Thermal injuries in Atlantic salmon in a pilot laboratory trial

Kristine Gismervik[a,*], Siri K. Gåsnes[a], Jinni Gu[a], Lars H. Stien[b], Angelico Madaro[b], Jonatan Nilsson[b]

[a] Norwegian Veterinary Institute, Pb 750 Sentrum, 0106 Oslo, Norway
[b] Research Group Animal Welfare, Institute of Marine Research, P.O box 1870 Nordnes, NO-5817 Bergen, Norway

ABSTRACT

Thermal delousing is a new method for removing sea lice from farmed Atlantic salmon (*Salmo salar* L). We investigated thermally-related tissue injuries in Atlantic salmon in a pilot laboratory trial to describe the acute effect of high water temperatures (34–38 °C). Acute tissue injuries in gills, eyes, brain and possible also nasal cavity and thymus were seen in salmon exposed to water temperatures of 34 - 38 °C in 72 to 140 s. This implies that exposing salmon to such water temperatures is a welfare risk, not only due to the direct tissue injuries that may also be dependent on exposure time, but also due to risk of thermal pain and aversion, including flight reactions.

1. Introduction

Thermal delousing is a new method for removing sea lice from farmed Atlantic salmon (*Salmo salar* L) (Grøntvedt et al., 2015). Although launched as an environmentally and fish welfare friendly technology, it can lead to injuries and acute fish mortality (Hjeltnes, Bang-Jensen, Bornø, Haukaas, & Walde, 2018; Overton et al., 2018). The salmon are crowded and pumped into a chamber with heated seawater for about 30 s, before returning to the sea cage (Noble et al., 2018). The treatment temperature varies between 28 and 34 °C, dependent on sea temperature and delousing effect (Hjeltnes et al., 2018; Roth, 2016). However, extreme temperatures of 36 °C or higher have been reported in anonymous surveys (Hjeltnes et al., 2018).

After commercial thermal delousing, pathologists have observed gill and brain hemorrhages, scale and skin loss and affected thymic and nasal tissues in salmonids in mortality diagnostics (Poppe, Dalum, Røislien, Nordgreen, & Helgesen, 2018). Still, it may be hard to distinguish thermal injuries from other factors like pumping, crowding and the panic reaction in salmon seen during thermal de-licing (Hjeltnes et al., 2018; Noble et al., 2018; Poppe et al., 2018). It has been debated whether it is the heat itself, or panic behavior and collisions in the treatment chambers that causes the injuries. It is known that salmonids have nociceptors (pain receptors) that respond to heat. Ashley, Sneddon, and McCrohan, (2007) reported an average heat threshold temperature of ∼29 °C for polymodal and ∼33 °C for mechanothermal nociceptors in rainbow trout.

High water temperatures are reported as strongly aversive and mortal in salmonids (Beitinger, Bennett, & McCauley, 2000; Elliott, 1991; Frechette, Dugdale, Dodson, & Bergeron, 2018). Lethal water temperature for Atlantic salmon varies according to acclimation temperature, exposure time and life stage (Elliott, 1991; Huntsman, 1942). However, acclimation above ∼28 °C is not considered possible for salmon (Anttila et al., 2014; Elliott & Elliott, 2010). In experiments, parr and smolts die within 10 min at 30 - 33 °C (Elliott & Elliott, 2010). During thermal death of wild salmon, large salmon died first while parr survived (Huntsman, 1942).

Basic knowledge of upper temperatures and holding times that may give acute tissue damage in live salmon is lacking. Here, we investigate thermally-related tissue injuries in Atlantic salmon to describe the acute effect of high water temperatures (34–38 °C).

2. Material and method

The pilot was performed in connection with a behavior trial at the Institute of Marine Research in Matre in May 2018 (reported in Nilsson et al., 2019). The Atlantic salmon smolts were 234 ± 52 g (mean ± SD) and acclimated to 8.5 °C. In the behavior trial the salmon were individually transferred by careful dip-netting to a treatment tank (ø = 1.5 m) with different temperatures until they reached the behaviorally defined humane endpoint (laying on the side for two seconds). At this point the salmon were picked up and euthanized by an overdose

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* Corresponding author.

Table 1

Overview over the key macroscopic and histological injuries of gills, eyes, brain, snout, nasal cavity and thymus in Atlantic salmon exposed to different water temperatures. Macro; macroscopic bleedings or injuries scored directly after treatment. Opacity scored 0–4 according to Bass and Wall (Undated). Histology: gills; sparse to severe bleedings or epithelial injuries/edema, and/ or epithelial cell necrosis, PGI = proliferative gill infection (non-specific) were seen in all samples including controls, eye; partly to almost totally loss of corneal eye epithelium, brain; sparse to severe focal bleedings in brain and/or meninges, nasal cavity; sparse blood congestion and focal/multifocal bleeding in lamina propria and/or edema in nasal cavity.

T reatments		Gills			Eye			Brain		Snout damage	Nasal cavity	Thymus
°C	Sec.	Macro	Histology		Opacity		Hist.	Macro	Hist.	Macro	Hist.	Hist.
				PGI	Left	Right	Left					
34	90–140	–	Spa.	D	–	–	ND	–	ND	–	D	ND
34	90–140	–	Spa.	D	–	–	Partly	–	ND	–	ND	ND
34	90–140	–	Spa.	D	–	–	ND	–	ND	–	ND	ND
34	90–140	–	Spa.	D	–	–	ND	–	Sev.	–	ND	Spa. e. c. necrosis
36	99	Sev.	Mod.	D	1	1	Partly	ND	Spa.	Mod.	D	ND
36	100	Sev.	Mod.	D	0	0	Partly	D	Mod.	Mod.	–	Sparse bleeding
36	105	Sev.	Mod.	D	0*	1	ND	D	Spa.	Mod.	D	ND
36	115	Sev.	Spa.	D	1	2	Partly	D	Spa.	Mod.	–	ND
36	117	Sev.	Spa.	D	0	1	ND	ND	ND	Sev.	×	ND
36	130	Sev.	Mod.	D	0	0	Partly	ND	ND	Spa.	×	–
37	114	Sev.	Sev.	D	1	0	Partly	ND	Spa.	Mod.	D	–
37	117	Sev.	Mod.	D	0	2	Partly	ND	ND	Spa.	D	–
38	72	Sev +	Sev.	D	1	0	Partly	ND	ND	Spa.	D	–
38	95	Sev +	Sev.	D	3	1	Total	ND	Spa.	Mod	×	×
8.5	0	ND	ND	D	0	0	ND	ND	ND	ND	–	ND
8.5	0	ND	ND	D	0	0	ND	ND	ND	Mod.	–	–
8.5	0	ND	ND	D	0	0	ND	ND	ND	Spa.	–	ND
8.5	0	ND	ND	D	0	0	ND	ND	ND	ND	–	ND
8.5	0	ND	ND	D	0	0	ND	ND	ND	ND	–	ND
8.5	0	ND	–	–	0	0	–	–	–	Spa.	–	–
8.5	0	ND	–	–	0	0	–	–	–	Spa.	–	–
8.5	0	ND	–	–	0	0	–	–	–	Spa.	–	–
8.5	0	ND	–	–	0	0	–	–	–	Spa.	–	–
8.5	0	ND	–	–	0	0	–	–	–	Spa	–	–

Spa. = sparse. Mod. = moderate. Sev. = severe. ND = not detected. D = detected. Spa. e. c. necrosis = sparse epithelial cell necrosis. × = sample not suitable for analysis. - = not investigated. *Small bleeding/injury detected.

of tricaine methanesulfonate (Finquel vet.). At treatment temperatures of 36–38 °C, tissue injuries were immediately examined as described below. In addition to individually exposed salmon, the 34 °C group of salmon in the pilot was treated simultaneously. As all individuals in this group could not be netted out in the same netting event, exact exposure times are not known at an individual level but were within 90–140 s for all individuals. These individuals were euthanized and examined in the same manner.

Ten salmon were scored for macroscopic injuries after hot water (36–38 °C) exposure. As a control ten salmon kept at 8.5 °C were individually dip-netted, euthanized and macroscopically scored. Temperatures and exposure time are given in Table 1, also showing the key injuries discovered. The scoring was done on a 0–3 scale; none, mild, moderate, severe (Gismervik, Nielsen, Lind, & Viljugrein, 2017; Grøntvedt et al., 2015; Hjeltnes, Bornø, Jansen, Haukaas & Walde, 2017), for eye damage/bleeding, snout injury, scale loss, skin bleeding, wounds, gill bleeding and paleness and fin injuries. Bleedings in brain, thymus, and palate were scored as present or not. Eye opacity was scored 0–4 (Bass & Wall, Undated; Wall & Bjerkås, 1999), total gill score 0–5 (Grøntvedt et al., 2015; Taylor, Muller, Cook, Kube & Elliott, 2009).

Histology examination and scoring was performed in fourteen salmon exposed to hot water (34–38 °C), and five of the control fish. The following organs were sampled and fixed in 10% phosphate-buffered formalin: gills, left eye, thymus, skin and skeletal muscle, brain,

nasal cavity, pseudobranch, heart, liver, spleen, kidney, pyloric caeca with pancreas. Skeletal muscle samples were obtained ventral to the dorsal fin, by transverse section in the lateral line area, covering both red and white muscle. Thymus and nasal cavity were not sampled in all fish (see Table 1 for details). After formalin fixation, organs were prepared for histology by standard paraffin wax embedding and stained with haematoxylin and eosin. Pathological lesions were graded from none detected, mild, moderate and severe by light microscopy. All macroscopic and histological scorings were conducted by authorized fish health personnel (Norwegian Veterinary Institute), including full autopsy.

The experiment was approved according to the Norwegian animal welfare act by the Norwegian Food Safety Authority, permit number 15,383.

3. Results and discussion

Key findings were that acute tissue injuries were seen in salmon exposed to water temperatures of 34 - 38 °C in 72 to 140 s (Table 1). Gill injuries were macroscopically seen as gill bleedings and coagulated clots histologically seen as bleedings or congestions, epithelial damage or edema and/or necrosis; the higher the temperature the more severe damage (Table 1 and Fig. 1). That the gill tissue is most affected is not surprising, as gills are extremely delicate, reacting quickly to an
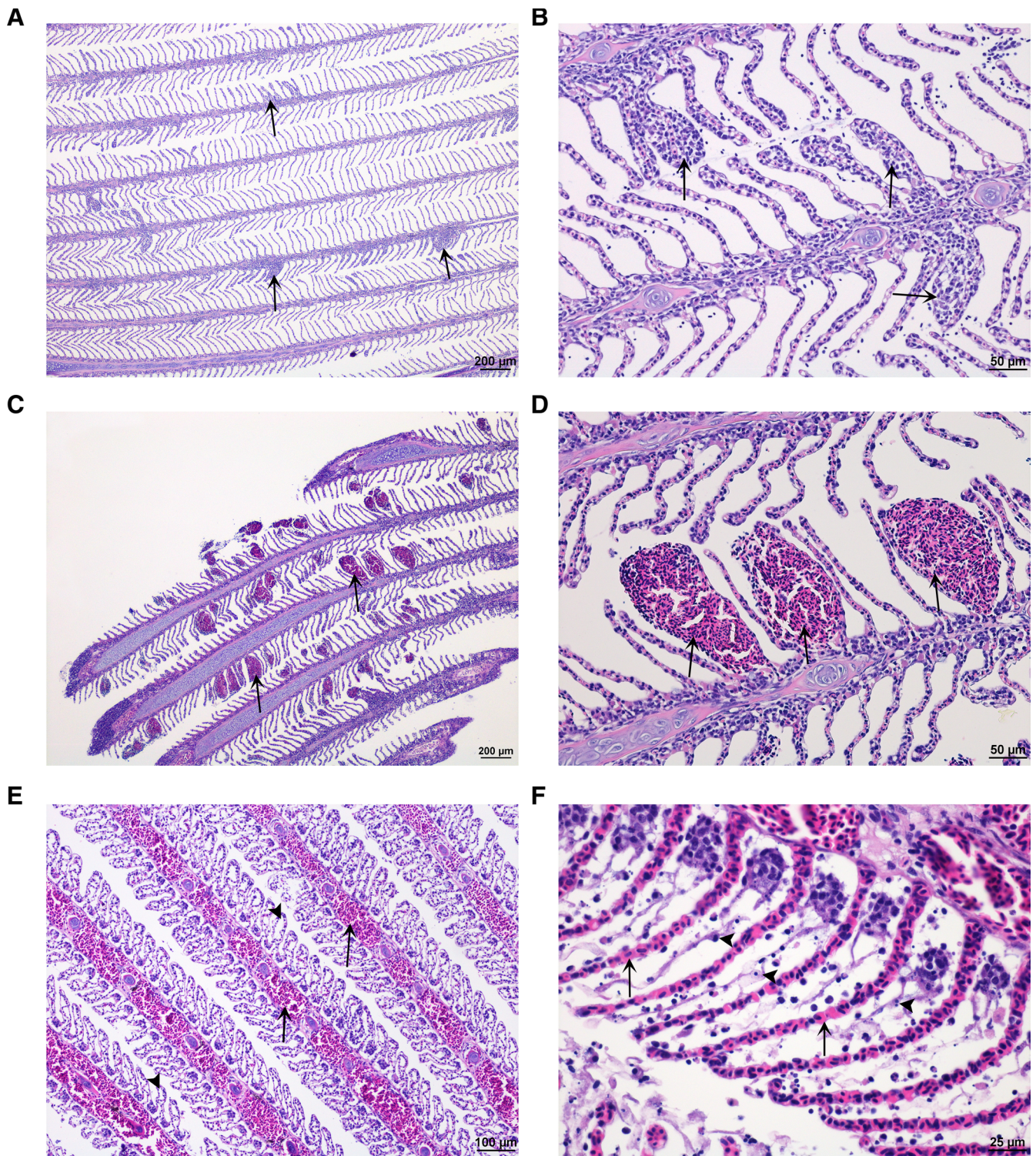
Fig. 1. Gill tissue. (A and B) Normal gill tissue apart from non-specific multifocal proliferative gill inflammation (arrow) in Atlantic salmon in control group. (C and D) Moderate lamellar bleeding in Atlantic salmon (arrow) exposed to 36 °C water. (E and F) Severe filamental (E) and lamellar congestion (F) (arrow) and severe epithelial lifting/ edema (E, arrow head) and cellular necrosis of epithelial cells (F, arrow head) in Atlantic salmon exposed to 38 °C water. (H&E stain).
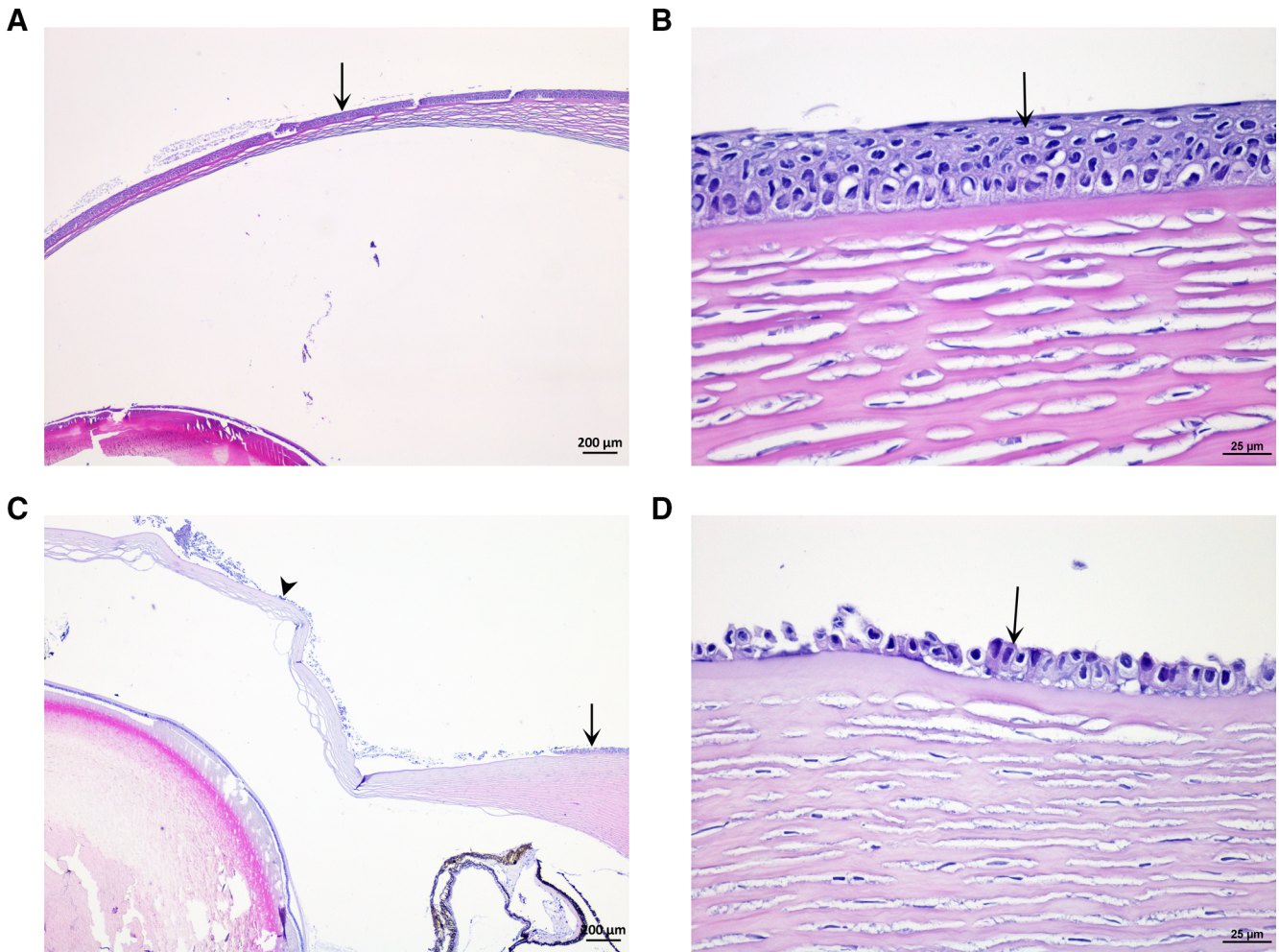
Fig. 2. Eye. (A and B) Normal cornea of Atlantic salmon (arrow: epithelium) in control group. (C) Partial loss of corneal epithelium in Atlantic salmon exposed to 38 °C water. (arrow: normal epithelium; arrow head: loss of epithelium). (D) Partial loss of epithelial cells (arrow) of cornea. (H&E stain).

unfavorable environment (Strzyzewska, Szarek, & Babinska, 2016).

Opacity in one or both eyes was seen in eight of the ten salmon exposed to 36–38 °C. One salmon exposed to 38 °C had a score 3 in one eye (Table 1 and Fig. 2). According to the histology, the opacity discovered was not cataract (clouding) in the eye lens, which macroscopically easily can be a mix up (Bass & Wall, Undated), but partly or almost total loss of the corneal eye epithelium assumed painful (Ashley, Sneddon, & McCrohan, 2006). Eyes are vulnerable and can also be damaged by dip-netting of the fish. Still, none of the ten controls showed macroscopic eye damage, five of them also controlled to be negative by histology.

Brain bleedings were discovered macroscopically in three of ten salmon and by histology in seven of 13 salmon after hot water exposure (Fig. 3). No brain bleeding was seen in controls. This pilot suggests that hot water itself or the stress may give brain bleedings at the temperatures and holding times examined (Table 1). Going through video recordings from the 36–38 °C groups, collisions with the tank walls cannot be ruled out for all salmon with brain bleedings. Still, one

salmon had no clear wall collisions, but a macro- and microscopic bleed, and at least four individuals had no diagnosed brain bleedings despite visible tank wall collisions, suggesting colliding with the walls was not a major contributor to brain injuries in the experimental set up. There was also no macroscopic skin bleedings or wounds detected on the salmon body in either the treated or control group, with the exception of snout and fin damage seen in both groups. The most severe damage appeared in the group exposed to warm water (Table 1).

At least one fish exposed to 38 °C water temperature showed macroscopic bleedings in nostrils, but this was not registered systematically on all. Histologically sparse blood congestion and focal or multifocal bleeding were seen in lamina propria and/or edema in the nasal cavity of fish exposed to water temperatures of 36–38 °C, and from one of four salmon exposed to water temperature of 34 °C (Table 1 and Fig. 4). Nasal cavity is an organ normally not sampled, so interpretations of the results must be taken with care as it may be difficult to judge whether the changes are artifacts associated with sampling, also since it was not sampled in the controls. Sparse epithelial cell necrosis was found in the
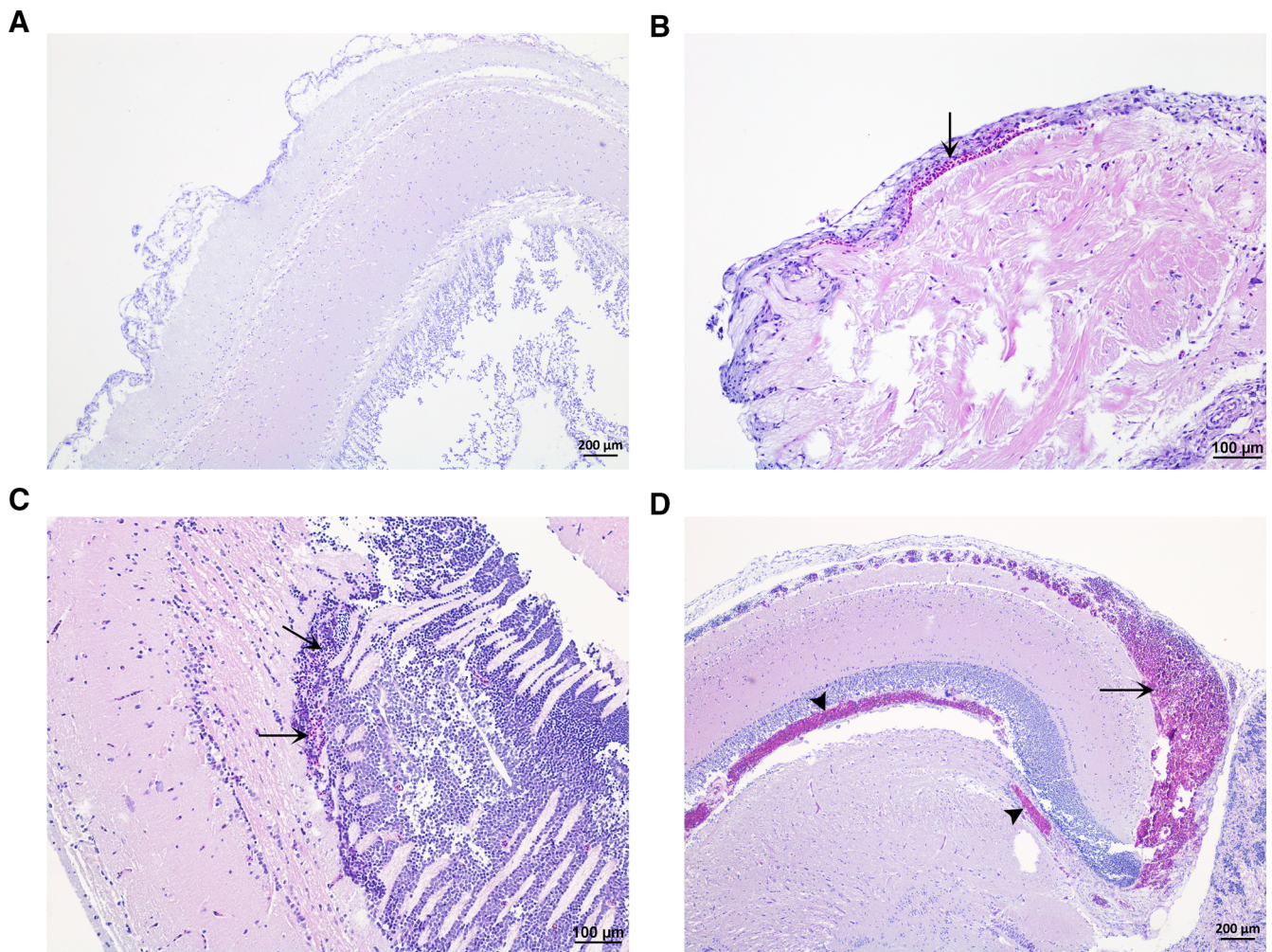
Fig. 3. Brain. (A) Normal brain of Atlantic salmon in control group. (B) Sparse focal bleeding (arrow) in meninges in Atlantic salmon exposed to 37 °C water. (C) Sparse focal bleeding (arrow) in brain in Atlantic salmon exposed to 38 °C water. (D) Severe focal bleeding in meninges (arrow) and brain (arrow head) in Atlantic salmon exposed to 34 °C water. (H&E stain).

thymus of one salmon, and sparse bleeding in another (Fig. 5). The remaining fish, including four controls, showed no thymus pathology.

No histopathological changes were detected on pseudobranch, heart, liver, spleen, kidney, pyloric caeca, pancreas or muscle from hot water exposed fish or five controls. Concerning skin health, epidermis was partly missing, scales exposed and/or edemas in scale pockets were seen in almost all treated and control fish. This may be due to artifacts during sampling or processing, but it was observed that the salmon generally had "loose scales".

We have here shown that a sudden exposure to water at 34–38 °C can be lethal after 72 to 140 s in salmon acclimated to 8.5 °C. We used equilibrium loss and cease of movements for two seconds as a humane endpoint. Such behavior has also been suggested by others as a sign of irreversible thermal shock (Beitinger et al., 2000; Elliot, 1991), and wild salmon would be an easy meal lying on their sides. Still, we did not check reflexes like the eye roll (Kestin, Van de Vis, & Robb, 2002). Acute injuries, especially in gills but also eyes and other tissue, were

detected in the salmon. In humans, the cornea is acknowledged as the most richly innervated body structure when it comes to nerves (Al-Aqaba, Dhillon, Mohammed, Said & Dua, 2019), 40 times more sensitive than dental pulp, 400 times more than the skin (Bonini, Rama, Olzi, and Lambiase, (2003). Polymodal nociceptors in corneas of cats are excited by temperatures over 37–38 °C, and are sensitized to repeated heating (Belmonte & Giraldez, 1981; Gallar, Pozo, Tuckett, & Belmonte, 1993). Cats have a normal rectal temperature ranging from 36.7–38.9 °C (Levy, Nutt, & Tucker, 2015). Ashley et al. (2006) identified corneal nociceptors in rainbow trout, polymodal temperature threshold 31.70 ± 2.09, still ranging from 21.4 °C. Ashley et al. (2007) reported an average heat threshold temperature of ∼29 °C for polymodal and ∼33 °C for mechanothermal nociceptors on the head of the rainbow trout, still ranging from 20.1–22.0 °C. Ashley et al. (2007) argues that a lower noxious thermal level than mammals found in rainbow trout, can be explained by the function nociception has, and evolved to match habitat temperatures. Lethal limit for trout is
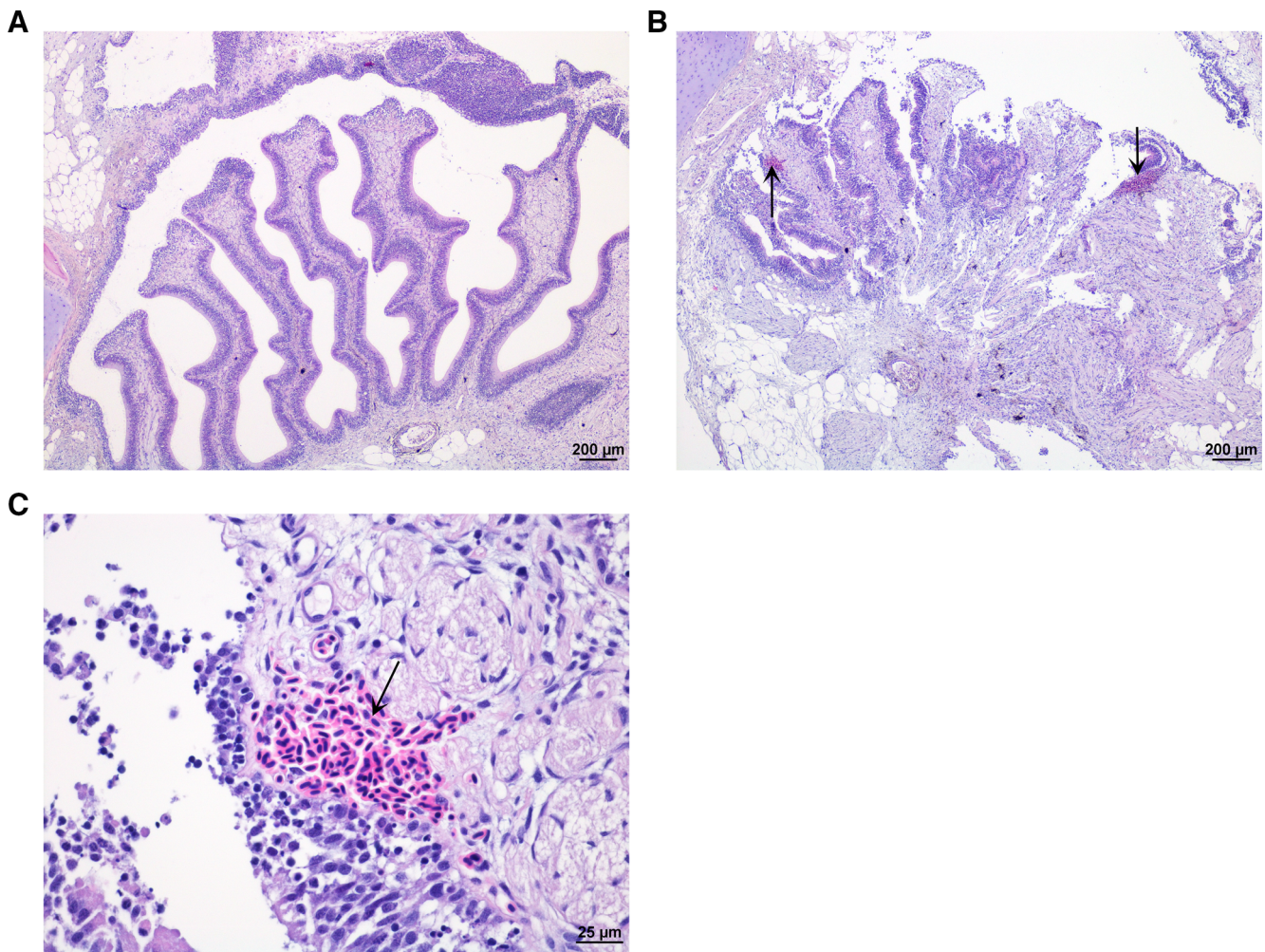
Fig. 4. Nasal cavity. (A) Normal nasal cavity in Atlantic salmon. (B and C) Focal bleeding (arrow) in lamina propria of mucosa in Atlantic salmon exposed to 37 °C water. (H&E stain).

described to be > 25 °C, where trout actively avoide such temperatures, even at the risk of hypoxia (Matthews & Berg, 1997). Research on more thermally tolerant fish species like the goldfish supports thermal nociception levels in accordance with thermal limits. Nordgreen et al. (2009) found a mean heat noxious level of 38 °C in goldfish based on behaviour, that correspond to their critical maximal limit of 37,5–38,3 °C (Ford & Beitinger, 2005). Adult wild Atlantic salmon are found to maintain a narrow range of body temperatures (16–20 °C) using behavioral thermoregulation via use of cool refuge in rivers (Frechette et al., 2018). Elliot (2010) states that salmon will soon die with water temperatures exceeding 22–28 °C.

Based on the biological function of nociception and pain keeping an animal alive (Sneddon, 2017), it is likely that water at 34–38 °C, here shown to give acute tissue injuries within minutes, also is potentially painful and strongly aversive to Atlantic salmon. Fish pain in general is reviewed elsewhere (ie. Chatigny, 2018; EFSA, 2009; Pouca & Brown, 2017; Sneddon, 2009). The salmon showed immediate strong aversive behaviors like bursts, colliding in tank walls, headshake and increased swimming speed at these temperatures (reported in details in Nilsson et al., 2019 and at ensured oxygen conditions). Others have reported similar aversive behavioral responses to high water temperatures (Elliot, 1991). Heat stress is also found to give physiological responses, like elevation of glucose and lactate when raising the temperature from 15 to 26 °C over 15 h (Gallant, LeBlanc, MacCormack & Currie, 2017).

4. Conclusion

Exposure to water at 34–38 °C in 72 to 140 s can lead to acute tissue injuries in gills, eyes, brain and possible also nasal cavity and thymus in Atlantic salmon. This implies that exposing salmon to such water temperatures is a welfare risk, not only due to the direct tissue injuries that may also be dependent on exposure time, but also due to risk of thermal pain and aversion, including flight reactions.
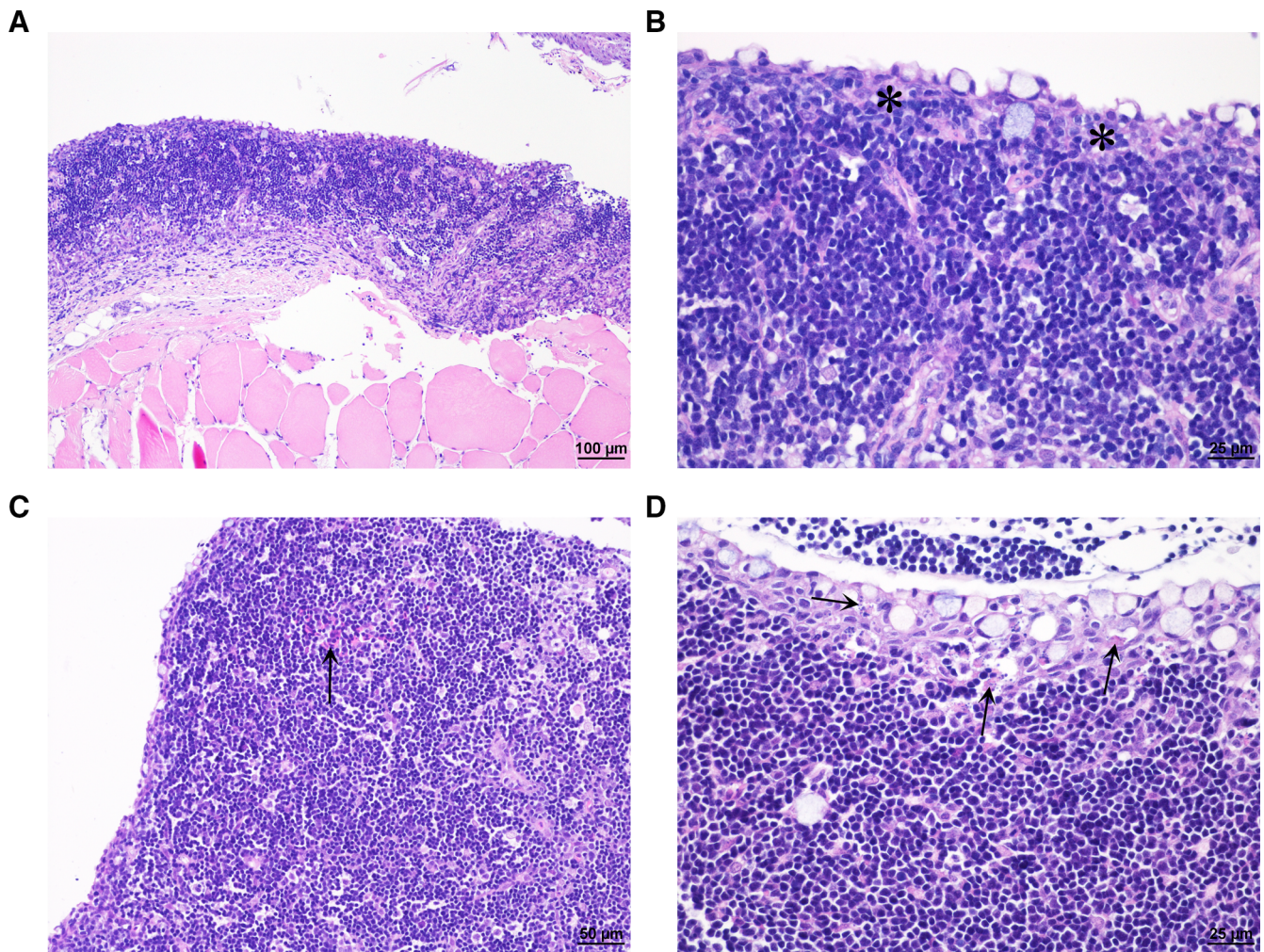
Fig. 5. Thymus. (A and B) Normal thymus of Atlantic salmon in control group (asterisk: epithelium). (C) Sparse focal bleeding (arrow) in thymic parenchyma in Atlantic salmon exposed to 36 °C water. (D) Sparse cellular necrosis of epithelial cells (arrow) in Atlantic salmon exposed to 34 °C water. (H&E stain).

Ethical statement

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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