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Technical note: Modifying Atlantic salmon (*Salmo salar*) jumping behavior to facilitate innovation of parasitic sea lice control techniques[1]

T. Dempster,*[2] T. S. Kristiansen,† Ø. J. Korsøen,†‡ J. E. Fosseidengen,† and F. Oppedal†

*Sustainable Aquaculture Laboratory–Temperate and Tropical (SALTT), Department of Zoology, University of Melbourne, 3010 Victoria, Australia; †Institute of Marine Research, NO-5984 Matredal, Norway; and ‡Centre for Research-Based Innovation in Aquaculture Technology (CREATE), SINTEF Fisheries and Aquaculture, NO-7465 Trondheim, Norway

ABSTRACT: Industrial salmon farms are reservoirs of parasitic sea lice (*Lepeophtheirus salmonis* and *Caligus* spp.), which causes both production inefficiencies and contributes to population-level declines of wild salmon and trout. Current control methods vary in effect and stimulate controversy by the discharge of chemicals into the environment. An alternate control method uses a thin, chemical-infused oil layer on the sea surface. As farmed salmon jump through the surface, the treatment makes contact with the lipophilic carapace of sea lice and kills them. To enhance the effectiveness of this method, we tested whether the natural jumping behavior of salmon could be increased and directed. In a 2,000-m³ experimental sea-cage, we removed the ability of groups of salmon to access the surface for different periods (0 to 48 h) and measured their surface behaviors after the surface became accessible again. Surface removal for 24 and 48 h induced 93% of salmon to jump in the 2 h after surface access was reinstated, a result that differed ($P < 0.001$) from the shorter duration (0 to 12 h) treatments. Salmon without surface access for 24 and 48 h jumped 2 to 3 times more often ($P < 0.001$), and made their first jump 2 to 3 times sooner ($P = 0.003$) on average after surface access became available than salmon in the shorter duration treatments. Our results indicate that removal of surface access for short periods may lead to loss of air from the physostomous swim bladder and cause negative buoyancy. This creates a behavioral drive for salmon to jump, swallow air and fill their swim bladders once surface access is reinstated. By combining the increased jumping behavior induced by this technique with a floating, oil-infused treatment, efficiency of sea lice treatments may be improved and treatment chemicals can be recollected, thus decreasing environmental pollution.

Key words: aquaculture, *Caligus*, *Lepeophtheirus*, lice, salmon, trout

INTRODUCTION

Salmon aquaculture in open net pens is widespread, yet controversial due to its significant environmental impacts (Naylor et al., 2000; Krkošek et al., 2007). The parthenogenetic copepod parasites *Lepeophtheirus salmonis* and *Caligus* spp., or "sea lice," infest the skin of farmed salmon, causing costly outbreaks (Costello, 2009a). Salmon farms act as reservoirs of sea lice and thereby increase their infection levels on wild salmon and trout, contributing to population declines in Europe and North America (Krkošek et al., 2007; Costello, 2009b). Controlling sea lice in salmon farms is thus critical for both farmed salmon production and wild salmon conservation.

Current methods to remove sea lice, including medicated feeds and chemical baths, are partially effective. Both techniques involve the loss of toxic chemicals to the environment, which affect nontarget organisms (Burridge et al., 2010). An alternate sea lice control method uses a thin chemical-infused oil layer placed on the surface (Boxaspen and Holm, 2001). As farmed salmon display their natural behavior and jump through the oil layer on the surface, the treatment makes contact with the lipophilic carapace of sea lice. Collection
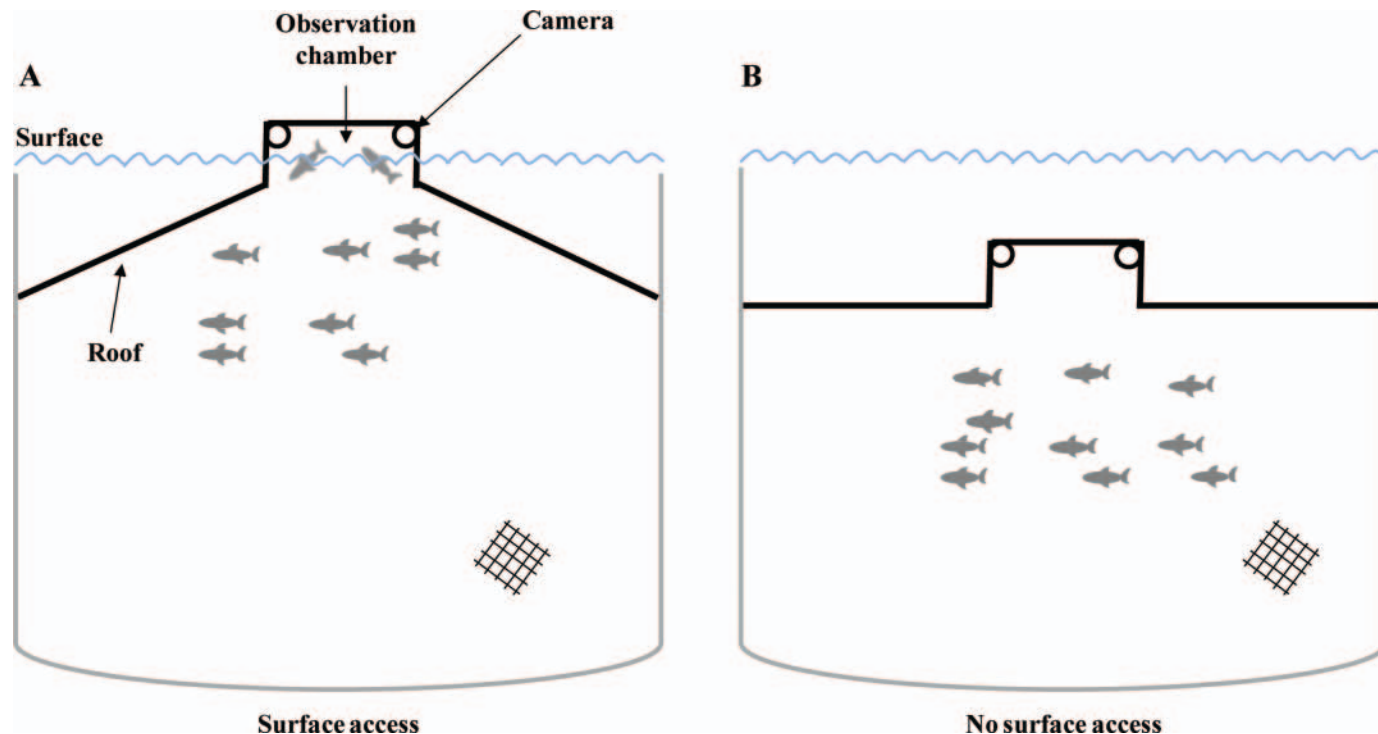
Figure 1. Schematic diagram of the 2,000-m³ net cage (12 m × 12 m × 14 m depth) used as the experimental unit in the experiment conducted in Masfjorden, Norway. The net cage was modified by the inclusion of a roof sewn to the cage walls at 5 m depth. A circular observation chamber (3.5 m diameter, 1.5 m high) instrumented with 4 video cameras was sewn into the middle of the roof. In the surface access position (diagram A), salmon could jump within the area of the observation chamber. In the no-surface-access position (diagram B), the roof and observation chamber were lowered and no jumping was possible. Color version available in the online PDF.

of the oil after treatment is possible using an oil boom and skimmers. However, this method is currently ineffective because farmed salmon jump too infrequently to enable efficient application (Furevik et al., 1993; Boxaspen and Holm, 2001).

The objective of this research was to test whether the natural jumping behavior of salmon can be increased and directed for effective treatment of sea lice. Salmon have a physostomous swim bladder system that is used for buoyancy regulation (Berenbrink et al., 2005). To fill the swim bladder, salmon jump and break the surface, and swallow air in the process (Furevik et al., 1993). We tested the hypothesis that removing the ability of salmon to access the surface for short periods would motivate salmon to jump once the surface became accessible again.

MATERIALS AND METHODS

All experimental work using fish was conducted in accordance with the laws and regulations controlling experiments and procedures on live animals in Norway, following the Norwegian Regulation on Animal Experimentation 1996.

Location and Experimental Setup

The experiment was conducted in Masfjorden, western Norway (60° N) from August 11 to September 16, 2010 (hereafter referred to as the experimental period).

A standard commercial-scale net cage (12 m × 12 m × 14 m depth; ≈2,000 m³) was modified for use as the experimental net cage. The experimental net cage was modified by adding a roof of netting 5 m below the top of the cage. A circular observation chamber (3.5 m diameter, 1.5 m high) was sewn into the middle of the roof. In the "surface access" position (Figure 1A), only the area inside the observation chamber was open to the surface. In the "no surface access" position (Figure 1B), the midpoint of the cage roof was located at approximately 5 m depth, and cage volume was reduced to approximately 1,500 m³. All treatments were made within the single experimental net cage, which was the experimental unit.

Experimental Design

To test whether different periods without access to the sea surface modified the extent and frequency of salmon jumping behavior, we conducted an experiment where different groups of fish were subjected to surface removal for different periods within the experimental net cage. The design included a control (**CN**), where fish had access to the surface at all times; a procedural control (**PC**), where surface access was removed and immediately reinstated; a 2-h surface access removal (**2H**); a 12-h surface access removal during the daytime (**12HD**); a 12-h surface access removal including 1 night (**12HN**); a 24-h surface access removal (**24H**); and a 48-h surface access removal (**48H**). All 7 experi-

mental treatments were replicated 3 times with a group of 10 new fish used in each replicate (i.e., 21 different groups of 10 fish). Treatment replicates were interspersed across the 6-wk experimental period to avoid confounding due to possible environmental variability. The order of treatment replicates was 2H, CN, 24H, 48H, PC, 48H, 2H, 2H, PC, 24H, 48H, 12HN, 24H, 12HD, CN, 12HN, PC, 12HD, CN, 12HN, 12HD.

Experimental Fish

Atlantic salmon (*Salmo salar* L.) of the Aquagen strain were on average $1{,}075 \pm 18$ g in BW (range: 535 to 1,840 g) and 46.2 ± 0.2 cm in total length (range: 36 to 55 cm), with no difference in average BW or lengths among treatments (1-way ANOVA; df = 6, 203; $P >$ 0.1). All fish were sourced from a standard 2,000-m^3 production net cage 40 m distant from the experimental net cage. A total of 210 fish were used in the experiment (7 treatments × 3 replicates × 10 fish per replicate). For each replicate, 10 fish were captured by dip net from the production net cage, anaesthetized with Benzoak VET (dose: 10 mL/100 L of seawater), and tagged with individually color-coded external tags (11 cm T-bar tags, Hallprint, Adelaide, Australia). The 10 fish were transferred to the experimental cage, released, and allowed to recover for 18 to 24 h before a treatment was made. Fish were fed in the production cage until the time they were removed from the production cage and transferred to the experimental net cage. Thus, fish within any treatment replicate were off feed only for the recovery period and the duration of that specific treatment replicate. Sea lice (*Lepeophtheirus salmonis* or *Caligus* spp.) were not present on the experimental fish.

Surface Jumping Behaviors

Jumping behavior at the surface was monitored with 4 cameras (VNSVUC-Z10, Scan Secure AS, Horten, Norway, http://www.secure.no) mounted to the inside of the observation chamber with all 4 videos viewed simultaneously using Geovision (Scan Secure AS, Oslo, Norway) software. Cameras gave 100% coverage of the water surface within the observation chamber and provided multiple angles from which to view jumping salmon so that all 10 individual fish could be accurately identified from their unique color-coded tag. Each time an individual jumped, the tag color and the time elapsed after access to the sea surface was reinstated were recorded.

Environmental Variables and Statistical Analyses

Throughout the experimental period, an online probe (YSI model 30–50 ft, YSI, Yellow Springs, OH) was used to determine salinity and temperature from 0 to 15 m depth at a reference point close to the experimental net cage. One-way ANOVA was used to compare the percentage of salmon that jumped and the number of times salmon jumped in the 2-h period after surface access was reinstated among the 7 experimental treatments. One-way ANOVA was used to compare the average time to first jump among the 2H, 12HD, 12HN, 24H, and 48H experimental treatments because fish did not jump in the CN and PC treatments. Multivariate ANOVA tests were used to compare temperate and salinity at 1, 5, 10, and 15 m among the 7 experimental treatments.

RESULTS AND DISCUSSION

Surface removal for 24 and 48 h caused 93% of salmon to jump (Figure 2A) in the 2 h after surface access was reinstated, which was greater ($P < 0.001$) than the percentage of salmon that jumped in the shorter duration (<24 h), PC, and CN treatments (Figure 2B). Salmon without surface access for 24 and 48 h jumped 2 to 3 times more often (Figure 2C; $P < 0.001$) and made their first jump 2 to 3 times sooner (Figure 2D; $P = 0.003$) on average after surface access became available compared with salmon in the shorter duration treatments. Salmon did not jump after the CN and PC treatments, suggesting that time without surface access caused the response. No mortality was recorded in the experiment. Environmental variables did not confound the experiment because temperate ($P = 0.18$) and salinity ($P = 0.73$) did not differ among the 7 experimental treatments at 1, 5, 10, and 15 m. The results support the hypothesis that removing the ability of salmon to access the surface for short periods stimulates salmon to jump once the surface becomes accessible again.

We speculate from these results that salmon lost sufficient air from the swim bladder to become negatively buoyant. This created a strong motivation to jump and swallow air once the surface became accessible. Surface removal for 24 to 48 h is unlikely to negatively affect salmon because appetite, growth, and condition are unaffected by surface removal for up to 22 d (Dempster et al., 2009). The most common current methods of removing lice from large salmon involve enclosing sea cages with tarpaulins or pumping the fish into wellboats, or both, and adding the chemical treatment. In both methods, fish are crowded, oxygen content is decreased, water quality degrades rapidly, fish are starved in advance, and they are exposed to the potentially irritating and harmful chemical treatment for 30 to 40 min. Salmon display a clear avoidance reaction to these medicines (Oppedal et al., 2011). Oxygen is normally added to prevent hypoxia, but sea lice removal is generally a stressful and potentially harmful procedure (Børu, 2009; Fridell and Alexandersen, 2009; Nilsen and Brun, 2009; Oppedal and Vigen, 2009a,b). After lice removal, many farmers have reported poor performance of the fish exemplified as poor appetite and growth, disease outbreaks, and increased mortality.
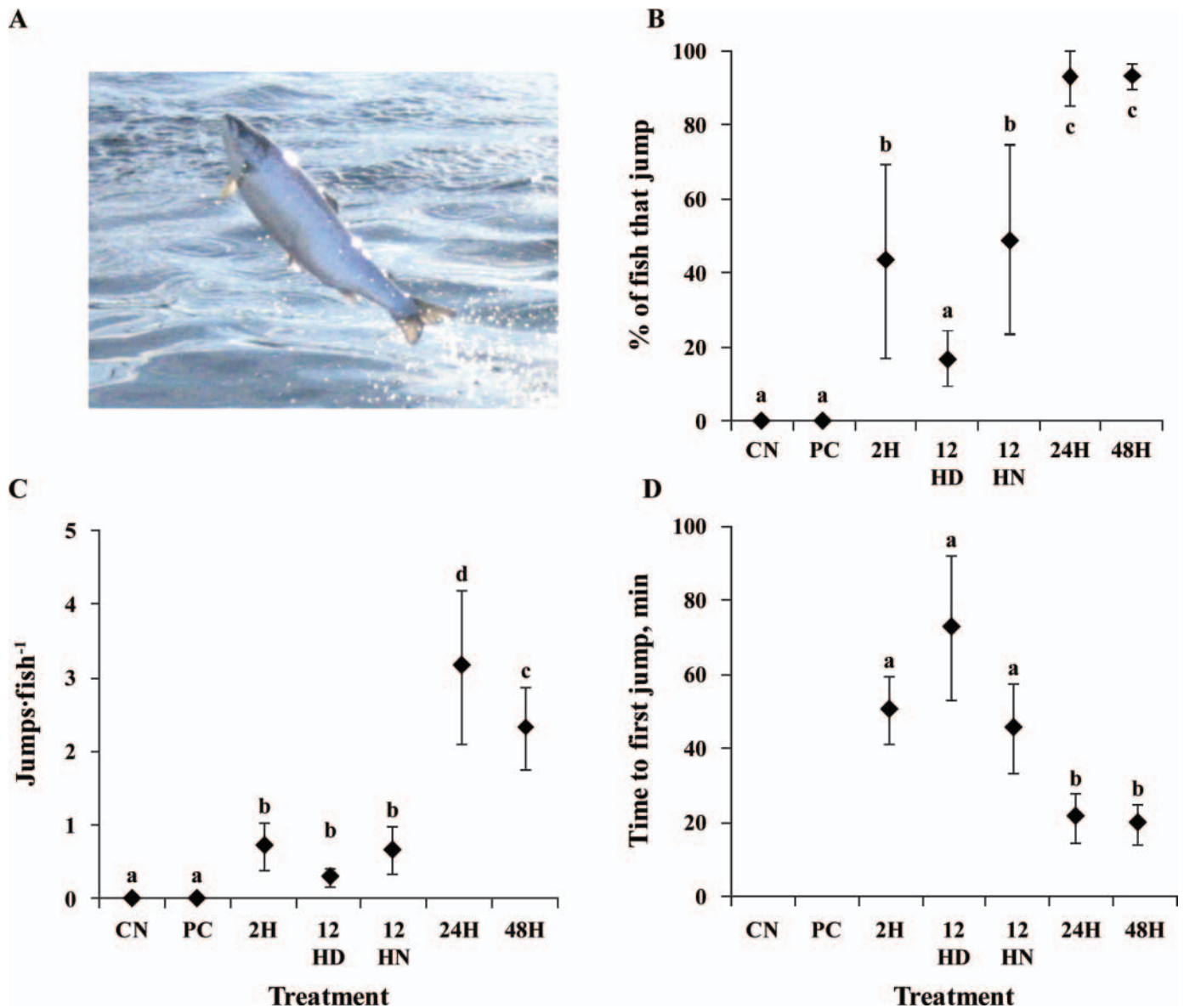
Figure 2. Typical jump of a farmed Atlantic salmon (*Salmo salar*; panel A). Percentage of individuals that jumped at the surface within a 2-h period after each treatment (panel B): CN = control, where fish had access to the surface at all times; PC = procedural control, where surface access was removed and immediately reinstated; 2H = 2-h surface access removal; 12HD = 12-h surface access removal during the daytime; 12HN = 12-h surface access removal including 1 night; 24H = 24-h surface access removal; 48H = 48-h surface access removal [mean ± SE; 3 replicates; different letters (a–d) indicate differences among treatments at $P < 0.05$, 1-way ANOVA with post hoc Student-Newman-Keuls tests]. Panel C shows the number of jumps per salmon (mean ± SE) during the 2-h period after each treatment, and panel D shows the time to first jump (mean ± SE) after surface access was reinstated. No data are shown for the CN and PC treatments in panel D because no salmon jumped. Color version available in the online PDF.

Combining the intense jumping response we have elicited with an oil-infused treatment (Boxaspen and Holm, 2001) provides a new method for sea lice removal. Salmon heavily infested with sea lice jump more frequently than unaffected salmon (Furevik et al., 1993), which may further increase the strength of the behavioral response. An existing oil-infused treatment using pyrethrum kills 90% of attached sea lice when salmon are exposed to it for 2 s (Boxaspen and Holm, 2001). Sea lice have a lipid layer in the cuticle (Kabata, 1974), meaning that lice will be effectively exposed to oil-based compounds, whereas salmon skin is lipophobic and will repel an oil-based treatment. The brief con-

tact with the oil, which occurs when salmon break the surface through jumping, leads to uptake of the oil and the treatment chemical by the lipophilic carapace of sea lice (Boxaspen and Holm, 2001). This effect makes the delivery technique specific with reduced potential to affect salmon compared with traditional methods. In addition to the existing pyrethrum-infused oil, new, effective topical treatments could also be adapted to the oil-based delivery technique. Although sea lice evolve resistance to chemical treatment agents (Lees et al., 2008), development of resistance should slow if treatment efficiency improves. Further, the new technique has no requirement to starve fish before treatment or

crowd salmon in poor water quality with decreased oxygen saturation, thus alleviating these negative effects on fish welfare.

Thus, our results provide the conceptual basis for a technique that may drive greater uptake of surface-based, oil-infused treatment chemicals by parasitic sea lice on farmed salmon. Greater use of this technique by the salmon farming industry may reduce the loss of harmful chemicals to the environment. Ultimately, more efficient treatment of sea lice on farmed salmon may reduce economic burdens to the industry and diminish sea lice burdens in wild salmon and trout.

LITERATURE CITED

Berenbrink, M., P. Koldkjaer, O. Kepp, and A. R. Cossins. 2005. Evolution of oxygen secretion in fishes and the emergence of a complex physiological system. Science 307:1752–1757.

Børu, B. 2009. Challenges related to dosages of bath treatment in net-cages. Norsk Fiskeoppdrett 6a:77–79. (In Norwegian).

Boxaspen, K., and J. C. Holm. 2001. The development of pyrethrum-based treatments against the ectoparasitic salmon lice *Lepeophtheirus salmonis* in sea cage rearing of Atlantic salmon *Salmo salar* L. Aquacult. Res. 32:701–707.

Burridge, L., J. S. Weis, F. Cabello, J. Pizarro, and K. Bostick. 2010. Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. Aquaculture 306:7–23.

Costello, M. J. 2009a. The global economic cost of sea lice to the salmonid farming industry. J. Fish Dis. 32:115–118.

Costello, M. J. 2009b. How sea lice from salmon farms may cause wild salmonid declines in Europe and North America and be a threat to fishes elsewhere. Proc. Biol. Sci. 276:3385–3394.

Dempster, T., Ø. Korsøen, F. Oppedal, O. Folkedal, and J. E. Juell. 2009. Submergence of Atlantic salmon (*Salmo salar*) in sea-cages: A potential short-term solution to poor surface conditions. Aquaculture 288:254–263.

Fridell, F., and A. Alexandersen. 2009. De-lice treatment in large net-cages—How should it be done? Norsk Fiskeoppdrett 6a:81–83. (In Norwegian).

Furevik, D., Å. Bjordal, I. Huse, and A. Fernö. 1993. Surface activity of Atlantic salmon (*Salmo salar* L.) in net pens. Aquaculture 110:119–128.

Kabata, Z. 1974. Mouth and mode of feeding of Caligidae (Copepoda) parasites of fishes, as determined by light and scanning EM. J. Fish. Res. Board Can. 31:1583–1588.

Krkošek, M., J. S. Ford, A. Morton, S. Lele, R. A. Myers, and M. A. Lewis. 2007. Declining wild salmon populations in relation to parasites from farm salmon. Science 318:1772–1775.

Lees, F., M. Baillie, G. Gettinby, and C. W. Revie. 2008. The efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* on farmed Atlantic salmon (*Salmo salar* L.) in Scotland, 2002–2006. PLoS ONE 3:e1549. doi:10.1371/journal.pone.0001549.

Naylor, R. L., R. Goldburg, J. Primavera, N. Kautsky, M. Beveridge, J. Clay, C. Folke, J. Lubchenco, H. Mooney, and M. Troell. 2000. Effect of aquaculture on world fish supplies. Nature 405:1017–1024.

Nilsen, A., and E. Brun. 2009. Mapping of the practical methods during delousing treatments. Norsk Fiskeoppdrett 6a:72–73. (In Norwegian).

Oppedal, F., T. Dempster, and L. H. Stien. 2011. Environmental drivers of Atlantic salmon behaviour in sea-cages: A review. Aquaculture 311:1–18.

Oppedal, F., and J. Vigen. 2009a. Atlantic salmon avoid the delicing treatments—if there is an option. A.-L. Agnalt, I. E. Bakketeig, T. Haug, J. A. Knutsen, and I. og Opstad, ed. Fisken og Havet 2–2009:157–159. (In Norwegian).

Oppedal, F., and J. Vigen. 2009b. Oxygen conditions during delousing. Norsk Fiskeoppdrett 6a:74–76. (In Norwegian).

References

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