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Preliminary investigation of an idiopathic muscle disease in farmed burbot *Lota lota*

Constanze Pietsch[1,2,*], Fridolin Tschudi[1], Stefan Baumann[3], Heike Schmidt-Posthaus[4]

[1]Institute of Natural Resource Sciences (IUNR), Zurich University of Applied Sciences (ZHAW), Grüental, PO Box, 8820 Wädenswil, Switzerland

[2]Institute for Agronomy, Hochschule für Agrar-, Forst- und Lebensmittelwissenschaften (HAFL), Applied University Bern (BFH), 3052 Zollikofen, Switzerland

[3]Basis57 Nachhaltige Wassernutzungs AG, Frauenmattweg 30, 6472 Erstfeld, Switzerland

[4]Centre for Fish and Wildlife Health (CFWH), Vetsuisse Faculty, University Bern, 3012 Bern, Switzerland

ABSTRACT: The rearing of less established fish species in recirculating aquaculture systems (RASs) is increasing, but may require adaptations of the rearing facilities if health impairments occur. We observed several health issues in burbot *Lota lota* reared for up to 2 yr in a RAS and used microbiological, histological and molecular-biological methods to identify the causative agents. Minor skin trauma led to the development of ulcers. In addition, several fillets of burbot showed pronounced granulomatous inflammation and calcification with signs of muscle fiber degeneration which resembled a condition called 'sandy flesh disease' in North American walleye. Several infectious agents were able to be excluded as a cause for the disease. *Carnobacterium maltaromaticum* was isolated in high numbers in some of the affected muscle tissue. However, the role of this bacterium or other causative agents or husbandry conditions remains to be elucidated.

KEY WORDS: Aquaculture · Rearing conditions · Skin alterations · Sandy flesh disease

1. INTRODUCTION

Burbot *Lota lota* are not commonly used in aquaculture, but their growth performance and flesh quality may contribute to their growing value in fish farming in the future. However, to date, rearing of burbot in recirculating aquaculture systems (RASs) has seldom been conducted.

The burbot is the only freshwater species in the Lotidae family. Its most prominent relative is the Atlantic cod *Gadus morhua*. The burbot is carnivorous and has a benthic and nocturnal lifestyle (Holzer et al. 2011), and its preference for bottom-dwelling and hiding behavior requires adaptations of standard rearing facilities used for more common freshwater aquaculture species. The present study focuses on the effects of these adapted rearing conditions on burbot health conditions.

Suboptimal husbandry conditions, causing e.g. swimming against pipes or boxes that have been supplied as hiding places, or fish handling such as grading, may cause mechanical skin damage that can lead to inflammatory responses and secondary infections with environmental microorganisms like bacteria or fungi, which can have severe consequences for the health of fish (Gonzalez et al. 2007). Superficial external fungal infections in fish have been shown to involve *Saprolegnia, Aphanomyces* and *Achlya* species (Richards & Pickering 1978, Singhal et al. 1987, Afzali et al. 2015) and, less often, *Fusarium* species (Cutuli et al. 2015). Co-infection with *F. oxysporum* and *Aeromonas hydrophila* has

*Corresponding author: pietsch.constanze@gmail.com

been reported in Nile tilapia *Oreochromis niloticus* (Cutuli et al. 2015). *Saprolegnia* infection may have lethal consequences for the affected fish (Lone & Manohar 2018). *Fusarium* infections may lead to isthmus and gill infection in fish (Kulatunga et al. 2017).

Suboptimal husbandry practices may also cause changes in subdermal tissues, such as skeletal muscle. Muscle changes have been reported in fish in relation to nutritional deficiencies (including vitamin imbalances and a lack of minerals), vaccine administration or viral diseases (Cowey et al. 1977, Miyazaki 1986, Coustans et al. 1990, Koppang et al. 2005, Garseth et al. 2018, Kotzamanis et al. 2018, Ruiz García et al. 2019).

In wild walleye *Stizostedion vitreum*, a disease known as myofibrogranuloma or sandy flesh disease (SFD) has been described (Holloway & Smith 1982, Holloway & Shoemaker 1993). SFD is a degenerative disease of the skeletal muscle of unknown etiology. Walleye with SFD generally show no external symptoms or abnormal behavior. The disease is characterized by severe alterations of the trunk musculature due to extensive hypertrophy of the muscle fibers. Two types of lesions have been described: coagulation necrosis of muscle fibers accompanied by granulomatous inflammation or a non-inflammatory process characterized by focal acute myolysis. Additionally, massive deposition of calcium occurs in affected muscle areas (55-fold increase of calcium compared to non-affected areas). Macroscopically, opaque, yellowish-brown muscle areas with a sandy texture can be seen in fish with advanced forms of the myopathy. SFD has been recognized to occur most frequently in mature specimens (Economon 1975) and is suspected to be of hereditary nature. So far, SFD is of concern primarily in the USA, particularly in North and South Dakota, Minnesota and Nebraska. However, this disease shows several similarities in macroscopic and histological appearance to the syndrome we describe here in burbot.

The aim of this study was to characterize a fillet lesion in aquaculture-raised burbot which influences the economic value of these animals and which could compromise the success of this new species in intensive aquaculture.

2. MATERIALS AND METHODS

2.1. Rearing conditions

The study was carried out at the facilities of Basis57 Nachhaltige Wassernutzungs AG, Switzerland, in pilot-scale cold-water RASs. These consisted of 11 fiberglass fish tanks (1.5 × 1.5 m, 2 m^3 each) discharging into a 30 micron drum filter (HDF 803, Hydrotech), upon which water entered a 600 W UV disinfection subunit (Kathadyn Group), followed by 3 serial 2 m^3 moving bed biofilters (each filled with 600 l IAS BioElements™) with degassing. Water was then pumped into a low-head oxygenator and flowed back into the fish tanks. The recirculation rate was 44 $m^3\ h^{-1}$, and flow into each tank was adjusted to the biomass. Temperature was set to 15 ± 1°C by adjusting the water exchange. The measurement and dosing of oxygen was automatically controlled (BlueBox, Go-Systemelektronik). Oxygen was kept at 100 ± 5% saturation by adding oxygen-enriched air (85% O_2) by pressure swing adsorption (OXYLINE, Diamond Lite). The electrical conductivity was kept between 400 and 500 µS cm^{-1} during normal operation, and increased to 8000 µS cm^{-1} (4 ppt NaCl) for some cases of salt treatment after handling. pH ranged between 7.0 and 7.3, depending on the water exchange rate. Daytime (8 h) light intensity was set at 1.92 ± 0.03 lx at the water surface und 0.17 ± 0.01 lx at the tank bottom (90 cm below the water surface). Nighttime (14 h in total) included 1 h each of 1 h linearly increasing and decreasing twilight and 12 h of complete darkness. Fish of different age classes (1 yr old fish hatched in March 2015, 2 yr old fish hatched in March 2014, 2 yr old fish hatched in March 2013) were sourced from a local hatchery with stock originating from Lake Constance and were used at different stocking densities and with different pipes (110 mm) and grids made of polyethylene. The stocking densities varied between 20 and 60 kg m^{-3}. Fish were fed ad libitum at intervals of 20 min during the first 6 h of nighttime with Aller Metabolica 4.5 mm (Emsland Aller Aqua). The feed intake was controlled by using feed traps installed in the outflow of each tank. Burbot showed very low activity at all different densities. Except during feeding events, burbot lay on the tank bottom or in the provided cavities on the tank wall. No unusual swimming behavior was detected.

2.2. Sampling of the fish

Fish fillets were collected during the normal slaughtering processes in 2015 to 2017 by ZHAW as well as by an external processor using electrical stunning for 5 min at 63 V AC with a 'Fish Stunner Mini' (FIAP) or a blow on the head and killing by a gill cut. The fillets were then stored on ice. Fillets showing macroscopic changes in texture and color were noted, and 3 whole fish (24 to 27 cm in length) and 9 separate fillets were sent to the Centre for Fish and

Wildlife Health (CFWH), Vetsuisse Faculty, University of Bern, for further analyses. Whole fish were inspected for external skin lesions.

2.3. Parasitology

Wet mounts of several randomly selected skin areas and small pieces of gills of all 3 examined whole fish were analyzed under the microscope to detect external parasites. Additionally, the gut contents of the 3 animals were analyzed by microscopy for intestinal parasites.

2.4. Bacteriology

Samples of skin, liver, spleen, kidney and musculature were sampled aseptically from all 3 fish independently using an inoculation loop and cultured on blood agar plates (Biomerieux), and on bromothymol blue-lactose-agar plates (Merck) supplemented with 0.5% sucrose for 48 h. Samples from each fillet were additionally cultured independently on blood agar and on bromothymol blue-lactose-agar plates. Skin and gill samples from all 3 animals were cultured independently on special agar plates to favor growth of flavobacteria (Anacker & Ordal 1959) for 5 d. Cultures were checked daily for bacterial growth for 48 h for blood agar plates and bromothymol blue-lactose-agar plates and for 5 d to screen for flavobacteria, respectively. Bacterial colonies were differentiated, the predominant cultures were identified, and subcultures were performed and identified to species level by the Institute for Bacteriology, University of Bern, using microflex-LT MALDI-TOF MS (Bruker Daltonics).

2.5. Histopathology

Skin, fillet and inner organs were investigated for macroscopic changes, and small pieces (1 × 1 cm) of liver, kidney and skin and several pieces of macroscopically changed muscle tissue were removed and immediately fixed in 10% buffered formalin for 24 to 48 h for histopathological examination. Fixed samples were paraffin-embedded and routinely processed for histological examination, and sections of 3 µm thickness were cut and stained with hematoxylin-eosin (H-E). The muscle sections were additionally stained with Gram, acid-fast stain (Ziehl Neelsen), Giemsa and silver stain (Grocott) to detect possible infectious agents. Additionally, muscle sections were stained with von Kossa and Congo Red to demonstrate mineralization and amyloid deposition, respectively (Armed Forces Institute of Pathology 1994). All slides were examined by light microscopy. The sum of histopathological changes of the muscle tissue (necrosis, granulomatous inflammation, fibrosis, deposition of amorphous material) was graded as 0 (no microscopic findings), 1 (minimal), 2 (mild), 3 (mild to moderate), 4 (moderate), 5 (moderate to severe) or 6 (severe).

2.6. PCR analyses

In order to detect a possible *Fusarium* spp. infection of the skin, ulcerated skin areas of 5 different individuals were sampled, and the genomic fungal DNA was isolated using a DNeasy Plant Mini Kit (Qiagen). Following the method of Hue et al. (1999), we used the primers f1 (5′-AGT ATT CTG GCG GGC ATG CCT GT-3′) and r1 (5′-ACA AAT TAC AAC TCG GGC CCG AGA-3′) for PCR at 10 µM concentrations each, yielding a PCR product of 329 bp identifying *Fusarium* spp. or closely related species (*F. moniliforme, F. solani, F. oxysporum, F. proliferatum*, *F. dimerum*, *F. semitectum, F. subglutinans*, *F. nivale* and *Neocosmospora vasinfecta*). Genomic DNA (4.3 to 9 ng DNA μl^{-1}) was used for PCR (Mastermix Sybr PCR kit, Qiagen), applying the following conditions: initial denaturation at 95°C for 15 min, followed by 40 cycles at 95°C for 15 s, 58°C for 30 s and 72°C for 30 s. The obtained PCR products were mixed with Orange Gel and transferred to a 1% agarose gel containing Gel Red (20 µl in 50 ml gel). Separation of PCR products was achieved by gel electrophoresis for 30 min at 80 V. Thereafter, the PCR products were visualized using UV light, extracted from the agarose gel using a QIAquick Gel Extraction kit (Qiagen), Sanger-sequenced (Microsynth AG, Balgach, Switzerland) and blasted in GenBank (BLAST-n, NCBI, www.ncbi.nlm.nih.gov).

In order to detect a possible infection with *Mycobacterium* sp., two 20 µm sections of paraffin-embedded material of muscle tissue showing granuloma were prepared. Each section was deparaffinized and lysed, and DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's protocol. Samples were incubated with Proteinase K at 56°C overnight in a shaking incubator. The DNA yield was determined by spectrophotometry using the NanoDrop photometer (NanoDrop Technologies). Conventional PCR was performed

using HotStarTaq DNA Polymerase (Qiagen) according to the manufacturer's instructions. Amplification of a 200 to 300 bp portion of the 16S-23S spacer was performed with primers Sp1 (5′-ACC TCC TTT CTA AGG AGC ACC-3′), whereby AAGGA corresponds to the beginning of the spacer sequence, and Sp2 (5′-GAT GCT CGC AAC CAC TAT CCA-3′) (Roth et al. 2000). A positive control sample (*M. marinum*, 218 bp) obtained from confirmed cases in a group of guppies and a negative control using water were included in the PCR procedure. To confirm the specificity of the PCR products, they were purified with WIZAR RD®SV Gel and the PCR Clean-Up System (Promega). The products were checked on a 1.5% agarose gel for amplification and molecular weight. In parallel, histological material of 3 individuals was deparaffinized and lysed, and DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) and analyzed by PCR in order to allow further fungal species identification. For this, kapa polymerase (Kapa Biosystems, South Africa) was used according to the manufacturer's instructions. To analyze the beta tubulin region and the internal transcribed spacer region of the ribosomal RNA of ascomycetes, we included the previously published primer pairs Bt2 (5′ GGT AAC CAA ATC GGT GCT GCT TTC 3′ and 5′ ACC CTC AGT GTA GTG ACC CTT GGC 3′) and the primers ITS3 (5′ GCA TCG ATG AAG AAC GCA GC 3′) and ITS4 (5′ TCC TCC GCT TAT TGA TAT GC 3′) according to Glass & Donaldson (1995) and White et al. (1990). The PCR products were visualized on a 2% agarose gel containing GelRed™ (Biotium, obtained from Chemie Brunschwig) after electrophoresis for 40 min at 80 V. The PCR products were sent for sequencing by Sanger cycle sequencing/capillary electrophoresis (Microsynth AG, Switzerland). The sequencing results were compared to known sequences by BLAST-n based on a search in the GenBank database (NCBI).

3. RESULTS

3.1. Incidence and macroscopic appearance of skin and muscle lesions

Skin ulcers were usually localized at the head (Fig. 1) and sides of the body (Fig. 1), characterized by hemorrhagic, round depressed areas of up to 10 mm in diameter. In some cases, more extensive and deeper ulcers could be observed on the body (Fig. 1). In the fillet underlying the skin ulcers, irregular areas were present, replacing up to 70% of the normal fillet musculature with a yellow discoloration and a gritty texture (Fig. 2).

Fig. 1. Gross appearance of ulcers in different locations on the skin of burbot

Fig. 2. Macroscopic appearance of sandy flesh disease in 2 burbots in December 2016. Note the focal extensive rough, yellow to brownish-colored areas, with a gritty appearance (indicated by the asterisks). Each fillet weighted approximately 900 g

Table 1. Incidence of sandy fillets (sandy flesh disease, SFD) in burbot in different sampling batches in 2016 and 2017, recorded by an external processor

Sampling date	Age (mo) at slaughter (hatch year)	Incidence of SFD (%)	No. fish processed batch^{-1}
22.06.2016	27 (2014)	9.2	87
02.08.2016	29 (2014)	22.5	102
17.08.2016	17 (2015)	15.5	58
07.09.2016	18 (2015)	0	91
14.09.2016	18 (2015)	0	63
20.09.2016	19 (2015)	9.1	77
14.11.2016	21 (2015)	7.6	66
28.11.2016	33 (2014) and 21 (2015)	9.7	62
12.12.2016	34 (2014) and 22 (2015)	15.9	63
12.12.2016	34 (2014) and 22 (2015)	24.0	25
19.12.2016	34 (2014)	8.1	148
19.12.2016	34 (2014) and 22 (2015)	11.4	35
01.02.2017	23 (2015)	22.3[a]	67
01.02.2017	23 (2015)	8.1[b]	70

[a]In fish with a mean weight of 750 g; [b]In fish with a mean weight of 450 g

These changes were first detected in 2015, where 2% (6 out of 309) of the fish (hatched in 2013) were affected, and musculature changes were only observed in burbot older than 2 yr. Body condition in all animals was normal.

In 2016, in addition to older fish (hatched in 2014), 1.5 yr old animals that hatched in 2015 were affected (Table 1). Incidence reached a level of 10.3% with a total number of 114 affected individuals reported by one processing company. Seven fillets of affected fish (approximately 900 g each) were sent to the CFWH for histology. An association between age at slaughtering or sex and incidence of lesions was not observed.

3.2. Parasitology and bacteriology

No parasites were detected on skin, gills and in the intestine. Bacteriological analyses of inner organs revealed no growth of bacteria. Skin samples of the 3 animals examined showed severe growth of mixed bacterial culture including *Aeromonas hydrophila*. *A. hydrophila* was considered a secondary pathogen, as it is frequently isolated in the environment and can easily colonize pre-damaged skin areas (Austin & Austin 2007). Culture of affected muscle tissue revealed the presence of moderate numbers of *Carnobacterium maltaromaticum* colonies in 2 of the 7 fillets after 24 h of cultivation. No flavobacteria were identified on skin or gills.

3.3. Histopathology

Histology revealed no pathology in liver and kidney. Skin lesions were characterized by multiple severe ulcers. The underlying dermis was multifocally necrotic with multiple hemorrhages of various sizes. In several fish, the dermis was infiltrated with high numbers of macrophages, and fewer lymphocytes and plasma cells (Fig. 3d). In adjacent areas, the epidermis was hyperplastic with reduced numbers of goblet and alarm cells.

Macroscopically affected muscle tissue was examined histologically and showed several well to poorly circumscribed areas of necrosis and granulomatous inflammation. Affected muscle fibers were hypertrophic, multifocally replaced by eosinophilic to amphophilic amorphous material (necrosis) (Fig. 3a,b) and adjacent calcification which was confirmed by von Kossa and Congo Red staining (Fig. 3c). The necrotic areas were surrounded by high numbers of mainly macrophages and fewer lymphocytes. Special staining (Gram, Ziehl Neelsen, Giemsa and silver) did not reveal any infectious agents in the affected areas. The von Kossa and Congo Red stains revealed no specific staining for the presence of amyloid (data not shown).

3.4. Presence of *Fusarium* spp. or *Mycobacterium* sp. DNA in ulcerated skin areas

PCR revealed the presence of *Fusarium* DNA in skin taken from ulcerated areas (Fig. 4). However, on histological material, no fungal material could be identified by PCR and subsequent sequencing.

The PCR for *Mycobacterium* sp. showed a weak band in one of the fillets showing signs of SFD on the agarose gel. The subsequent sequencing revealed an 88% match to *Micrococcus luteus*.

4. DISCUSSION

Although burbot culturing is promising in RAS, here we report a syndrome affecting muscle tissue that results in a complete loss in value of the affected fillets. Since affected muscle tissue is only detectable after deskinning of the fish, full production costs are incurred. Given that aquaculture has relatively low margins, losses in the late stages of production can be very severe for the producers. Beside adverse economic effects, skin changes of the fish may also indicate health problems. Mechanical damage can lead to skin ulcers, which become infected with secondary pathogens, like fungi and bacteria. Infections with *Fusarium* species have rarely been reported (Cutuli et al. 2015, Ke et al. 2016). Thus, we recommend characterizing skin infections in farmed fish in more detail in the future. Up to now, it has not been assumed that these fungal infections are responsible for skin or fillet changes, and further experiments are needed to fulfill Koch's postulates.

Co-occurrence of skin ulcers and muscle necrosis may lead to a higher susceptibility to infectious agents in already weakened animals. *Carnobacterium maltaromaticum* was isolated from examined fillets. However, the role of this bacterium in the development of the described lesions remains unclear. *C. maltaromaticum* are ubiquitous lactic acid bacteria, formerly known as *Lactobacillus piscicola* and *Carnobacterium piscicola* (Hiu et al. 1984, Collins et al. 1987, Mora et al. 2003). *C. maltaromaticum* is known to systemically infect lake whitefish

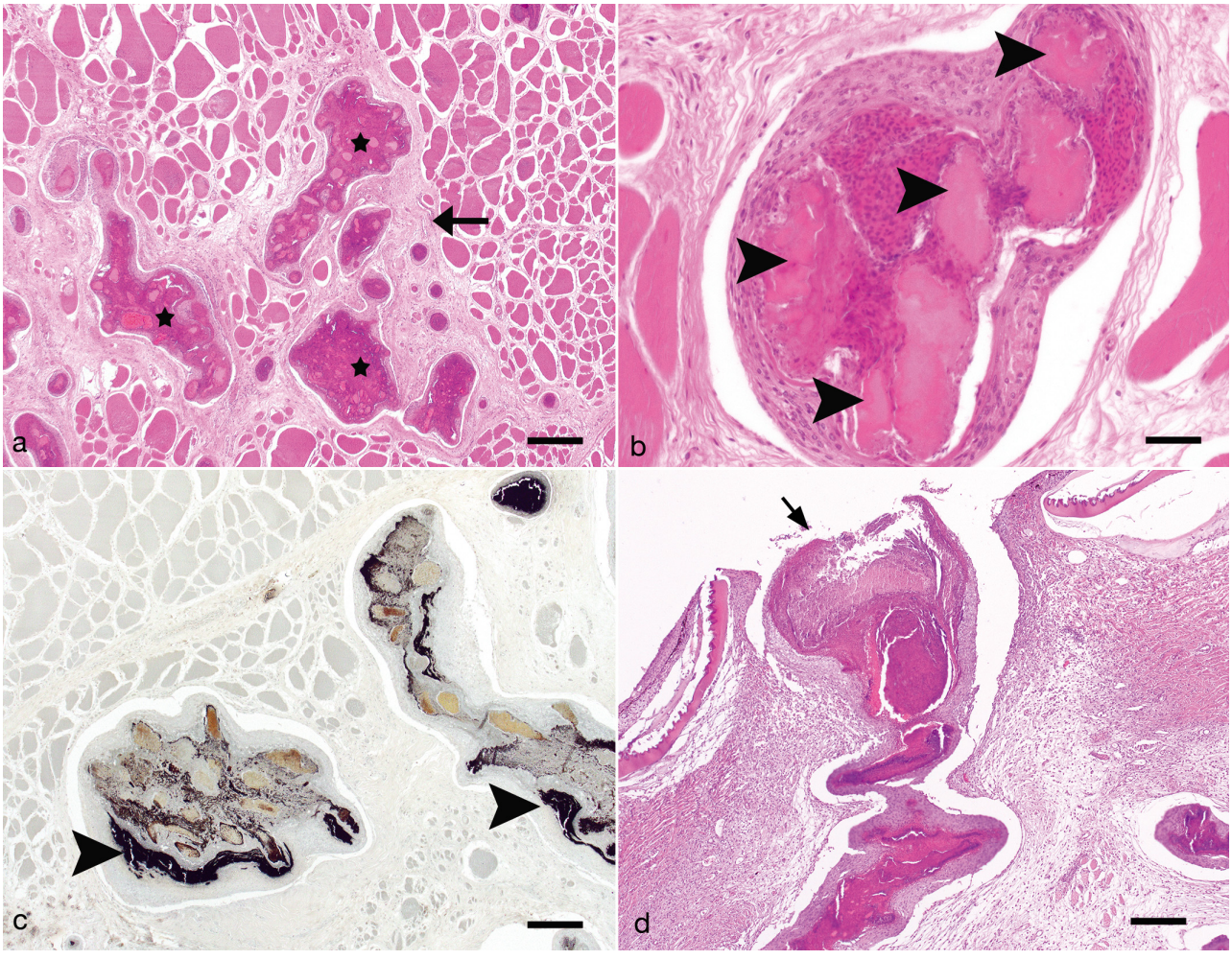

Fig. 3. Histological appearance of muscle sections of a burbot in 2015. (a) Several well circumscribed areas of necrosis and inflammation in muscular tissue. Affected muscle fibers multifocally replaced by eosinophilic to amphophilic amorphous material (stars), surrounded by a small rim of basophilic material (calcification) and a thick rim of fibrosis (arrow); HE stain. (b) Necrotic areas (arrowheads) surrounded by high numbers of mainly macrophages and fewer lymphocytes (granulomatous inflammation); HE stain. (c) Necrotic material shows no specific staining, whereas calcified areas present as black staining (arrowheads); Kossa stain. (d) Skin, focal ulceration with underlying necrotic and calcified areas (arrow). Scale pockets are dilated by edema and infiltration with inflammatory cells; dermis and subcutis are distended by severe infiltration with inflammatory cells and hemorrhage; HE stain. Scale bars = 100 µm (a,d), 25 µm (b), 50 µm (c)

Coregonus clupeaformis, whereby kidney, spleen and swim bladder are mostly infected, leading to congestion and hyperplastic swim bladder changes (Loch et al. 2008). Stressed fish seem to be more susceptible (i.e. associated with postspawning morbidity). In addition, these bacteria have also been found in salmonids (Cone 1982, Hiu et al. 1984, Loch et al. 2011), striped bass *Morone saxatilis* and brown bullhead catfish *Ameiurus nebulosus* (Baya et al. 1991). Changes described in these reports were acute, whereas burbot showed granulomatous muscle lesions with calcification indicating a chronic, long-lasting process.

Granulomatous inflammations may have different causes, such as mycobacterial infections, fungi or metazoans, or the exposure to heavy metals, feed binders and oil adjuvants in vaccines (Ferguson 2006). In our study, mycobacteria, fungi and parasites were investigated by special stains, but could not be demonstrated in the affected tissues. Mineral imbalance has also been reported to cause granulomas in multiple organs including epaxial skeletal musculature of Atlantic salmon *Salmo salar* (Good et al. 2016). These fish also showed nephrocalcinosis, but to exclude a bacterial cause, only *Mycobacterium* species were tested in that study.

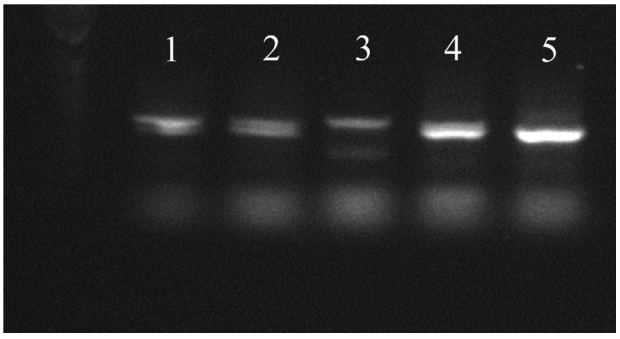

Fig. 4. Confirmation of *Fusarium* spp. DNA in the 5 samples originating from skin of different burbot (1–5), whereby strongly infected areas (samples 4 and 5) give stronger bands in the gel electrophoresis of the PCR products than less strongly infected locations (samples 1 to 3)

SFD and the resulting myopathy have up to now only been described for adult walleye as early as in the 1980s (Holloway & Smith 1982). Typical for the disease in walleye is that there are no gross signs in the musculature. Lakes in Nebraska have been reported to contain 20% of diseased walleyes, whereas the populations in other lakes were less affected. Based on the incidence findings, a genetic disposition for this syndrome was assumed, which also cannot be excluded for the burbot in our study since all fish originated from the same fish farm. In spawning walleye, muscle degeneration was found to be connected to increased serum calcium levels (Shoemaker & Holloway 1997). In addition to increased calcium levels, changed levels of other minerals have been observed in diseased fish (Kelly et al. 1987). However, it remains unclear if reduced mineral levels are the cause of disease or a secondary finding.

It is still unknown if this is a species-specific syndrome for burbot. Interestingly, the burbot rearing tanks were close to several tanks containing up to 3500 pikeperch *Sander lucioperca* since 2015, but none of the pikeperch showed any signs of skin ulcers or myopathies.

5. CONCLUSIONS

The rearing conditions for burbot should likely be further improved and standardized, since it cannot be excluded that the conditions selected for the present study favored the occurrence of skin damage and subsequent infections as well as the myopathy. On the skin, only *Fusarium* species were identified. The myopathy that occurred in the fillet of a number of fish may be related to the presence of *Carnobacterium maltaromaticum*, but further research is needed to support this hypothesis. Myopathy in burbot has an economic impact due to the need to discard affected fillets. Therefore, in order to establish healthy fish stocks, future research should be conducted to determine if this is an inherited disease or a disease that can be transmitted from one fish species to another.

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LITERATURE CITED

Afzali SF, Mohd Daud HH, Sharifpour I, Afsharnasab M, Shankar S (2015) Experimental infection of *Aphanomyces invadans* and susceptibility in seven species of tropical fish. Vet World 8:1038–1044

Anacker RL, Ordal EJ (1959) Studies on the myxobacterium *Chondrococcus columnaris*. I. Serological typing. J Bacteriol 78:25–32

Armed Forces Institute of Pathology (1994) Laboratory methods in histotechnology. American Registry of Pathology, Washington, DC

Austin B, Austin DA (2007) Bacterial fish pathogens: diseases of farmed and wild fish, 4th edn. Springer, Chichester

Baya AM, Toranzo AE, Lupiani B, Li T, Roberson BS, Hetrick FM (1991) Biochemical and serological characterization of *Carnobacterium* spp. isolated from farmed and natural populations of striped bass and catfish. Appl Environ Microbiol 57:3114–3120

Collins MD, Farrow JAE, Phillips B, Ferusu AS, Jones D (1987) Classification of *Lactobacillus divergens, Lactobacillus piscicola*, and some catalase-negative, asporogenous, rod-shaped bacteria from poultry in a new genus, *Carnobacterium*. Int J Syst Bacteriol 37:310–316

Cone DK (1982) A *Lactobacillus* sp. from diseased female rainbow trout, *Salmo gairdneri* Richardson, in Newfoundland, Canada. J Fish Dis 5:479–485

Coustans MF, Guillaume J, Metailler R, Dugornay O, Messager JL (1990) Effect of an ascorbic acid deficiency on tyrosinemia and renal granulomatous disease in turbot (*Scophthalmus maximus*) interaction with a slight polyhypovitaminosis. Comp Biochem Physiol Part A Physiol 97:145–152

Cowey CB, Knox D, Adron JW, George S, Pirie B (1977) The production of renal calcinosis by magnesium deficiency in rainbow trout (*Salmo gairdneri*). Br J Nutr 38:127–135

Cutuli MT, Gibello A, Rodriguez-Bertos A, Blanco MM, Villarroel M, Giraldo A, Guarro J (2015) Skin and subcutaneous mycoses in tilapia (*Oreochromis niloticus*) caused by *Fusarium oxysporum* in coinfection with *Aeromonas hydrophila*. Med Mycol Case Rep 9:7–11

Economon PP (1975) Myofibrogranuloma, a muscular dystrophy-like anomaly of walleye (*Stizostedion vitreum vitreum*). Special publication 113. Minnesota Division of Fish and Wildlife, Environment Section, St Paul, MN

Ferguson HW (2006) Systemic pathology of fish: a text and atlas of normal tissues in teleosts and their responses to disease, 2nd edn. Scotian Press, London

Garseth ÅH, Fritsvold C, Svendsen JC, Bang Jensen B, Mikalsen AB (2018) Cardiomyopathy syndrome in Atlantic salmon *Salmo salar* L.: a review of the current state of knowledge. J Fish Dis 41:11–26

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330

Gonzalez SF, Huising MO, Stakauskas R, Forlenza M and others (2007) Real-time gene expression analysis in carp (*Cyprinus carpio* L.) skin: inflammatory responses to injury mimicking infection with ectoparasites. Dev Comp Immunol 31:244–254

Good C, Marancik DP, Welch TJ, May T, Davidson J, Summerfelt S (2016) Systemic granuloma observed in Atlantic salmon *Salmo salar* raised to market size in a freshwater recirculation aquaculture system. Aquacult Res 47: 3679–3683

Hiu SF, Holt RA, Sriringanathan N, Seidler RJ, Fryer JL (1984) *Lactobacillus piscicola*, a new species from salmonid fish. Int J Syst Bacteriol 34:393–400

Holloway HL Jr, Shoemaker CA (1993) Humoral creatinine in walleye (*Stizostedion vitreum*). Great Basin Nat 53: 199–202

Holloway HL, Smith CE (1982) A myopathy in North Dakota walleye *Stizostedion vitreum* (Mitchill). J Fish Dis 5: 527–530

Holzer G, Unfer G, Gumpinger C, Hinterhofer, M, Guttmann S, Pinter K (2011) Der Verein 'Die Bewirtschafter' stellt den Fisch des Jahres 2011 vor: die Aalrutte (*Lota lota*). Österr Fisch 64:254–268

Hue FX, Huerre M, Rouffault MA, De Bievre C (1999) Specific detection of *Fusarium* species in blood and tissues by a PCR technique. J Clin Microbiol 37:2434–2438

Ke X, Lu M, Wang J (2016) Identification of *Fusarium solani* species complex from infected zebrafish (*Danio rerio*). J Vet Diagn Invest 28:688–692

Kelly RK, Klaverkamp JF, Hunt RV, Nielsen O (1987) Chemical analysis of muscle from walleye (*Stizostedion vitreum vitreum*) with myofibrogranuloma, a chronic myopathy. Can J Fish Aquat Sci 44:1425–1431

Koppang EO, Haugarvoll E, Hordvik I, Aune L, Poppe TT (2005) Vaccine-associated granulomatous inflammation and melanin accumulation in Atlantic salmon, *Salmo salar* L., white muscle. J Fish Dis 28:13–22

Kotzamanis Y, Kouroupakis E, Ilia V, Haralabous J and others (2018) Effects of high-level fishmeal replacement by plant proteins supplemented with different levels of lysine on growth performance and incidence of systemic noninfectious granulomatosis in meagre (*Argyrosomus regius*). Aquacult Nutr 24:1738–1751

Kulatunga DC, Dananjaya SH, Park BK, Kim CH, Lee J, De Zoysa M (2017) First report of *Fusarium oxysporum* species complex infection in zebrafish culturing system. J Fish Dis 40:485–494

Loch TP, Xu W, Fitzgerald SM, Faisal M (2008) Isolation of a *Carnobacterium maltaromaticum*-like bacterium from systemically infected lake whitefish (*Coregonus clupeaformis*). FEMS Microbiol Lett 288:76–84

Loch TP, Kumar R, Xu W, Faisal M (2011) *Carnobacterium maltaromaticum* infections in feral *Oncorhynchus* spp. (Family Salmonidae) in Michigan. J Microbiol 49: 703–713

Lone SA, Manohar S (2018) *Saprolegnia parasitica*, a lethal oomycete pathogen: demands to be controlled. J Infect Mol Biol 6:36–44

Miyazaki T (1986) A histopathological study on the carp fed α-tocopherol deficient diets including oxidized methyl linolate. Fish Pathol 21:73–79

Mora D, Scarpellini M, Franzetti L, Colombo S, Galli A (2003) Reclassification of *Lactobacillus maltaromicus* (Miller et al. 1974) DSM 20342T and DSM 20344 and *Carnobacterium piscicola* (Collins et al. 1987) DSM 20730T and DSM 20722 as *Carnobacterium maltaromaticum* comb. nov. Int J Syst Evol Microbiol 53:675–678

Richards RH, Pickering AD (1978) Frequency and distribution patterns of *Saprolegnia* infection in wild and hatchery-reared brown trout *Salmo trutta* L. and char *Salvelinus alpinus* (L.). J Fish Dis 1:69–82

Roth A, Reischl UDO, Streubel A, Naumann L and others (2000) Novel diagnostic algorithm for identification of mycobacteria using genus-specific amplification of the 16S-23S rRNA gene spacer and restriction endonucleases. J Clin Microbiol 38:1094–1104

Ruiz García MA, Hernández-Cruz CM, Caballero MJ, Fernández-Palacios H and others (2019) Incidence of systemic granulomatosis is modulated by the feeding sequence and type of enrichment in meagre (*Argyrosomus regius*) larvae. Aquacult Res 50:284–295

Shoemaker CA, Holloway HL Jr (1997) Effects of myofibrogranuloma on serum calcium levels in walleye (*Stizostedion vitreum*). Great Basin Nat 57:268–272

Singhal RN, Jeet S, Davies RW (1987) Experimental transmission of *Saprolegnia* and *Achlya* to fish. Aquaculture 64:1–7

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, NY, p 315–322

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