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Thermal delousing in anaesthetised small Atlantic salmon (Salmo salar) post-smolts: A case study showing the viability of anaesthesia prior to delousing for improved welfare during treatment for salmon lice

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Abstract

*Delousing treatment for salmon sea lice (*Lepeophtheirus salmonis*) is considered a significant welfare concern in farming of Atlantic salmon (*Salmo salar*), where both industry and legislative bodies prompt for better methods. Currently, the most common method is thermal delousing, where fish are crowded, pumped into a vessel and exposed to ~28–34°C for ~30 s. Physical collisions occurring as a result of a loss of behavioural control lead to acute stress. Crowding triggers vigorous escape behaviour as salmon respond not only to treatment but also to being channeled to and from the treatment zone. A sequence of events considered to cause mortality and poor welfare. The present case study was motivated by an urgent need for delousing in groups of small salmon post-smolts in experimental research. For this purpose, a simple, small-scale system for thermal delousing was constructed, including anaesthesia to alleviate behavioural responses. The anaesthetised fish showed little behavioural response to thermal treatment, strong appetite within hours, and negligible mortality. The described method is regarded as a welfare-friendly alternative to industrial delousing in smaller fish groups, for example, in experimental research. We would encourage detailed research aimed towards gaining a deeper understanding of the welfare effects of anaesthesia prior to treatment for delousing.*

Keywords: *anaesthesia, animal welfare, Atlantic salmon, behaviour, handling, thermal delousing*

Introduction

Over recent decades, the salmon louse (*Lepeophtheirus salmonis*) has become resistant to chemical therapeutants used for topical delousing of Atlantic salmon (*Salmo salar*) within sea cages (Torrisen *et al* 2013). Delousing has therefore shifted to mechanical treatments which require transfer of fish into treatment vessels. Today, the most common delousing method in Norway is thermal treatment, where salmon and rainbow trout (*Oncorhynchus mykiss*) are bathed in water with temperatures of 28–34°C for 30 s (Overton *et al* 2018; Stien *et al* 2019). The risk of elevated mortality after thermal treatment is higher than after non-thermal mechanical delousing (Overton *et al* 2018). The applied water temperatures seen during commercial thermal delousing will, if extended for minutes, induce severe tissue damage and death (Gismervik *et al* 2019; Nilsson *et al* 2019), and appear to be acutely painful to salmonids as the fish panic with strong struggling behaviour (Nilsson *et al* 2019) which can result in severe injuries due to collisions with the treatment chamber (Poppe *et al* 2018).

Administration of drugs with anaesthetic properties to fish prior to thermal treatment should lessen their behavioural responses and potentially reduce discomfort from exposure to high water temperature and further handling. Anaesthesia is generally divided into levels that are dependent on dose and time of exposure (for a review, see Sneddon 2012). The first level is 'light sedation' and characterised by fish maintaining equilibrium but showing reduced activity and a demeanour of disorientation. Isoeugenol (eg AQUI-S) is commonly deployed in aquaculture to induce light sedation in salmonids to lessen their response to handling (Zahl *et al* 2011) and is applied prior to thermal delousing in vessels with a pre-treatment well. There is unpublished evidence that light sedation ameliorated cortisol, glucose and lactate levels and adverse behaviour before, during and after simulated thermal delousing of small Atlantic salmon (Adams 2019). Maintaining equilibrium and locomotory behaviour in the fish is a requirement in the currently used constructional designs for thermal treatment, both for transportation and avoiding physical damage. However, deeper anaesthesia, as commonly and effectively induced in salmonids by metacaine (eg Finquel) or benzocaine (eg Benzoak) should, by default, further reduce the escape responses during thermal treatment and handling but, to our knowledge, its use has never been reported. Following the second level of anaesthesia, 'excitation', fish enter level three as characterised by three planes of 'anaesthesia.' Plane one, 'light anaesthesia', is commonly used for aquaculture procedures, such as the weighing of fish and close visual inspection (Sneddon 2012). Apart from loss of equilibrium and no activity, as common of all three planes of anaesthesia, under light anaesthesia fish will maintain regular heart rate, display reflex responses and show reduced gill ventilation rate and muscle tone. Thus, induction of light anaesthesia could be effective for improving salmonid welfare during thermal treatment.

In experimental work, the use of thermal treatment vessels can jeopardise the scientific outcome, as the effect of delousing may override that of the experimental treatment in question. This study describes a manual thermal delousing technique undertaken to treat groups of small salmon post-smolts in an experimental trial conducted in sea cages. Based on previous testing of single fish in thermal treatment, a simple small-scale system for thermal delousing was constructed and tested, in which light anaesthesia was applied prior to thermal exposure.

Materials and methods

The experiment was carried out at the Austevoll research station of the Institute of Marine Research, Norway, between 7th and 9th November 2018, and was approved by the Norwegian Food Authorities as an act of emergency due to high lice load on the fish. The experiment was supervised by a trained aquaculture veterinarian.

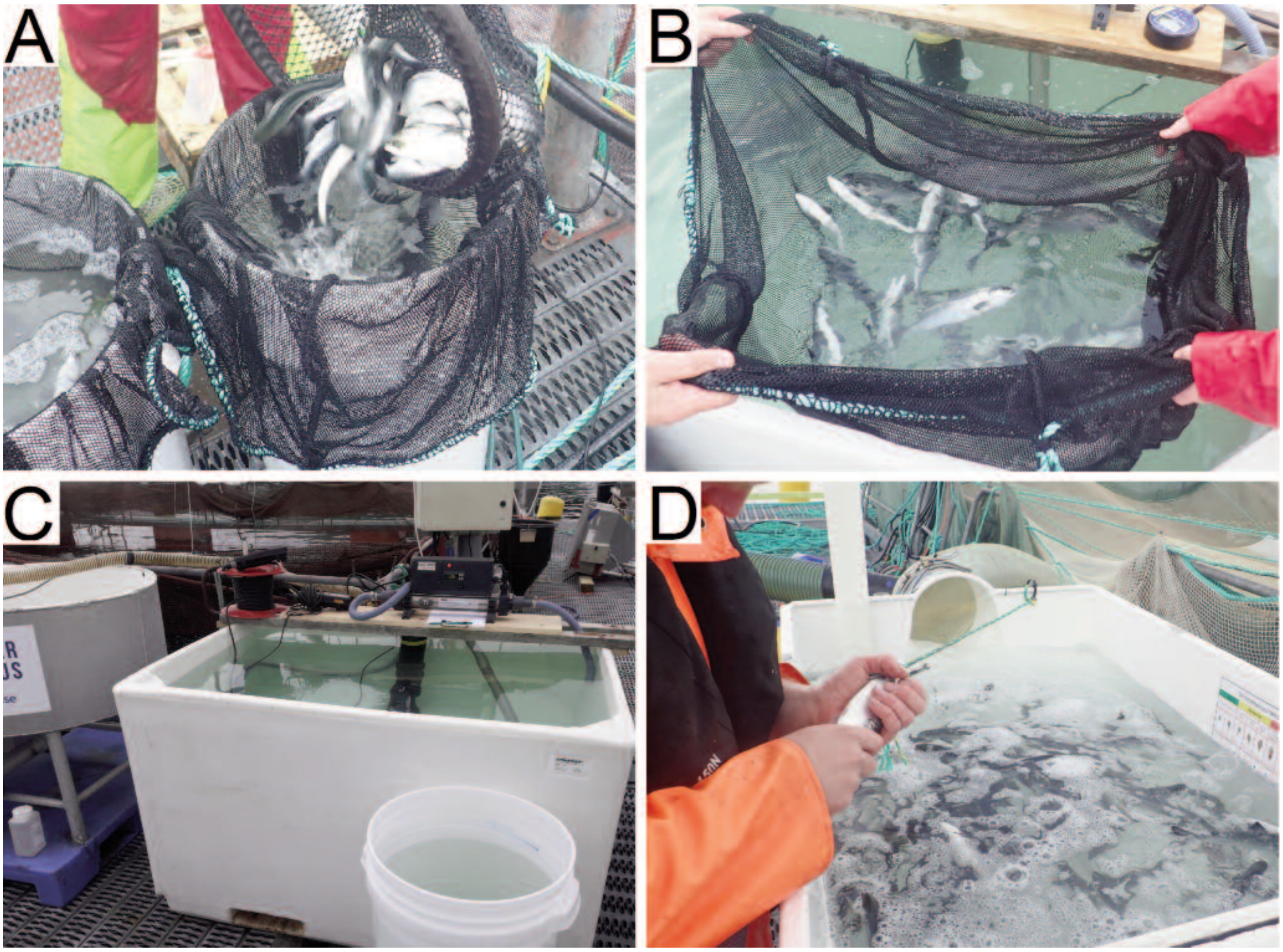
Salmon of the Salmobreed strain had been sea-transferred as 0+ smolts on the 5th October 2018 with 1,110 fish in each of four cages measuring 12 × 12 × 14 m (length × width × depth) as part of a growth experiment. On the 5th November it was discovered that the fish (n = 20 fish from one cage counted) had a mean number of 6.4 mobile lice, including 1.1 adult females, and damage to the mucus layer was observed for most fish. Delousing was carried out between the 7th and the 9th of November via manual labour and equipment which most aquaculture research facilities have available.

The fish were fasted for two days prior to thermal treatment. The first part of the procedure entailed crowding the fish by raising the cage net (12 × 12 × 14 m) to facilitate netting. Groups of ~30 fish were netted over to an anaesthetic tank (100 l) with seawater of ambient sea temperature (10°C) and the recommended anaesthetic dose of metacaine (FinquelVet, 100 mg l^{-1}; Western Chemical Inc, Washington DC, USA) was administrated (Figure 1[A]). A net (1 × 1 m; mesh width: 8 mm) was lowered into the anaesthetic tank to allow easy transfer and holding of the fish during thermal treatment. When the fish were anaesthetised, after ~1 min, the net containing the fish was lifted and lowered into the nearby thermal treatment tank (1.5 × 1 × 0.80 m; length × width × depth; Figure 1[B]). The water for thermal treatment was heated and temperature maintained by a circulating pump connected to a heat exchanger with thermostat set to 34°C (Figure 1[C]), and oxygen levels were maintained at > 100% saturation by continuous diffusion of oxygen into the water. The fish were kept in thermal treatment for 30 s at temperatures logged in the range between 33.5–34°C, before being transferred and released into a recovery tank with running seawater of ambient temperature (Figure 1[D]). During recovery, research and veterinary personnel scrutinised the fish for lice content and bodily damage, in accordance with the Salmon Welfare Index Method (SWIM; Stien *et al* 2013). At full recovery, the fish were channeled back into the sea cage through a pipe (water slide). The delousing procedure itself for one sea-cage group of 1,100 fish lasted for approximately 3 h when alternating between two anaesthetic tanks. Lice were counted both before and after treatment in two of the cages.

Results

The fish showed normal escape responses to being netted and confined during anaesthesia. When anaesthetised to a level where the fish were motionless and showed reduced respiration rate, they were transferred to the thermal treatment tank. The fish were rendered motionless during the initial 20 s of the thermal treatment, and showed mild burst behaviour during the last 10 s. A small number of fish that were only partly anaesthetised (loss of equilibrium but with minor motions of the tail) showed burst behaviour immediately after being introduced to the thermal treatment. The fish recovered equilibrium within a minute in the recovery tank. Lice counts confirmed that the thermal treatment had been effective at removing lice at the mobile life-stages. Immediately prior to thermal treatment, fish were randomly sampled from cage 1 (n = 8) and cage 2 (n = 20) and inspected for sea lice. Mean number per fish of pre-adult lice and adult males were 3.3 and 5.3 for cages 1 and 2, respectively, and the mean number of adult female lice was 1.5 per fish in both cages. To assess the delousing effect, randomly sampled fish from cage 1 (n = 80) and 2 (n = 18) were inspected for sea lice immediately after thermal treatment. For cages 1 and 2, the mean number of pre-adult lice and adult males per fish were, respectively, 0.05 and 0.1, with 0.05 and 0.06 adult females per fish for the same two cages. This corresponds to mean success rates of 98% for pre-adult lice and adult males and 96% for adult females. Minor scale loss (SWIM level 3) observed in the anaesthetic tank, as inflicted by netting, was the only observed bodily damage to the fish. Hand-feeding was carried out approximately 3 h after delousing finished, and a strong appetite response was observed visually with fish swimming to the surface to feed. This was also repeated at subsequent meals over the following days, ie reflecting behaviour seen prior to delousing and using an intensive feeding rate (250 g per ton fish per min), fish sustained high feeding activity levels at the surface beyond their estimated table ration. Mortality in the week post-treatment was two fish per cage in two of the four cages. Mean (± SEM) accumulated mortalities the month before vs after delousing was 0.64 ± (0.12) vs 0.75 (± 0.33) percent of the total number of fish per cage.

Figure 1



Steps of small-scale thermal delousing, showing (A) fish netted from the sea-cage being placed into anaesthesia, (B) anaesthetised fish being held in thermal treatment, (C) the thermal treatment tank with the heat exchanger for maintenance of temperature, with the anaesthetic tank and the recovery tank situated in close proximity to minimise exposure of fish to air, and (d) thermal-treated fish from the recovery tank being scrutinised for lice and physical damage prior to release back into the sea-cage.

Discussion

Unsurprisingly, the behavioural response to thermal treatment in anaesthetised salmon was much lower than that described in fully awake or sedated salmon (Adams 2019; Nilsson *et al* 2019), suggesting that anaesthesia can relieve stress and collisions caused by thermal treatment. The apparent fast recovery and low mortality after delousing suggest that the fish coped well with the full procedure, including handling and anaesthesia.

Metacaine was used in the present study, as it is a common anaesthetic used for farmed salmon in Norway and provides more effective immobilisation and recovery time in Atlantic salmon compared with anaesthesia by isoeugenol (AQUI-S) (Kiessling *et al* 2009). Anaesthetics do trigger physiological stress in Atlantic salmon and reduce respiration and circulation, with metacaine triggering a faster and stronger cortisol response than other common anaesthetic agents, but also a faster recovery rate (Kiessling *et al* 2009; Zahl *et al* 2010). Still, anaesthesia is assumed to alleviate severe handling stress in salmonids (Zahl *et al* 2011), which is in line with the present observation. Whether use of local anaesthetics (block nerve activity) which immobilise fish will also act as an analgesic (block nociceptive transmission) remains debated (Chatigny *et al* 2018), but compelling evidence is shown in rainbow trout (Mettam *et al* 2011) and, analogous to fish, in xenopus tadpoles (*Xenopus laevis*) (Ramlochansingh *et al* 2014). The salmon in the present study began to recover during the last 10 s of the thermal treatment, which suggests a need for anaesthetic refinement. Specifically, this might entail metacaine dosage adjustment, use of other anaesthetic agents, combination anaesthesia preferably with a known analgesic agent for fish, or pre-anaesthetic sedation (Zahl *et al* 2011; Sneddon 2012). Regarding the environmental implications of treating potentially large quantities of fish, waste management of anaesthetic water needs to take into account the type of anaesthetic agent and the applied volume.

For use in smaller fish groups as common in experimental trials, the current set-up for thermal treatment proved very useful as well as cost effective compared with hiring a commercial treatment vessel. The set-up may be easily scaled up to effectively include larger and greater numbers of fish, and automation in fish transfer between the various treatment baths (anaesthesia, thermal treatment and recovery). For use in commercial farming (up to 200,000 fish and 1,000 tons per cage), anaesthesia can be technically challenging and time consuming. There are difficulties associated with safe anaesthesia of very large numbers of fish, as well as active transfer in and out of treatment, and post-treatment recovery from anaesthesia prior to entry to the cage, to avoid fish piling up either onboard the vessel or on the bottom of the sea-cage. In other words, use of anaesthesia in commercial delousing would demand a rethink of existing methodologies and constructions at all procedural levels.

Animal welfare implications

Based on our demonstration of a simple set-up for thermal treatment, which was successful with regards to removing mobile sea lice and avoiding negative welfare effects, we would encourage further use and refinement of thermal delousing in anaesthetised experimental fish, including methods and choice of anaesthesia. Such work will aid in refining experimental set-ups and thereby promote the scientific outcome for research trials in which salmonids are susceptible to sea lice infestation. Further, we encourage detailed and controlled experimental studies on the effect of anaesthesia prior to treatment for lice, and innovation towards use in commercial delousing vessels.

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