymphocytes. Between 1 and 3 *T. bryosalmonae* extrasporogonic stages were present per high power field in the control fish (Fig. 4), while 0 to 2 *T. bryosalmonae* were detected in fish from the PAA treatment group. These results indicate that a continuous application of 0.1 mg PAA l^{-1} can be considered insufficient to protect fish from PKD. In future, it should be assessed if higher PAA dosages or similar PAA concentrations at longer contact times are potentially able to inactivate the waterborne pathogens or prevent PKD-outbreaks, respectively.

In the UV-C treatment group, only 2 out of 30 individuals (7%) showed mild kidney swelling, while in histology one out of 10 individuals revealed mild renal interstitial proliferation and infiltration with inflammatory cells. Histologically, no *T. bryosalmonae*

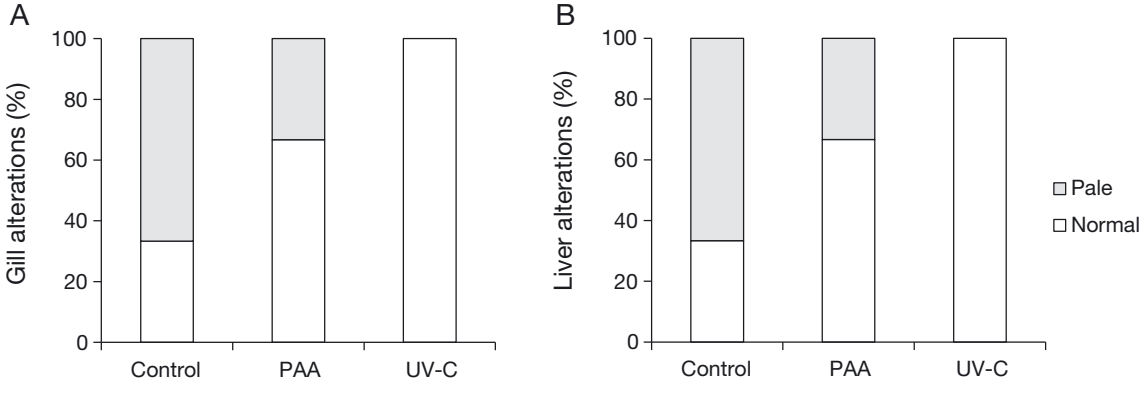

Fig. 2. (A) Gill and (B) liver alterations (normal vs. pale) of individual rainbow trout (n = 30) in the control, peracetic acid (PAA) and ultraviolet C light (UV-C) treatment groups

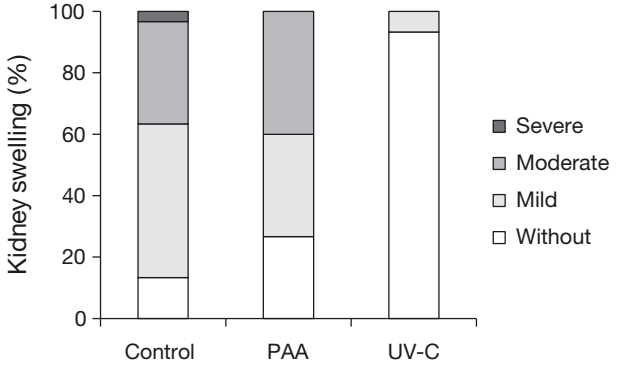

Fig. 3. Degree of kidney swelling of individual rainbow trout (n = 30) in the control, peracetic acid (PAA) and ultraviolet C light (UV-C) treatment groups

were found. Although this does not rule out subclinical infection, fish in the UV-C treatment group showed improved overall health status. A continuous UV-C treatment of the inflowing water seems be a promising tool to protect rainbow trout from suffering from clinical PKD. However, further research is needed to investigate suitability and applicability for aquaculture systems. Furthermore, it is still unclear, if fish from the UV-C treatment group are able to develop protective immunity.

UV-C irradiation is described as an effective tool to prevent infections by *Myxobolus cerebralis*, causing whirling disease in rainbow trout (Hedrick et al. 2000, 2007). UV dosages of 40 mW s cm^{-2} and higher resulted in a complete inactivation of waterborne actinospores (Hedrick et al. 2007). In our experiment, the UV-C dosage applied decreased from 323.5 to 158.6 mW s cm^{-2}. Even without considering the ultraviolet transmittance rate of the inflowing surface water, which will particularly vary in flow-through aquaculture, this UV-C dose range can be considered valuable for further PKD research and initial systems applications. However, there is definitely a need to determine the minimum UV-C dosage required for an effective PKD control in the future. This would help to determine pathogen targeting as well as potentially decrease acquisition and maintenance costs for UV-systems.

4. CONCLUSIONS

Whereas rainbow trout in control and PAA groups showed clinical signs of PKD, fish from the UV-C treatment group seemed to be largely unaffected by the disease. This is the first report showing that fish exposed to UV-C-irradiated *Tetracapsuloides bryosalmonae* spores did not or only faintly develop signs of PKD and that no parasitic cells were found in the

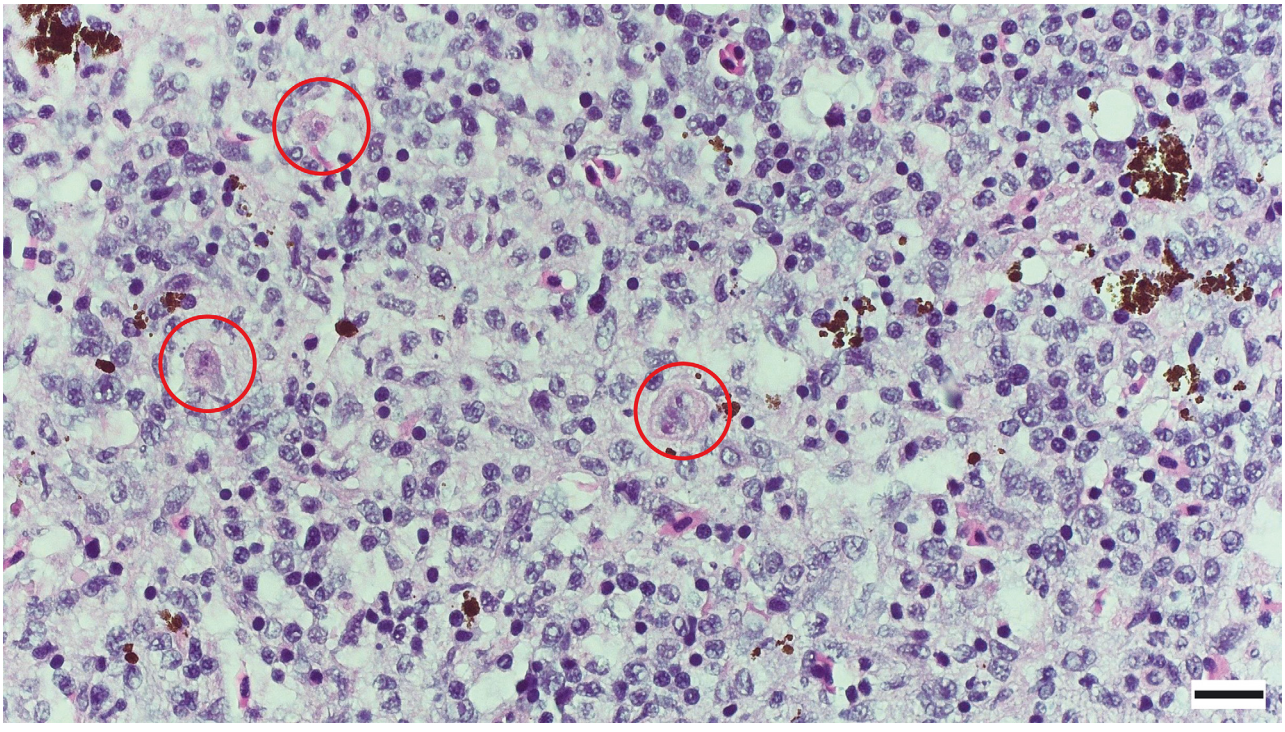

Fig. 4. Histological kidney section of a rainbow trout from the control group. Interstium is severely distended by infiltration with mainly macrophages and fewer lymphocytes and scattered *Tetracapsuloides bryosalmonae* (circled in red). H/E stain, magnification: 400×. Scale bar = 20 µm

kidney. Further research is needed to identify the minimal dosage for inactivation of spores in order to reduce acquisition and maintenance costs for UV-systems. Furthermore, other techniques such as ozone (cf. Summerfelt et al. 2008), ultrasound (Wolber & Pietrock 2004) or filtration (Hedrick et al. 1992) would be worthy of investigation.

Acknowledgements. The study was supported by the European Maritime and Fisheries Fund (EMFF) and the federal state of Thuringia (Ministry of Infrastructure and Agriculture) (Fund No.: 54-7932/23-19-72273/2021).

LITERATURE CITED

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

Bettge K, Segner H, Burki R, Schmidt-Posthaus H, Wahli T (2009b) Proliferative kidney disease (PKD) of rainbow trout: temperature- and time-related changes of *Tetracapsuloides bryosalmonae* DNA in the kidney. Parasitology 136:615–625

Gay M, Okamura B, de Kinkelin P (2001) Evidence that infectious stages of *Tetracapsula bryosalmonae* for rainbow trout *Oncorhynchus mykiss* are present throughout the year. Dis Aquat Org 46:31–40

Ghittino C, Latini M, Agnetti F, Panzieri C, Lauro L, Ciappelloni R, Petracca G (2003) Emerging pathologies in aquaculture: effects on production and food safety. Vet Res Commun 27:471–479

Grabner DS, El-Matbouli M (2010) *Tetracapsuloides bryosalmonae* (Myxozoa: Malacosporea) portal of entry into the fish host. Dis Aquat Org 90:197–206

Hedrick RP, Kent ML, Rosemark R, Manzer D (1984) Proliferative kidney disease (PKD) in Pacific salmon and steelhead trout. J World Maric Soc 15:318–325

Hedrick RP, Kent ML, Smith CE (1986) Proliferative kidney disease in salmonid fishes. US Fish Wildl Publ 136:1–11

Hedrick RP, Monge D, de Kinkelin P (1992) Transmission of PKX, the causative agent of proliferative kidney disease (PKD), to rainbow trout *Oncorhynchus mykiss* following filtration of water and sediments. Dis Aquat Org 14: 237–240

Hedrick RP, MacConnell E, De Kinkelin P (1993) Proliferative kidney disease of salmonid fish. Annu Rev Fish Dis 3:277–290

Hedrick RP, McDowell TS, Marty GD, Mukkatira K and others (2000) Ultraviolet irradiation inactivates the waterborne infective stages of *Myxobolus cerebralis*: a treatment for hatchery water supplies. Dis Aquat Org 42: 53–59

Hedrick RP, Petri B, McDowell TS, Mukkatira K, Sealey LJ (2007) Evaluation of a range of doses of ultraviolet irradiation to inactivate waterborne actinospore stages of *Myxobolus cerebralis*. Dis Aquat Org 74:113–118

Liu D, Straus DL, Pedersen LF, Meinelt T (2017) Pulse versus continuous peracetic acid applications: effects on rainbow trout performance, biofilm formation and water quality. Aquacult Eng 77:72–79

Pedersen LF, Henriksen NH (2017) Semi-continuously addition of peracetic acid to a flow-through fish farm. J Clean Prod 142:2606–2608

Plehn M (1924) Praktikum der Fischkrankheiten. Schweizerbart'sche Verlagsbuchhandlung (Erwin Nägele), Stuttgart. https://hvilested.dk/onewebmedia/ReolenTYSK/1924_PraktikumDerFischkrankheiten.pdf

Ros A, Schmidt-Posthaus H, Brinker A (2022) Mitigating human impacts including climate change on proliferative kidney disease in salmonids of running waters. J Fish Dis 45:497–521

Sudhagar A, Kumar G, El-Matbouli M (2019) The malacosporean myxozoan parasite *Tetracapsuloides bryosalmonae*: a threat to wild salmonids. Pathogens 9:16

Summerfelt S, Bebak-Williams J, Fletcher J, Carta A, Creaser D (2008) Description and assessment of the surface water filtration and ozone treatment system at the Northeast Fishery Center. Am Fish Soc Symp 61:000–000

Wolber JE, Pietrock M (2004) Ultrasonic water treatment as an alternative means of controlling fish mortality caused by *Bucephalus polymorphus* cercariae. Bull Eur Assoc Fish Pathol 24:153–160

Editorial responsibility: Thomas Braunbeck, Heidelberg, Germany
Reviewed by: 3 anonymous referees

Submitted: December 20, 2022
Accepted: June 14, 2023
Proofs received from author(s): August 28, 2023