Aquaculture 532 (2021) 736048

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Does the thermal component of warm water treatment inflict acute lesions on Atlantic salmon (*Salmo salar*)?

L. Moltumyr [a,b,*], K. Gismervik [c], J. Gu [c], S.K. Gåsnes [c], T.S. Kristiansen [a], I. Rønnestad [b], J. Nilsson [a], L.H. Stien [a]

[a] Institute of Marine Research, PO Box 1870, Nordnes, NO-5817 Bergen, Norway
[b] Department of Biological Sciences, University of Bergen, PO Box 7803, NO-5020 Bergen, Norway
[c] Norwegian Veterinary Institute, PO Box 5695, Torgarden, NO-7485 Trondheim, Norway

ARTICLE INFO

Keywords:
Delousing
Injuries
Tissue damage
Welfare indicator scoring
Histopathological examination

ABSTRACT

Warm water treatment, i.e. exposure to sea water at a temperature of 28–34 °C for 20–30 s, has in recent years been widely used for delousing of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) in Norwegian aquaculture. High mortality and various lesions (e.g. injuries and/or bleedings in skin, fins, eyes, brain, and gills) have, however, been reported after industrial warm water treatments. The objective of this study was to reveal whether the thermal component of warm water treatment inflicts acute lesions on Atlantic salmon. The study was conducted by exposing individual, sedated Atlantic salmon post-smolts ($\overline{w} = 1117 \pm 250$ g) to sea water at a temperature of 34 °C (warm water treatment, $n = 40$) or 9 °C (control treatment, $n = 20$) for 30 s, and subsequently conducting welfare indicator scoring and histopathological examination of their skin, fins, eyes, snout, nasal pits/mucosa, palate, gills, thymus, pseudobranch, brain, heart, liver, kidney, pyloric caeca, pancreas, and spleen. The results showed that the prevalence and severity of acute lesions were not significantly different between the two treatment groups, except for higher prevalence of injuries on the caudal ($p = 0.002$), dorsal ($p = 0.002$), and right pelvic fins ($p = 0.014$) in the warm water treatment group. The main cause of these fin injuries may have been a strong behavioural reaction displayed by the fish when exposed to warm water. Possible consequences of fin injuries, the use of anaesthetic, and statistical limitations were discussed. It was concluded that exposure of Atlantic salmon to sea water at a temperature of 34 °C for 30 s did not lead to any statistically significant change in the prevalence of acute lesions except an increase in minor, possibly behaviour-related, fin injuries. Detection of a lower lesion prevalence than was possible in this study, but which may concern many individuals in an industrial setting, requires examination of a larger number of fish.

1. Introduction

Warm water treatment has in recent years been widely used for delousing of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) in Norwegian aquaculture (Overton et al., 2018), despite effects on fish welfare not being adequately documented (Noble et al., 2018; Hjeltnes et al., 2019). There are different warm water treatment systems (e.g. Thermolicer® from Steinsvik, Inc., Førresfjorden, Norway, and Optilice® from Optimar, Inc., Valderøy, Norway), but the treatments mainly follow the same procedure: the fish are crowded in the sea cage and pumped past a dewatering strainer into a treatment chamber where they are exposed to sea water at a temperature of 28–34 °C for 20–30 s (Holan et al., 2017; Noble et al., 2018). Although water temperatures in this range are lethal within 10 min for Atlantic salmon, the fish can survive such temperatures for shorter periods of time (Elliott, 1991; Elliott, 2010; Elliott and Elliott, 2010; Nilsson et al., 2019). The delousing mechanism is assumed to be that the lice, due to their smaller size, are heated more rapidly to a harmful temperature than their hosts and detach from the fish (Brunsvik, 1996; Grøntvedt et al., 2015; Holan et al., 2017).

The delousing effect and impact on fish welfare from warm water treatment have been assessed by independent research institutions for the Thermolicer® (Grøntvedt et al., 2015) and the Optilice® (Roth, 2016) systems in the developmental stage of the technologies. In both reports, it is concluded that the respective systems are effective and safeguard fish welfare. The assessment of the final systems includes,

* Corresponding author.
E-mail address: Lene.Moltumyr@hi.no (L. Moltumyr).

https://doi.org/10.1016/j.aquaculture.2020.736048
Received 20 March 2020; Received in revised form 9 October 2020; Accepted 13 October 2020
Available online 14 October 2020
0044-8486/© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

however, few test replicates, and aspects like possible pain, recovery time, and treatment frequency are not discussed. Nevertheless, the systems quickly came into industrial use as alternatives to chemical treatments were urgently needed due to development of drug resistance in the salmon louse populations (Aaen et al., 2015).

High mortality (Overton et al., 2018; Stien et al., 2018) and various lesions (Hjeltnes et al., 2018; Poppe et al., 2018; Hjeltnes et al., 2019) have been reported after industrial warm water treatments of salmonids, and concern has been raised that exposure to water at the temperatures used during the treatments is painful for the fish (Ashley et al., 2007; Nilsson et al., 2019).

Overton et al. (2018) reviewed the delousing methods for Atlantic salmon in Norwegian aquaculture and found that warm water treatments accounted for the largest increases in post-treatment mortality, followed by mechanical treatments and then chemical treatments with hydrogen peroxide, azamethiphos, deltamethrin, and cypermethrin, respectively. The post-treatment mortality was influenced by ambient sea water temperature, fish size, and pre-treatment mortality (Overton et al., 2018). Lesions reported in Atlantic salmon after industrial warm water treatments include skin wounds, scale losses, fin injuries, degeneration of nasal mucosa and bleedings in eyes, palate, gills, thymus, and brain (Hjeltnes et al., 2018; Poppe et al., 2018). Many of the reported lesions are in tissues and organs that are not standard sampling material in fish health examinations (i.e. nasal mucosa, thymus, palate, and brain) and that were not examined when Grøntvedt et al. (2015) and Roth (2016) assessed the respective warm water treatment systems. The reports of Hjeltnes et al. (2018) and Poppe et al. (2018) are, however, based on material from injured, sick, moribund, and dead fish sampled by fish health personnel after industrial warm water treatments, and not controlled studies. It is therefore uncertain whether all the various lesions are due to warm water treatment or if some lesions were already present before treatment.

Many of the components of warm water treatment can inflict lesions on fish. Crowding of the fish before pumping leads to stress and risk of hypoxic conditions (Skjervold et al., 2001; Oppedal et al., 2011; Erikson et al., 2016). The mechanical part of the treatment (crowding, pumping, straining, and transportation through the treatment systems) can lead to hard encounters with sharp bends and edges, shovels, other fish, etc., that may cause wounds, tears, and clamp and stroke injuries (Grøntvedt et al., 2015; Roth, 2016; Noble et al., 2018). Also, when exposed to warm water, salmonids display abnormal behaviours such as frequent direction changes and surface breaks, high swimming speeds, and collisions with enclosure walls, equipment, and other fish (Elliott, 1991; Elliott and Elliott, 1995; Ineno et al., 2005; Erikson et al., 2012; Roth, 2016; Nilsson et al., 2019). It has been discussed whether such collisions are the cause of many of the lesions observed after industrial warm water treatments (Hjeltnes et al., 2018). It is not known whether the warm water inflicts lesions per se.

Most of the understanding regarding the effects of acute water temperature change on salmonids, comes from studies where the fish are subjected to smaller and/or slower changes in temperature and/or longer exposure times than in industrial warm water treatment (e.g. DuBeau et al., 1998; Currie et al., 2000; Galloway and Kieffer, 2003; Hyvärinen et al., 2004; Fowler et al., 2009; Nakano et al., 2014). When fish acclimated to a specific temperature, or range of temperatures, are exposed to a rapid and large increase in water temperature, a so-called heat shock, a neuroendocrine stress response is initiated (Nakano et al., 2014). This stress response is characterised by an increase in stress hormones and consequent haematological, osmoregulatory, and metabolic changes (Wendelaar Bonga, 1997). Also, a cellular stress response is initiated: High temperature denatures proteins and can significantly perturb ligand binding and in consequence disrupt function (Somero, 1995). The appearance of damaged proteins triggers production of heat shock proteins that protect the cells against heat-induced damage (Hightower, 1991; Dietz and Somero, 1993; DuBeau et al., 1998).

In ectothermic animals like Atlantic salmon, the rate of internal body temperature change due to a rapid external temperature change empirically follows Newton's law of excess temperature, which states that the instantaneous rate of temperature change is proportional to the difference between the ambient temperature and the body temperature (Fry, 1967; Stevens and Fry, 1970, 1974). Dean (1976) found that when juvenile salmonids acclimated to a water temperature of 15 °C were abruptly transferred to water at 25, 27, 29, or 30 °C, their muscle mass required at least two minutes to attain 90% of the new temperature. Based on the relatively short exposure times used in warm water treatment (20–30 s), it seems reasonable to assume that potential lesions on external tissues and organs will be most relevant.

Mangor-Jensen et al. (2017) conducted a pilot laboratory trial where unanaesthetised Atlantic salmon post-smolts of 2–4 kg were exposed to sea water at 8, 20, 31, or 34 °C for 30 s and then recovered in sea water at 8–9 °C for 14 days. Analysis of blood electrolytes indicated a small stress response at the two highest water temperatures, but histological examination of mucus cells did not reveal any statistically significant changes in cell size or density. Gismervik et al. (2019) studied acute thermal injuries in unanaesthetised Atlantic salmon post-smolts ($\overline{w}$ = 234 ± 52 g) in a pilot laboratory trial, and found that exposure to sea water at 34–38 °C for 72–140 s caused injuries in the gills, eyes, brain, and possibly also the nasal cavity and thymus of the fish. Although salmonids with a body weight below one kilogram are rarely warm water treated in the aquaculture industry, this result implies that warm water treatment of Atlantic salmon at equal and somewhat higher water temperatures and longer exposure times than the current treatment standard (28–34 °C for 20–30 s), poses a serious risk to their health and welfare.

The objective of this study was to reveal whether the thermal component of warm water treatment (in this case 34 °C for 30 s) inflicts acute lesions on Atlantic salmon. Knowledge about this is important in order to identify the cause(s) of the high mortality and the various lesions that have been reported after industrial warm water treatments of salmonids.

2. Materials and methods

2.1. Ethics

The study was approved by the Norwegian Food Safety Authority (ID No. 15383) and adhered to the Norwegian Animal Welfare Act (Lovdata, LOV-2009-06-19-97) and the Norwegian Regulation on Use of Animals in Experiments (Lovdata, FOR-2015-06-18-761). In accordance with the 3Rs in humane experimental technique (Russell and Burch, 1959), the study was conducted with relatively few fish as warm water is aversive to Atlantic salmon (Nilsson et al., 2019) and the injury potential of the treatment was unknown.

2.2. Experimental animals and rearing conditions

The study included 60 unvaccinated, farmed Atlantic salmon post-smolts of mixed sexes (AquaGen® Atlantic QTL-innOva® PRIME strain, AquaGen, Inc., Trondheim, Norway). The fish were reared in indoor tanks at the Institute of Marine Research, Matre Research Station, Norway, and were first fed on the 22nd of February 2017. From the 1st of August 2017, the fish were subjected to a L12:D12 light regime for six weeks followed by a L24:D0 light regime for six weeks to become autumn smolts. On the 15th of September 2017, the fish were

transferred to an off-white, squared tank with rounded corners ($D = 1.5 \times 1.5 \times 0.8$ m, $V = 1350$ L, stocking density $\sim$ 259 fish m^{-3} until February 2018 when the density was reduced to ~111 fish m^{-3}). From the 1st of November 2017, the fish were kept in brackish water ($S \sim 25$ ppt, $T \sim 9$ °C) and subjected to a natural light regime. The fish were fed with dry feed (Spirit Supreme, Skretting, Inc., Stavanger, Norway) from a drum feeder during daytime. On the 6th of April 2018, the fish were evenly distributed into three tanks (41–42 fish per tank, stocking density $\sim$ 30 fish m^{-3}) with the same characteristics as the previous tank, but with full strength sea water ($S \sim 34$ ppt). The fish were fasted for two days before the experimental treatment. The treatment was conducted 16 months after the first feeding, when the fish had reached a mean body weight above one kilogram.

2.3. Experimental procedure

The study was conducted on the 26th of June 2018 at Matre Research Station. A two-stage sedation procedure was performed using Aqui-S® vet. (isoeugenol 540 mg mL^{-1}, Scan Aqua, Inc., Årnes, Norway). This anaesthetic has proven beneficial in reducing stress and adverse behaviour during warm water treatment of Atlantic salmon (Erikson et al., 2012; Adams, 2019). First, the fish were lightly sedated (stage 1, Schoettger and Julin, 1967) in the stock tanks by adding Aqui-S® vet. to a concentration of 3 μL L^{-1} of sea water (Fig. 1). Then, the fish were individually netted from the stock tanks with a knot-less, fine-meshed, and shallow hand net, and deeply sedated (stage 3a, Schoettger and Julin, 1967) in a rectangular cart ($D = 80 \times 68 \times 70$ cm, $V = 185$ L) containing Aqui-S® vet. at a concentration of 60 μL L^{-1} of sea water.

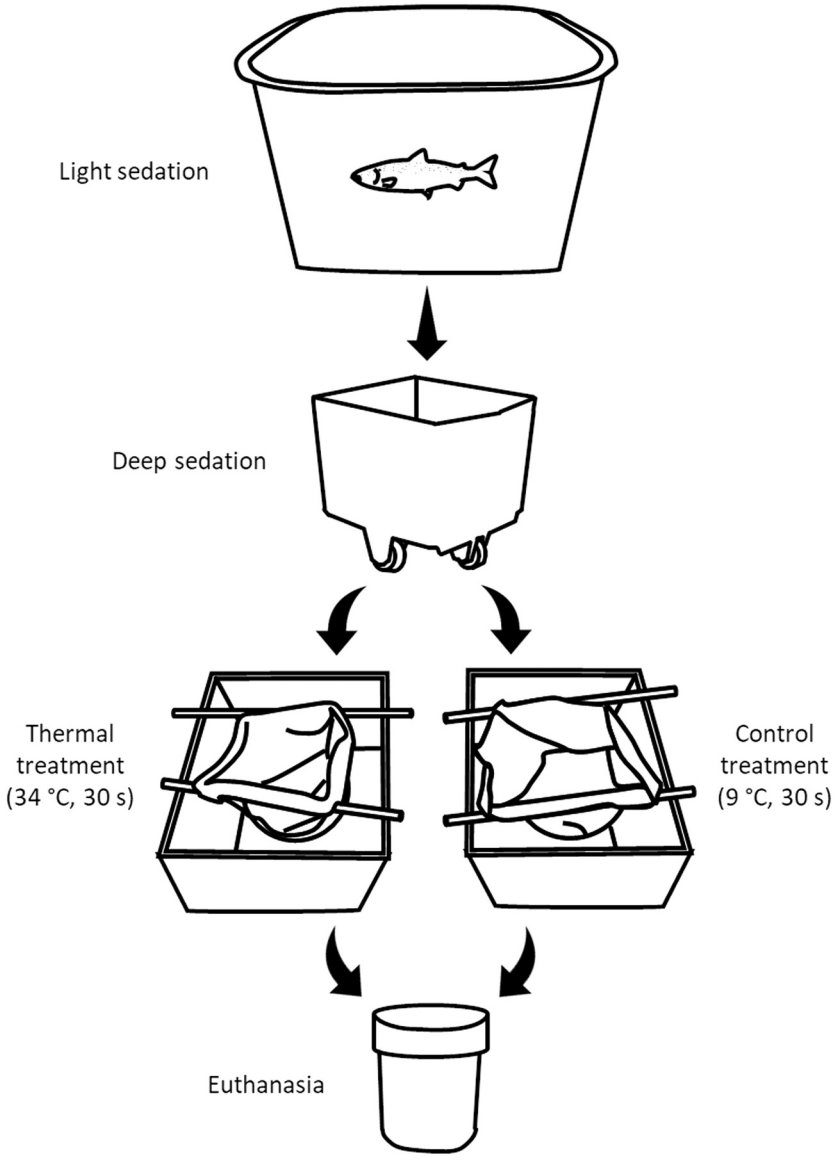

Fig. 1. Experimental procedure. After being lightly sedated in the stock tank, the fish were individually transferred to a cart for deeper sedation (stage 3a, Schoettger and Julin, 1967) before they were exposed to sea water at a temperature of 34 °C (warm water treatment) or 9 °C (control treatment) for 30 s. After exposure, the fish were immediately euthanised with an overdose of anaesthetic.

Thereafter, each fish was gently transferred by hand to a custom made, soft bag of non-slip fabric ($D = \varnothing$ ~60 × ~50 cm). The bag was submerged in a cart of similar type as the sedation cart, containing sea water at a temperature ± *SD* of either 34.0 ± 0.1 °C for warm water treatment or 9.2 ± 0.2 °C for control treatment. Immediately before each fish was introduced, the temperature (Testo® 176 T2 temperature data logger, Testo, Inc., Lenzkirch, Germany) and oxygen saturation (OxyGuard® Handy Polaris 2 oxygen meter, OxyGuard International, Inc., Farum, Denmark) of the water in the treatment carts were checked, adjusted if necessary, and manually logged. The mean oxygen saturation ± *SD* was 106 ± 6% in the warm water treatment cart ($n = 40$) and 109 ± 6% in the control treatment cart ($n = 19$). The order of exposure was: two fish (one at a time) in 34 °C water, one fish in 9 °C water, two fish in 34 °C water, and so on to a total of 60 fish (40 warm water treated fish and 20 control fish). The treatment bag was closed during exposure, and therefore direct observation of the fish was not possible. After 30 s in the treatment bag, the fish were transferred to a circular container ($D = \varnothing$ 43.5 × 39.7 cm, $V = 37.9$ L) containing Finquel® vet. (tricaine methanesulfonate 1000 mg g^{-1}, Scan Aqua, Inc., Årnes, Norway) at a concentration of 500 mg L^{-1} of sea water for euthanisation. The various tanks, carts, and containers were located next to each other to minimise the air exposure time of the fish. Caution was taken to handle the fish as gentle as possible to avoid inflicting lesions on the fish that were not directly caused by the treatment water.

2.4. Measurements and welfare indicator scoring

After euthanisation, the fish were gently lifted out of the container by hand and laid on a measuring board for weighing to the nearest gram and measuring of fork length to the nearest 0.1 cm. The mean body weight and fork length ± *SD* were 1137 ± 226 g ($n = 40$) and 47.8 ± 3.0 cm ($n = 40$) for fish in the warm water treatment group, and 1077 ± 293 g ($n = 20$) and 47.1 ± 4.2 cm ($n = 19$) for fish in the control group, respectively. Smooth and wet nitrile gloves were worn when handling the fish, and it was assured that the fish did not come into contact with irregular or absorbent surfaces.

After being measured, the fish were visually inspected for external, macroscopic lesions, and welfare indicators were scored. This was conducted by authorised fish health professionals who were blinded to treatment information on the fish. Skin bleedings, skin wounds, scale losses, fin injuries (all fins except the adipose fin), snout injuries, eye injuries/bleedings, gill bleedings, and gill paleness were scored from 0 to 3, denoting no, mild, moderate, and severe lesions, respectively (Grøntvedt et al., 2015; Gismervik et al., 2017; Noble et al., 2018). Eye opacity was scored from 0 to 4, where 0 = no, 1 < 10%, 2 = 10–50%, 3 = 50–75%, and 4 > 75% opaque covering of the eye (Wall and Bjerkås, 1999; Bass and Wall, 2008). This scoring schema was originally developed for cataracts, i.e. opacification of the lens, but it can be difficult by macroscopic inspection to decide whether the opaqueness is situated in the lens or in other parts of the eye (Gismervik et al., 2019). Total gill score, i.e. all macroscopic lesions on the gills, was quantified from 0 to 5, denoting no, very mild, mild, moderate, severe, and very severe lesions, respectively (Taylor et al., 2009; Grøntvedt et al., 2015; Persson et al., 2015). Bleedings in the nasal pits, palate, thymus, and brain were recorded as absent or present. Some welfare indicator scores for some fish were missing from the data set, and the exact number of fish at each score is therefore given in the figures.

2.5. Tissue and organ sampling

A full necropsy was conducted on all fish by authorised fish health professionals. Fish tissues and organs were macroscopically inspected, sampled, and fixed in a 10% phosphate-buffered formalin solution for later histopathological examination. The sampling included skin, skeletal muscle, fins, eyes, nasal mucosa, gills, thymus, pseudobranch, brain, heart, liver, kidney, pyloric caeca, pancreas, and spleen. All samples were taken from the left side of the fish if no visible lesions on the right side. Skin and skeletal muscle samples were taken ventrally to the dorsal fin by transverse section in the lateral line area, including both red and white muscle tissue. Due to time restraints, fin samples were only taken from those of the 30 last fish on which lesions were detected macroscopically. Gill samples were taken from the second gill arch, and kidney samples were taken from the mid-kidney.

2.6. Histopathological examination

Formalin fixed tissue and organ samples from 42 fish (28 warm water treated fish and 14 control fish) were prepared for histopathological examination by paraffin wax embedding and standard histological techniques (Bancroft and Gamble, 2008) at the Norwegian Veterinary Institute in Trondheim, Norway. The examination was limited to this number of fish due to time restraints. After staining with haematoxylin and eosin, the samples were examined for histopathological changes by means of light microscopy and, when appropriate, graded according to the categories «no remarks», «sparse», «moderate», and «pronounced». The examination was conducted by a veterinary pathologist who was blinded to treatment information on the fish.

2.7. Data analysis

Fisher's exact test was used for testing differences in welfare indicator scores and histopathological changes between the treatment groups. The tests were conducted using the «fisher.test» function in the «stats» package of RStudio® (version 1.1.463, R Core Team, 2019). The significance level was set at 0.05.

3. Results

3.1. Behaviour

Despite being deeply sedated (Section 2.3) and having lost their equilibrium, all fish in the warm water treatment group displayed a strong behavioural reaction (vigorous wriggling in the treatment bag) during treatment. Two fish even jumped immediately out of the treatment bag when introduced into the warm water. These fish were excluded from the study and replaced by excess fish from the stock tanks to a total of 60 correctly treated fish. All control fish remained calm throughout the treatment.

3.2. Welfare indicator scores

3.2.1. Skin

There were no statistically significant differences in the prevalence and severity of skin bleedings ($p = 1.00$) or scale losses ($p = 0.78$) between warm water treated fish and control fish (Fig. 2). No skin wounds were detected on the torso of any of the 60 examined fish.

3.2.2. Fins

Warm water treated fish had significantly higher prevalence of injuries on the caudal ($p = 0.002$), dorsal ($p = 0.002$), and right pelvic fins ($p = 0.014$) than control fish (Fig. 3b, c, and e). There were no statistically significant differences in the prevalence and severity of injuries on the anal fin ($p = 0.46$), the left and right pectoral fins ($p = 0.30$ and p

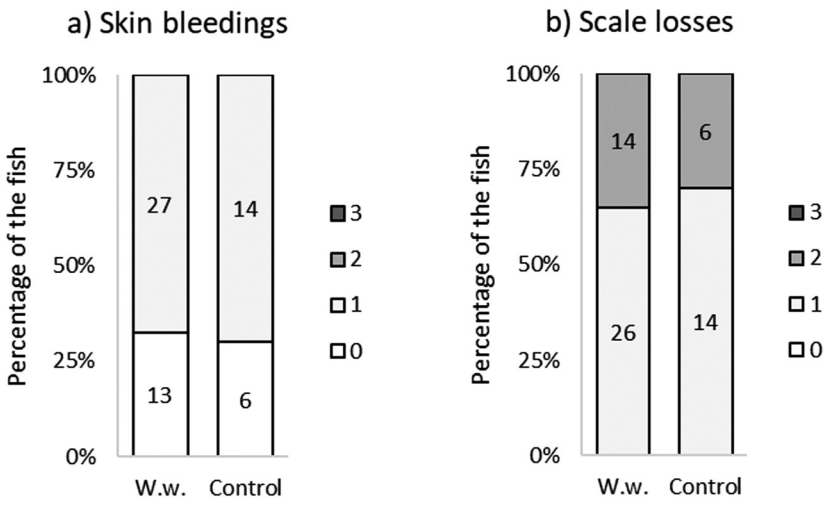

Fig. 2. Percentage of the fish with a) skin bleedings and b) scale losses in the warm water treatment group ($n = 40$) and the control group ($n = 20$). The legend indicates welfare indicator scores (0 = no, 1 = mild, 2 = moderate, 3 = severe lesions), and the digits inside the columns indicate the number of fish at each score.

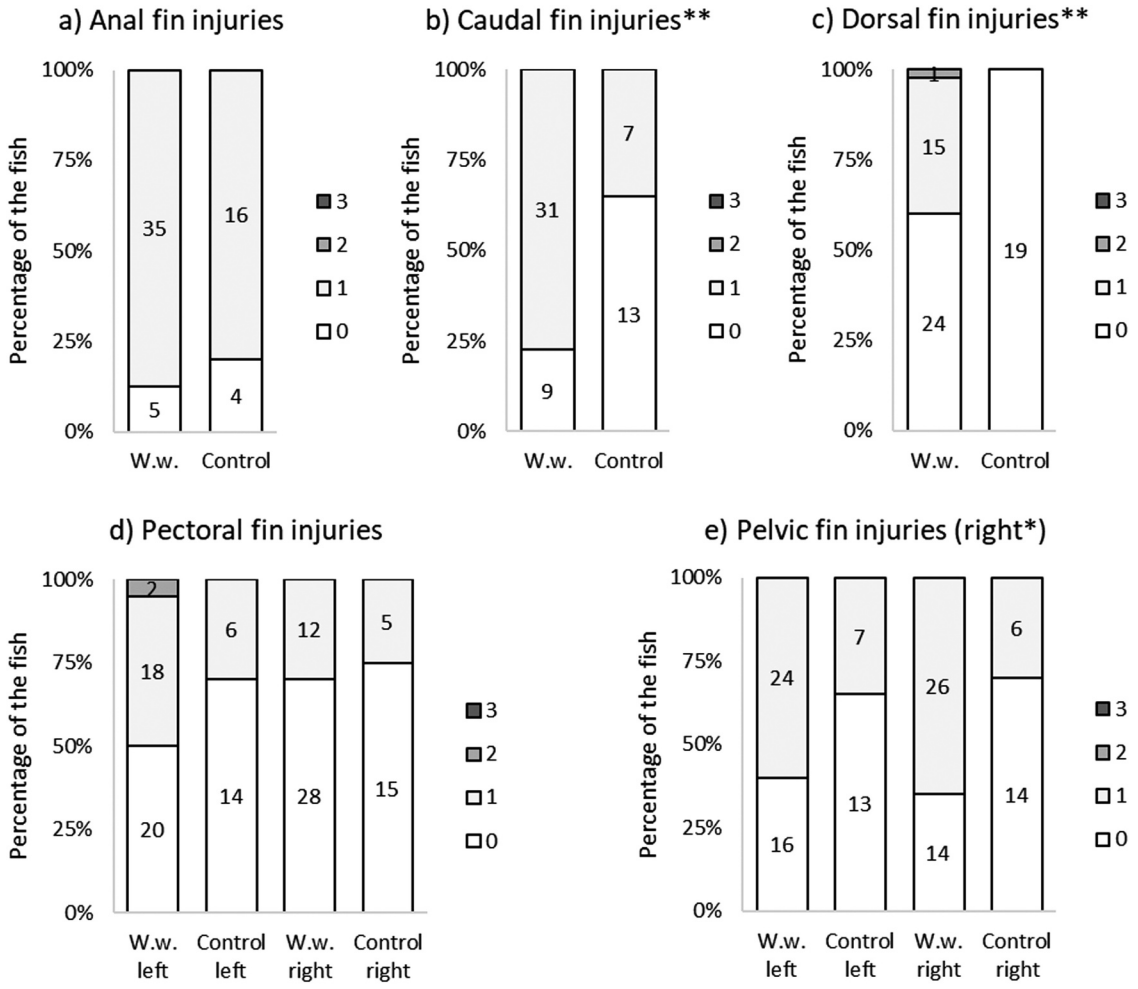

Fig. 3. Percentage of the fish with fin injuries in the warm water treatment group ($n = 40$) and the control group ($n = 20$ except for dorsal fin injuries where $n = 19$). The legend indicates welfare indicator scores (0 = no, 1 = mild, 2 = moderate, 3 = severe lesions), and the digits inside the columns indicate the number of fish at each score. Statistically significant differences are marked with asterisks, where *: $p \leq 0.05$ and **: $p \leq 0.01$.

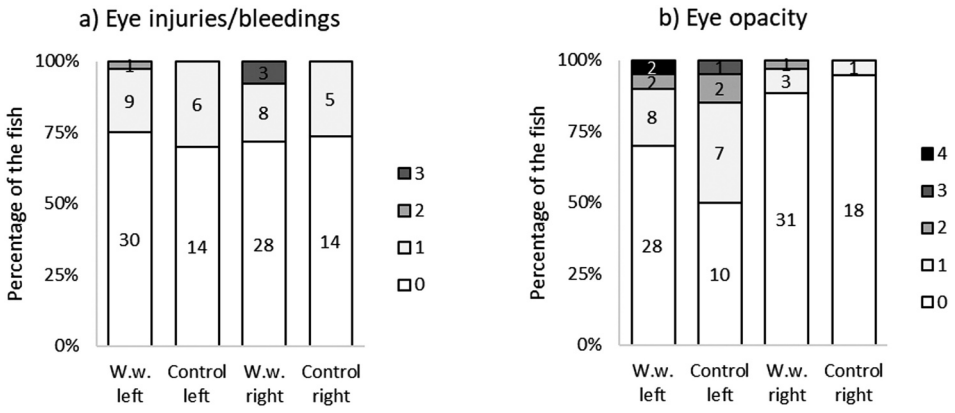

Fig. 4. Percentage of the fish with a) eye injuries/bleedings and b) eye opacity in the warm water treatment group and the control group. (Warm water left $n = 40$ and control left $n = 20$. Eye injuries/bleedings: warm water right $n = 39$ and control right $n = 19$. Eye opacity: warm water right $n = 35$ and control right $n = 19$.) The legend indicates welfare indicator scores (0 = no, 1 = mild, 2 = moderate, 3 = severe lesions for eye injuries/bleedings and 0 = no, 1 > 10%, 2 = 10–50%, 3 = 50–75%, 4 > 75% opaque covering of the eye for eye opacity), and the digits inside the columns indicate the number of fish at each score.

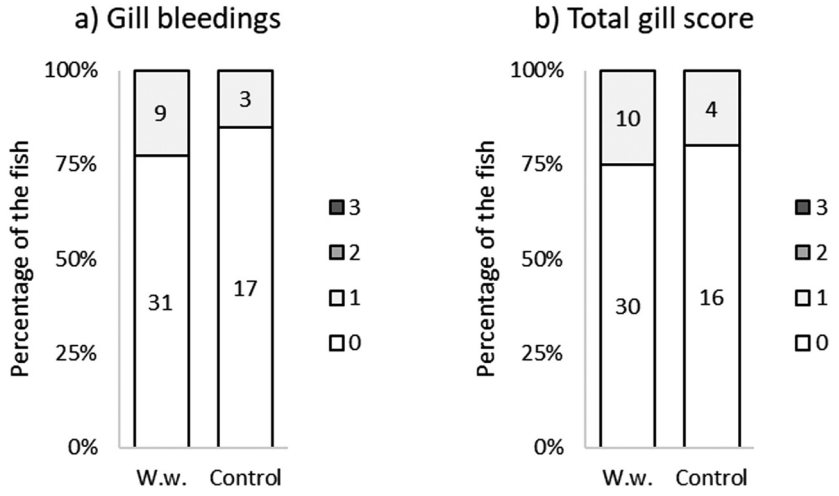

Fig. 5. a) Percentage of the fish with gill bleedings and b) macroscopically visible lesions (i.e. total gill score) in the warm water treatment group ($n = 40$) and the control group ($n = 20$). The legend indicates welfare indicator scores (0 = no, 1 = mild, 2 = moderate, 3 = severe lesions), and the digits inside the columns indicate the number of fish at each score.

= 0.77, respectively), and the left pelvic fin ($p = 0.10$, Fig. 3a, d, and e).

3.2.3. Snout, nasal pits and palate

There were no statistically significant differences in the prevalence and severity of snout injuries ($p = 0.30$) between the treatment groups, with 19 out of 40 (i.e. 48%) warm water treated fish and 6 out of 20 (i.e. 30%) control fish given score 2, and the remaining fish given score 1. No nasal pit bleedings or palate bleedings were detected among the 40 (39 for palate bleedings) warm water treated fish and the 20 control fish examined.

3.2.4. Eyes

There were no statistically significant differences in the prevalence and severity of eye injuries/bleedings (left eye: $p = 0.84$, right eye: $p = 0.61$) or eye opacity (left eye: $p = 0.22$, right eye: $p = 1.00$) between warm water treated fish and control fish (Fig. 4).

3.2.5. Gills

There were no statistically significant differences in the prevalence and severity of gill bleedings ($p = 0.73$) or macroscopically visible lesions ($p = 0.76$) between warm water treated fish and control fish (Fig. 5). One warm water treated fish had mildly pale gills (score 1).

3.2.6. Thymus and brain

There were no statistically significant differences in the prevalence of thymic bleedings ($p = 0.71$) between warm water treated fish (6 out of 40, i.e. 15%) and control fish (2 out of 20, i.e. 10%). No brain bleedings were detected among the 38 warm water treated fish and the 19 control fish examined.

3.3. Histopathological changes

Fisher's exact test did not reveal any statistically significant differences (all p-values >0.05) between warm water treated fish and control fish in the prevalence of any of the histopathological changes described in this section. Due to some samples not being analysable, the number of samples differ somewhat between tissues, and the exact number of samples in each treatment group is given in the text.

Skin samples from 27 warm water treated fish and 14 control fish were examined. In two of the warm water treated fish, there were partial loss of epidermis and sparse bleeding in scale pockets (Fig. 6a).

Fin samples from 10 warm water treated fish and 4 control fish

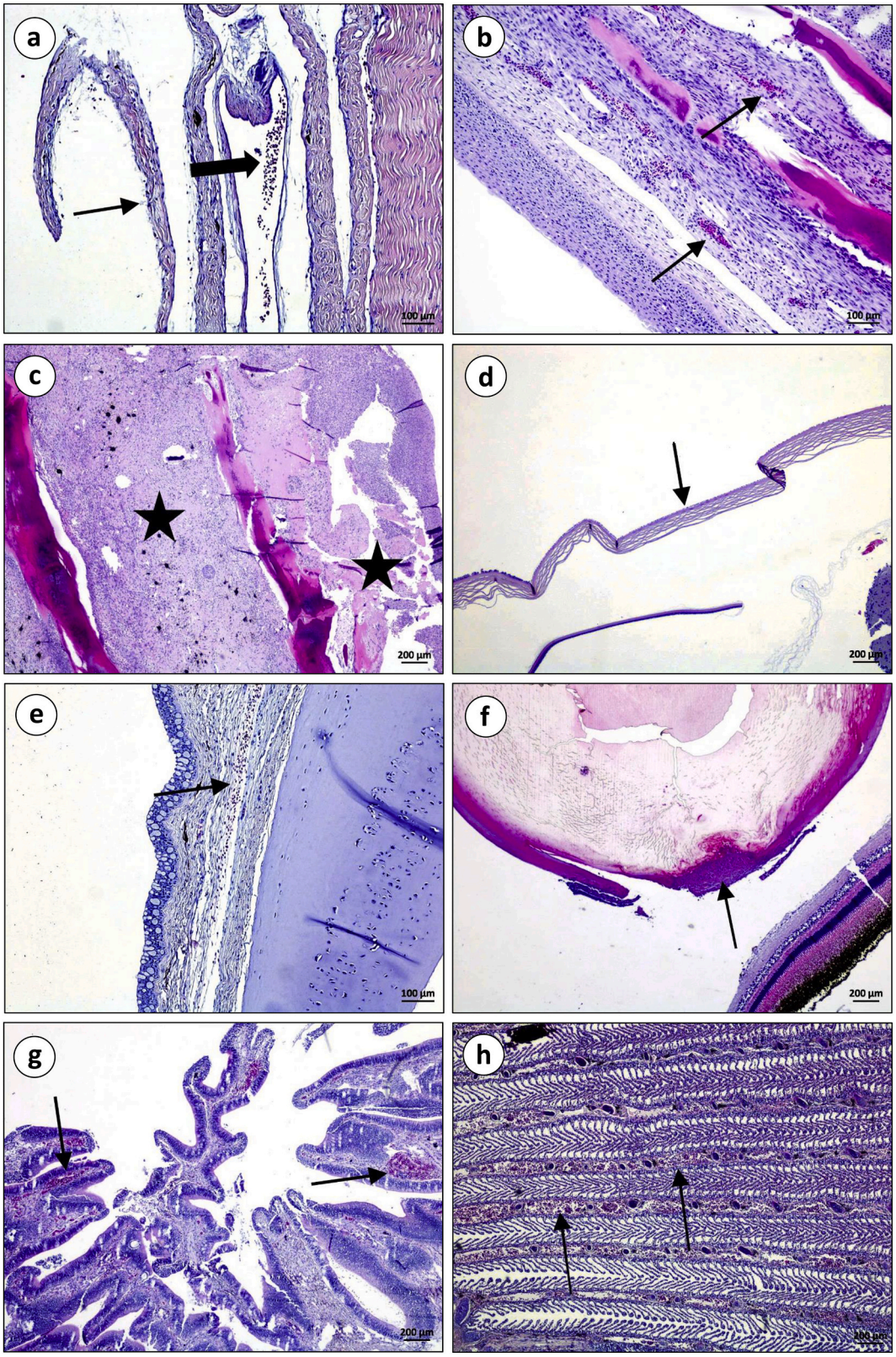

Fig. 6. Examples of histopathological changes in warm water treated fish and control fish. a) Loss of epidermis (thin arrow) and bleeding in scale pockets (thick arrow). b) Bleeding and congestion in fin (arrows). c) Granulomatous dermatitis in fin (stars). d) Loss of corneal epithelium (arrow). e) Bleeding in conjunctiva (arrow). f) Cataract (arrow). g) Bleeding and congestion in *lamina propria* of nasal mucosa (arrows). h) Congestion in central venous sinusoids in gills (arrow). i) Bleeding in thymus (arrow). j) Bleeding and congestion in pseudobranch (arrows). k) Bleeding in brain tissue (arrow). l) Bleeding in *stratum compactum* of cardiac ventricle (arrow). m) Bleeding in *bulbus arteriosus* (arrow). n) Bleeding in liver (arrow). o) Bleeding in lumen (thin arrow) and *lamina propria* (thick arrow) in collecting ducts in kidney.

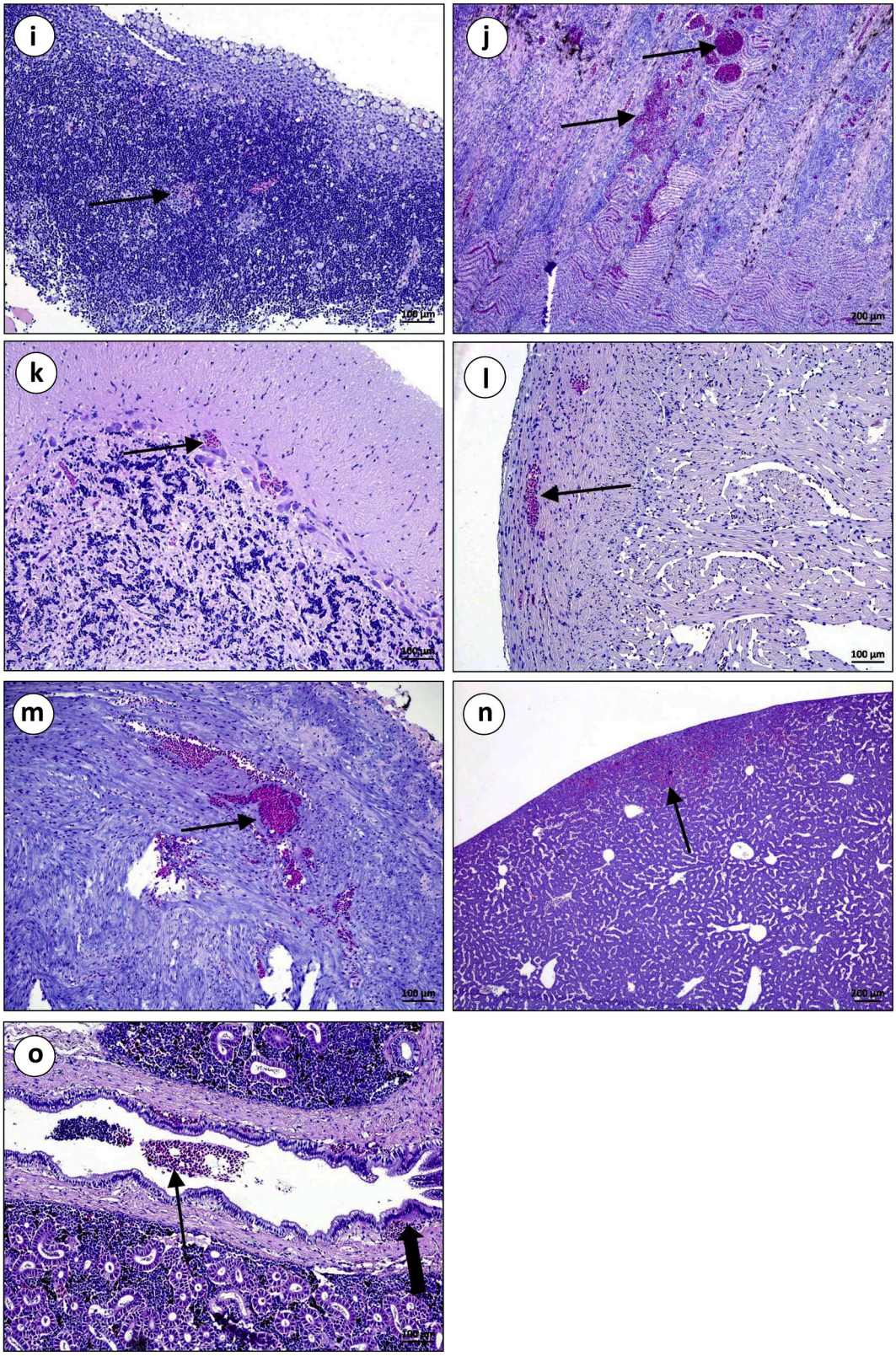

Fig. 6. (*continued*).

where lesions were detected macroscopically, were examined. In nine of the warm water treated fish and all four of the control fish, there were sparse to pronounced bleeding and/or congestion (Fig. 6b). In seven of the warm water treated fish and two of the control fish, there was also sparse to pronounced (granulomatous) dermatitis with or without epidermis and/or bone involved (Fig. 6c).

Eye samples from 28 warm water treated fish and 14 control fish were examined. In one of the warm water treated fish and one of the control fish, there were partial and pronounced loss of corneal epithelium, respectively (Fig. 6d). In one of the warm water treated fish, there

was sparse focal bleeding in the conjunctiva (Fig. 6e), and in another warm water treated fish, there were sparse pinpoint bleeding in the cornea and sparse cataract. Sparse cataract was also present in additionally three of the warm water treated fish and one of the control fish (Fig. 6f). In one of the warm water treated fish, there was pronounced keratitis (corneal inflammation) in which adjacent tissue was also involved. In three of the warm water treated fish and one of the control fish, there was sparse to moderate chronic keratitis.

Nasal mucosa samples from 19 warm water treated fish and 10 control fish were examined. In 13 of the warm water treated fish and all 10 of the control fish, there were sparse bleeding and/or congestion in the *lamina propria* (Fig. 6g). In further six of the warm water treated fish, the severity of this circulatory disturbance was moderate. In two of the warm water treated fish, there was sparse infiltration of inflammation cells in the nasal mucosa and submucosa, and in two of the control fish, there was focal inflammation of the nasal mucosa.

Gill samples from 28 warm water treated fish and 13 control fish were examined. In one of the warm water treated fish and one of the control fish, there was moderate congestion in central venous sinusoids (Fig. 6h). In all examined fish, there were sparse changes with varying numbers of lamellae with epithelial proliferation and inflammation.

Thymus samples from 27 warm water treated fish and 14 control fish were examined. In one of the warm water treated fish, there was a sparse, multifocal bleeding. In two of the warm water treated fish and five of the control fish, there were few or some bleeding foci (Fig. 6i).

Pseudobranch samples from 25 warm water treated fish and 13 control fish were examined. In two of the warm water treated fish, there were moderate and sparse bleeding and/or congestion in the lamellae, respectively (Fig. 6j).

Brain samples from 28 warm water treated fish and 14 control fish were examined. In nine of the warm water treated fish and four of the control fish, there were few bleeding foci in the meninges and/or the brain tissue (Fig. 6k).

Heart samples from 28 warm water treated fish and 14 control fish were examined. In two of the warm water treated fish, there was sparse multifocal and focal bleeding, respectively, in the *stratum compactum* of the ventricle (Fig. 6l). In another warm water treated fish, there was sparse focal bleeding in *bulbus arteriosus* (Fig. 6m). In ten of the warm water treated fish and five of the control fish, there was a varying extent of inflammation, melanisation, and/or malformation.

Liver samples from 28 warm water treated fish and 14 control fish were examined. In four of the warm water treated fish and three of the control fish, there was sparse focal bleeding along the edge of the liver (Fig. 6n). In six of the warm water treated fish and one of the control fish, there was sparse (fibrous) inflammation.

Kidney samples from 28 warm water treated fish and 14 control fish were examined. In nine of the warm water treated fish and three of the control fish, there was sparse bleeding in the lumen of the collecting ducts, whereas in one of the control fish, there was pronounced bleeding. In three of the warm water treated fish and one of the control fish with sparse bleeding inside the lumen, there was also sparse bleeding under the epithelium (*lamina propria*) of the collecting ducts (Fig. 6o).

Pyloric caeca and pancreas samples from 27 and 28 warm water treated fish, respectively, and 14 control fish were examined. No clear changes were found in any of the fish.

Spleen samples from 28 warm water treated fish and 14 control fish were examined. In 22 of the warm water treated fish and 12 of the control fish, there was a varying extent of subcapsular bleeding.

4. Discussion

Exposure of Atlantic salmon to sea water at 34 °C for 30 s did not lead to any statistically significant change in the prevalence of acute lesions except an increase in minor injuries on the caudal, dorsal, and right pelvic fins. The main cause of these fin injuries may have been the strong behavioural reaction displayed by the fish when exposed to warm water (Section 3.1). It cannot, however, be ruled out that the thermal component of the treatment was a contributing factor, for example by making the fins more fragile or leading to reopening of healing fin splits. Fin injuries have also been detected after warm water treatments of Atlantic salmon in an industrial setting (Grøntvedt et al., 2015).

Fin injuries can affect fish from a welfare, health, and production perspective (Noble et al., 2012). The consequences of fin injuries depend on the type and severity of injury, the type and number of affected fins, and the life stage of the fish (Arnold et al., 1991; Higham et al., 2005; Wagner et al., 2009). The primary functions of fins are to generate and control propulsion, and to help the fish control their posture (Arnold et al., 1991; Lauder, 2000; Drucker and Lauder, 2005; Standen and Lauder, 2005). Fin injuries can reduce fin function and thus affect the fish's swimming ability and postural control capacity (Barthel et al., 2003). As fin injuries are direct injuries to living tissue (Ellis et al., 2008), they can activate nociceptors and be painful for the fish (Chervova, 1997; Roques et al., 2010). Active fin injuries may also increase the susceptibility of the fish to infections caused by opportunistic pathogens (Harmache et al., 2006) and/or lead to osmoregulatory problems (Andrews et al., 2015). In addition, fin injuries may reduce the product quality and market value of the fish (Hoyle et al., 2007). On the other hand, fish have capacity for healing and regeneration of fins (Akimenko et al., 2003; Shao et al., 2011).

The behavioural reaction displayed by the Atlantic salmon in the treatment bag in this study, is in accordance with previously reported behaviour of salmonids exposed to warm water (Elliott, 1991; Elliott and Elliott, 1995; Ineno et al., 2005; Erikson et al., 2012; Roth, 2016; Nilsson et al., 2019). Nilsson et al. (2019) suggest that the behavioural reaction indicates nociception or pain. Rapid swimming and vigorous wriggling within a confined space is likely to increase the risk of mechanical injuries that may contribute to the high mortality associated with warm water treatment. In this study, the fish were held individually in a soft bag during the treatment, but if they had had the possibility to move freely in a chamber, the extent of damage could have been larger due to collisions with the chamber walls and other fish.

The use of anaesthetic in this study, may in itself have caused behavioural and physiological changes that might have affected the occurrence and/or extent of lesions. Isoeugenol, the active ingredient in Aqui-S® vet., has side effects that include impaired ventilation and depression of the cardiovascular system (Hill et al., 2002; Hill and Forster, 2004). A reduction in ventilation rate and extent of operculum opening may have led to reduced warm water exposure of the gills of the fish. It is therefore possible that the effect of warm water treatment on gill bleedings and/or gill paleness has been underestimated in this study. Such gill conditions have been reported after industrial warm water treatments (Grøntvedt et al., 2015; Hjeltnes et al., 2018; Poppe et al., 2018). In addition, a slower heartrate, decreased cardiac output, and reduced blood pressure may have led to potentially heated blood from the gills being transported more slowly through the body of the fish, thus reducing the risk of lesions on internal organs. Despite the possibility of impact on lesions, it was considered most appropriate to use anaesthetic to attempt avoiding behaviour-related injuries and to reduce stress in

the fish.

The discrepancy in the lesion prevalence between this study and reports from industrial warm water treatments can also have other causes: The fish usually examined after industrial warm water treatments are mainly injured, sick, moribund, and dead fish that are seldom compared to a control group. Details of fish health before treatment, execution of treatment, water quality, time between treatment and sampling, as well as type and number of treatments the fish have previously undergone, are often unknown. Thus, assumptions about causal relationships become uncertain. Furthermore, there is a possibility that potential lesions from warm water treatment may develop and become visible over time as indicated for eye injuries and cataracts in the report of Grøntvedt et al. (2015). The absence of any statistically significant change in the prevalence of acute lesions except fin injuries in this study, therefore, does not document the absence of potential long-term effects. It is, however, consistent with previous studies where warm water treatment of salmonids did not cause acute skin bleedings, skin wounds, eye injuries, cataracts (Grøntvedt et al., 2015), or changes in mucus cell size or density (Mangor-Jensen et al., 2017).

In the previously mentioned pilot trial by Gismervik et al. (2019, section 1), acute thermal injuries were found on Atlantic salmon at equal and somewhat higher water temperatures than in this study. In the pilot trial, however, smaller ($\overline{w} = 234 \pm 52$ g vs. 1117 ± 250 g), non-sedated fish swimming freely in the treatment tank for longer exposure times ($t = 72$–140 s vs. 30 s) than in this study were used. In addition to potential effects of partly higher temperature, it is to expect that the prevalence of thermal injuries will increase with exposure time as the warm water will have more time to exert its potential effects on the fish and a larger proportion of the fish's interior will be heated to a higher temperature (cf. Section 1). The smaller size of the fish in the pilot trial may possibly also have had an effect, as the internal temperature of smaller ectotherms responds more rapidly to changes in ambient temperature than the internal temperature of larger ectotherms due to a higher body surface area to volume ratio. On the other hand, Huntsman (1942) observed that large Atlantic salmon died before smaller salmon at high water temperatures ($T \sim 30$ °C), and Fowler et al. (2009) found that the heat shock response in rainbow trout is enhanced in juveniles compared to adults and may contribute to the larger thermal resistance that is often observed in young salmonid fish. Finally, the fact that the fish in the pilot trial of Gismervik et al. (2019) were not sedated and swam freely in the treatment tank enabled collisions with the tank walls and may have made it difficult to determine whether the detected lesions were caused by the collisions or the thermal component of the treatment.

As the number of fish in this study was limited due to the 3Rs (Section 2.1), a relatively high lesion prevalence was required for differences between the treatment groups to become statistically significant. In the ideal scenario with no lesions on the control fish, a lesion prevalence of at least 28% among the warm water treated fish (i.e. 11 out of 40 fish) would be required to obtain significance at the 0.05 level using Fisher's exact test. In this study, several lesions were detected also on the control fish, which further increased the required lesion prevalence. Detection of a lower lesion prevalence than was possible in this study, but which may concern many individuals in an industrial setting, requires examination of a larger number of fish. It is also conceivable that the basic lesion level in both treatment groups was so high that it concealed possible minor lesions inflicted by the thermal component of the treatment.

On the other hand, when screening for lesions by testing multiple parameters one-by-one, as in this study, it becomes increasingly likely that the groups being compared will appear to differ by chance in terms of at least one parameter as the number of comparisons increases. This «multiple comparisons problem» did, however, not have any major impact on the results in this study as all but the prevalence of injuries on the caudal, dorsal, and right pelvic fins were not statistically significant. The strong behavioural reaction displayed by the warm water treated fish, but not the control fish, substantiates that the difference in the prevalence of fin injuries between the treatment groups is not merely a chance effect.

5. Conclusion

Exposure of Atlantic salmon to sea water at a temperature of 34 °C for 30 s did not lead to any statistically significant change in the prevalence of acute lesions except an increase in minor fin injuries. The main cause of these fin injuries may have been the strong behavioural reaction displayed by the fish when exposed to warm water. Detection of a lower lesion prevalence than was possible in this study, but which may concern many individuals in an industrial setting, requires examination of a larger number of fish. Potential long-term effects of warm water treatment will be addressed in another study. Finally, it should be noted that industrial warm water treatment cannot currently be performed without the associated handling events, and therefore further work is also required to assess the potential for lesions during the entire treatment process.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Competing Interest

None.

Acknowledgements

This work was commissioned by the Norwegian Food Safety Authority and funded by the Institute of Marine Research (Project No. 14930-02) and the Norwegian Veterinary Institute through research-based governmental support. The authors thank the staff at Matre Research Station (IMR) and Cecilie Sviland Walde (NVI) for technical assistance.

References

Aaen, S.M., Helgesen, K.O., Bakke, M.J., Kaur, K., Horsberg, T.E., 2015. Drug resistance in sea lice: A threat to salmonid aquaculture. Trends Parasitol. 31, 72–81.

Adams, M., 2019. Performance of Atlantic Salmon Following Simulated Thermal Delousing with AQUI-S® Sedation, the Institute for Marine and Antarctic Studies. University of Tasmania, Australia.

Akimenko, M.A., Marí-Beffa, M., Becerra, J., Géraudie, J., 2003. Old questions, new tools, and some answers to the mystery of fin regeneration. Dev. Dyn. 226, 190–201.

Andrews, M., Stormoen, M., Schmidt-Posthaus, H., Wahli, T., Midtlyng, P.J., 2015. Rapid temperature-dependent wound closure following adipose fin clipping of Atlantic salmon *Salmo salar* L. J. Fish Dis. 38, 523–531.

Arnold, G.P., Webb, P.W., Holford, B.H., 1991. The role of the pectoral fins in station-holding of Atlantic salmon parr (*Salmo salar* L.). J. Exp. Biol. 156, 625–629.

Ashley, P.J., Sneddon, L.U., McCrohan, C.R., 2007. Nociception in fish: Stimulus–response properties of receptors on the head of trout *Oncorhynchus mykiss*. Brain Res. 1166, 47–54.

Bancroft, J.D., Gamble, M., 2008. Theory and Practice of Histological Techniques, 6 ed. Churchill Livingstone, Philadelphia, USA.

Barthel, B.L., Cooke, S.J., Suski, C.D., Philipp, D.P., 2003. Effects of landing net mesh type on injury and mortality in a freshwater recreational fishery. Fish. Res. 63, 275–282.
Bass, N., Wall, T., 2008. A Standard Procedure for the Field Monitoring of Cataracts in Farmed Atlantic Salmon and Other Species. Bord Iascaigh Mhara. Ireland's Seafood Development Agency, Dublin, Ireland, p. 2.
Brunsvik, P.S., 1996. Miljømessig Avlusing Av Laks, in: Småtrykk 261. Gildeskål forsøksstasjon, Inc, Inndyr, Norway.
Chervova, L.S., 1997. Pain sensitivity and behavior of fishes. J. Ichthyol. 37, 98–102.
Currie, S., Moyes, C.D., Tufts, B.L., 2000. The effects of heat shock and acclimation temperature on hsp70 and hsp30 mRNA expression in rainbow trout: *In vivo* and *in vitro* comparisons. J. Fish Biol. 56, 398–408.
Dean, J.M., 1976. Temperature of tissues in freshwater fishes. Trans. Am. Fish. Soc. 105, 709–711.
Dietz, T.J., Somero, G.N., 1993. Species- and tissue-specific synthesis patterns for heat-shock proteins HSP70 and HSP90 in several marine teleost fishes. Physiol. Zool. 66, 863–880.
Drucker, E.G., Lauder, G.V., 2005. Locomotor function of the dorsal fin in rainbow trout: Kinematic patterns and hydrodynamic forces. J. Exp. Biol. 208, 4479–4494.
DuBeau, S.F., Pan, F., Tremblay, G.C., Bradley, T.M., 1998. Thermal shock of salmon in vivo induces the heat shock protein hsp 70 and confers protection against osmotic shock. Aquaculture 168, 311–323.
Elliott, J.M., 1991. Tolerance and resistance to thermal stress in juvenile Atlantic salmon, *Salmo salar*. Freshw. Biol. 25, 61–70.
Elliott, A., 2010. A comparison of thermal polygons for British freshwater teleosts. Freshwater Forum 5, 178–184.
Elliott, J.M., Elliott, J.A., 1995. The effect of the rate of temperature increase on the critical thermal maximum for parr of Atlantic salmon and brown trout. J. Fish Biol. 47, 917–919.
Elliott, J.M., Elliott, J.A., 2010. Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: Predicting the effects of climate change. J. Fish Biol. 77, 1793–1817.
Ellis, T., Oidtmann, B., St-Hilaire, S., Turnbull, J., North, B., MacIntyre, C., Nikolaidis, J., Hoyle, I., Kestin, S., Knowles, T., 2008. Fin erosion in farmed fish. In: Fish Welfare. Blackwell Publishing, Ltd, Oxford, UK, pp. 121–149.
Erikson, U., Rosten, T.W., Rosten, C., Bruheim, T., 2012. Sedasjon med Aqui-S før avlusning med varmt vann. SINTEF Fiskeri og havbruk, Inc, Trondheim, Norway.
Erikson, U., Gansel, L., Frank, K., Svendsen, E., Digre, H., 2016. Crowding of Atlantic salmon in net-pen before slaughter. Aquaculture 465, 395–400.
Fowler, S.L., Hamilton, D., Currie, S., 2009. A comparison of the heat shock response in juvenile and adult rainbow trout (*Oncorhynchus mykiss*) – Implications for increased thermal sensitivity with age. Can. J. Fish. Aquat. Sci. 66, 91–100.
Fry, F.E.J., 1967. Responses of vertebrate poikilotherms to temperature. In: Thermobiology. Academic Press, New York, USA, pp. 375–409.
Galloway, B.J., Kieffer, J.D., 2003. The effects of an acute temperature change on the metabolic recovery from exhaustive exercise in juvenile Atlantic salmon (*Salmo salar*). Physiol. Biochem. Zool. 76, 652–662.
Gismervik, K., Nielsen, K.V., Lind, M., Viljugrein, H., 2017. Mekanisk avlusing med FLS-avlusersystem – dokumentasjon av fiskevelferd og effekt mot lus. In: Veterinærinstituttets Rapportserie 6–2017. Veterinærinstituttet, p. 43.
Gismervik, K., Gåsnes, S.K., Gu, J., Stien, L.H., Madaro, A., Nilsson, J., 2019. Thermal injuries in Atlantic salmon in a pilot laboratory trial. Vet. Anim. Sci. 8, 100081.
Grøntvedt, R.N., Nerbøvik, I.-K.G., Viljugrein, H., Lillehaug, A., Nilsen, H., Gjerve, A.-G., 2015. Termisk avlusing av laksefisk – dokumentasjon av fiskevelferd og effekt, In: Veterinærinstituttets rapportserie nr. 13/2015. Veterinærinstituttet, Oslo.
Harmache, A., LeBerre, M., Droineau, S., Giovannini, M., Brémont, M., 2006. Bioluminescence imaging of live infected salmonids reveals that the fin bases are the major portal of entry for Novirhabdovirus. J. Virol. 80, 3655–3659.
Higham, T.E., Malas, B., Jayne, B.C., Lauder, G.V., 2005. Constraints on starting and stopping: Behavior compensates for reduced pectoral fin area during braking of the bluegill sunfish *Lepomis macrochirus*. J. Exp. Biol. 208, 4735–4746.
Hightower, L.E., 1991. Heat shock, stress proteins, chaperones, and proteotoxicity. Cell 66, 191–197.
Hill, J.V., Forster, M.E., 2004. Cardiovascular responses of Chinook salmon (*Oncorhynchus tshawytscha*) during rapid anaesthetic induction and recovery. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 137, 167–177.
Hill, J.V., Davison, W., Forster, M.E., 2002. The effects of fish anaesthetics (MS222, metomidate and AQUI-S) on heart ventricle, the cardiac vagus and branchial vessels from Chinook salmon (*Oncorhynchus tshawytscha*). Fish Physiol. Biochem. 27, 19–28.
Hjeltnes, B., Bang Jensen, B., Bornø, G., Haukaas, A., Walde, C.S., (Eds.), 2018. The Health Situation in Norwegian Aquaculture 2017, In: Norwegian Veterinary Institute's Report Series 1b-2018. Norwegian Veterinary Institute, Bergen, Norway.
Hjeltnes, B., Bang Jensen, B., Bornø, G., Haukaas, A., Walde, C.S., (Eds.), 2019. The Health Situation in Norwegian Aquaculture 2018, In: Norwegian Veterinary Institute's Report Series 6b-2019. Norwegian Veterinary Institute, Bergen, Norway, p. 132.
Holan, A.B., Roth, B., Breiland, M.S.W., Kolarevic, J., Hansen, Ø.J., Iversen, A., Hermansen, Ø., Gjerde, B., Hatlen, B., Mortensen, A., 2017. Beste praksis for medikamentfrie metoder for lakseluskontroll (MEDFRI) – Faglig sluttrapport. In: Nofima rapportserie. Nofima, Inc, Tromsø, Norway.
Hoyle, I., Oidtmann, B., Ellis, T., Turnbull, J., North, B., Nikolaidis, J., Knowles, T.G., 2007. A validated macroscopic key to assess fin damage in farmed rainbow trout (*Oncorhynchus mykiss*). Aquaculture 270, 142–148.
Huntsman, A.G., 1942. Death of salmon and trout with high temperature. J. Fish. Board Can. 5, 485–501.
Hyvärinen, P., Heinimaa, S., Rita, H., 2004. Effects of abrupt cold shock on stress responses and recovery in brown trout exhausted by swimming. J. Fish Biol. 64, 1015–1026.
Ineno, T., Tsuchida, S., Kanda, M., Watabe, S., 2005. Thermal tolerance of a rainbow trout *Oncorhynchus mykiss* strain selected by high-temperature breeding. Fish. Sci. 71, 767–775.
Lauder, G.V., 2000. Function of the caudal fin during locomotion in fishes: kinematics, flow visualization, and evolutionary patterns. Am. Zool. 40, 101–122.
Mangor-Jensen, A., Stien, L.H., Fosseidengen, J.E., Mangor-Jensen, R., 2017. Varmebehandling av laks i forbindelse med avlusing. Rapport til Mattilsynet, In: Rapport fra Havforskningen. Institute of Marine Research, Bergen, Norway.
Nakano, T., Kameda, M., Shoji, Y., Hayashi, S., Yamaguchi, T., Sato, M., 2014. Effect of severe environmental thermal stress on redox state in salmon. Redox Biol. 2, 772–776.
Nilsson, J., Moltumyr, L., Madaro, A., Kristiansen, T.S., Gåsnes, S.K., Mejdell, C., Gismervik, K., Stien, L.H., 2019. Sudden exposure to warm water causes instant behavioural responses indicative of nociception or pain in Atlantic salmon. Vet. Anim. Sci. 8, 100076.
Noble, C., Jones, H.A.C., Damsgård, B., Flood, M.J., Midling, K.Ø., Roque, A., Sæther, B.-S., Cottee, S.Y., 2012. Injuries and deformities in fish: Their potential impacts upon aquacultural production and welfare. Fish Physiol. Biochem. 38, 61–83.
Noble, C., Nilsson, J., Stien, L.H., Iversen, M.H., Kolarevic, J., Gismervik, K., 2018. Welfare Indicators for Farmed Atlantic Salmon: Tools for Assessing Fish Welfare, 1 ed. Nofima, Inc, Tromsø.
Oppedal, F., Dempster, T., Stien, L.H., 2011. Environmental drivers of Atlantic salmon behavior in sea-cages: A review. Aquaculture 311, 1–18.
Overton, K., Dempster, T., Oppedal, F., Kristiansen, T.S., Gismervik, K., Stien, L.H., 2018. Salmon lice treatments and salmon mortality in Norwegian aquaculture: A review. Rev. Aquac. 1–20.
Persson, D., Kolstø, S., Pedersen, T.M., 2015. The use of a double gill scoring system for field evaluation of gill health in Atlantic salmon (*Salmo salar* L.), In: European Association of Fish Pathologists Conference. FoMAS, Inc., Las Palmas de Gran Canaria, Spain.
Poppe, T.T., Dalum, A.S., Røislien, E., Nordgreen, J., Helgesen, K.O., 2018. Termisk behandling av laks. Norsk veterinærtidsskrift 3, 148–156.
R Core Team, 2019. R: A language and environment for statistical computing, 1.1.463 ed. R Foundation for Statistical Computing, Vienna, Austria.
Roques, J.A.C., Abbink, W., Geurds, F., van de Vis, H., Flik, G., 2010. Tailfin clipping, a painful procedure: Studies on Nile tilapia and common carp. Physiol. Behav. 101, 533–540.
Roth, B., 2016. Avlusing av laksefisk med Optilice: Effekt på avlusing og fiskevelferd. Nofima, Inc, Tromsø, Norway.
Russell, W.M.S., Burch, R.L., 1959. The Principles of Humane Experimental Technique. Methuen & Co. Ltd, London.
Schoettger, R.A., Julin, A.M., 1967. Efficacy of MS-222 as an anesthetic on four salmonids. In: Investigations in Fish Control 13. U.S. Fish and Wildlife Service, Washington, D.C., USA, p. 15.
Shao, J., Chen, D., Ye, Q., Cui, J., Li, Y., Li, L., 2011. Tissue regeneration after injury in adult zebrafish: The regenerative potential of the caudal fin. Dev. Dyn. 240, 1271–1277.
Skjervold, P.O., Fjæra, S.O., Østby, P.B., Einen, O., 2001. Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*). Aquaculture 192, 265–280.
Somero, G.N., 1995. Proteins and temperature. Annu. Rev. Physiol. 57, 43–68.
Standen, E.M., Lauder, G.V., 2005. Dorsal and anal fin function in bluegill sunfish *Lepomis macrochirus*: Three-dimensional kinematics during propulsion and maneuvering. J. Exp. Biol. 208, 2753–2763.
Stevens, E.D., Fry, F.E.J., 1970. The rate of thermal exchange in a teleost, *Tilapia mossambica*. Can. J. Zool. 48, 221–226.
Stevens, E.D., Fry, F.E.J., 1974. Heat transfer and body temperatures in non-thermoregulatory teleosts. Can. J. Zool. 52, 1137–1143.
Stien, L.H., Gismervik, K., Tørud, B., Overton, K., Kristiansen, T.S., 2018. Dødelighet og fiskevelferd i laks- og regnbueørretproduksjon i sjø. In: Risikorapport norsk fiskeoppdrett 2018, Fisken og havet, særnummer 1-2018. Institute of Marine Research, Bergen, Norway, pp. 153–164.
Taylor, R.S., Muller, W.J., Cook, M.T., Kube, P.D., Elliott, N.G., 2009. Gill observations in Atlantic salmon (*Salmo salar*, L.) during repeated amoebic gill disease (AGD) field exposure and survival challenge. Aquaculture 290, 1–8.

Wagner, C.P., Einfalt, L.M., Scimone, A.B., Wahl, D.H., 2009. Effects of fin-clipping on the foraging behavior and growth of age-0 muskellunge. N. Am. J. Fish Manag. 29, 1644–1652.

Wall, T., Bjerkås, E., 1999. A simplified method of scoring cataracts in fish. Bull. Eur. Assoc. Fish Pathol. 19, 162–165.

Wendelaar Bonga, S.E., 1997. The stress response in fish. Physiol. Rev. 77, 591–625.