The use of freshwater to control infestations of the sea louse *Lepeophtheirus salmonis* K on Atlantic salmon *Salmo salar* L.

Aims and Objectives

This study has the objective of investigating a practical solution to control sealice infestations in an environmentally friendly way by using freshwater as part of an integrated approach:

- **In addition to the exposure test for Atlantic salmon, freshwater bioassays will be done concurrently on *Lepeophtheirus salmonis* to assess the potential effects of freshwater on unattached lice.**

- **Groups of Atlantic salmon will be transferred to small-scale plastic cages which have been enclosed within a tarpaulin and filled with freshwater.**

- **The study will assess the efficacy of short-term exposure to freshwater and the effects of handling in removing all stages of sea lice from infested salmon.**

Methods

System:

- 125 m^3 (5x5x5m) plastic cages each fitted with a standard net.
- Transferred to small-scale land base and fitted with an enclosed tarpaulin.
- System was then filled with approximately 140 000 L^{-1} of freshwater
- After filling, the tarpaulin and cages were transferred back to small-scale

Transferering of fish (first study):

- 200 fish counted out and transferred into one of the holding cages contained within the tarpaulin through pipe system fitted with a grid to remove seawater

Transferering of fish (second study):

- Approximately 400 fish were transferred into each of the four cages within the tarpaulin. One of the cages was used to study the effects of different exposure times in freshwater

Methods – cont.

Exposures:

First study:

Group	Transfer time for group (minutes)	Additional time after last fish transferred (minutes)	Exposure time (minutes)
1	5	60	60 to 65
2	5	120	120 to 125
3	5	180	180 to 185
4	5	240	240 to 245
5	5	300	300 to 305
5c	5	300	300 to 305
SW control	5	0	0

Second study:

Group	Transfer time for group (minutes)	Additional time after last fish transferred (minutes)	Exposure time (minutes)
1	5	60	60 to 65
2	5	120	120 to 125
3	5	180	180 to 185
4	5	240	240 to 245
4c	5	300	240 to 245

Methods – cont.

Lice counting:

- **Pre-counting before exposure and after each exposure time**

- **Lice registered in 5 categories:**

***Lepeophtheirus salmonis*: Adult female**
***Lepeophtheirus salmonis*: Adult male**
***Lepeophtheirus salmonis*: Preadult**
***Lepeophtheirus salmonis*: Chalimus**
Caligus elongatus

Revival:

- **All sea lice were counted and removed from every fish and assessed for health status**

- **Each lice was categorized as either swimming and/or attached (normal behavior); swimming erratically and/or poor attachment (affected) and no activity (dead). After being categorized, the lice were transferred into a container with seawater. The status of each louse was assessed every hour after transfer. After two hours recovery in seawater the lice were discarded.**

Methods – cont.

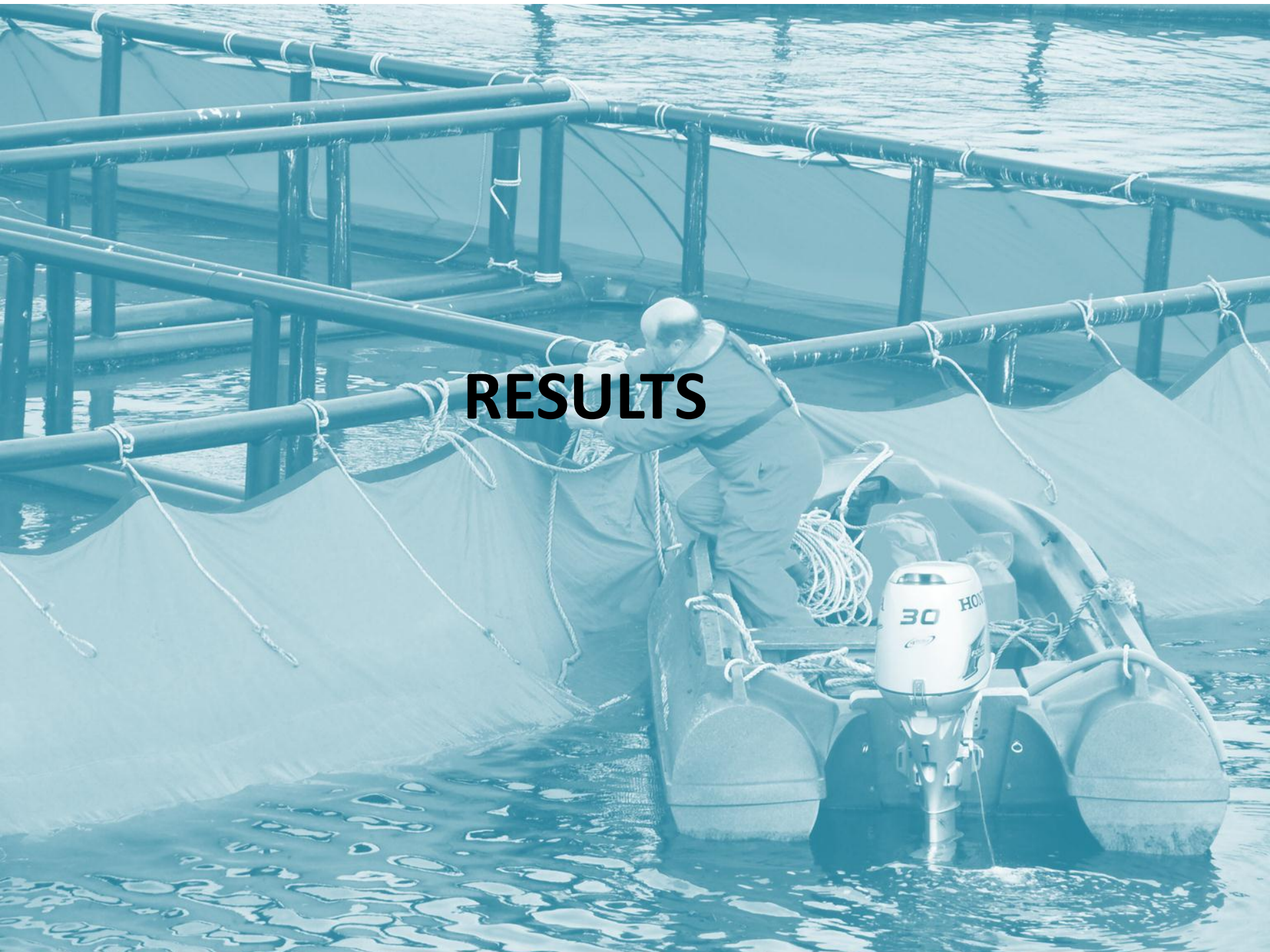
Bioassays:

- Lice collected the day before
- Minimum of 10 mature males and 10 mature females transferred into bioassay box supplied. Each box is composed of two separate chambers and a minimum of10 males were placed in one and 10 females in the other.
- Bioassays of 1, 2 and 3 hour exposure and seawater control
- After each exposure, lice were evaluated as follows:

Live: Normal behaviour, fast swimming if touched (- in a straight line). Capable of sticking to the wall of the dish.
Affected: Not normal behaviour. The swimming is slow, sometimes in circles, problems with sucking to the wall of the dish – they lose attachment and fall down if they are touched.
Dead: No response even when gently probed.

Water Quality:

- Oxygen saturation and concentration, salinity and temperature within the tarpaulin were continually monitored throughout both studies.

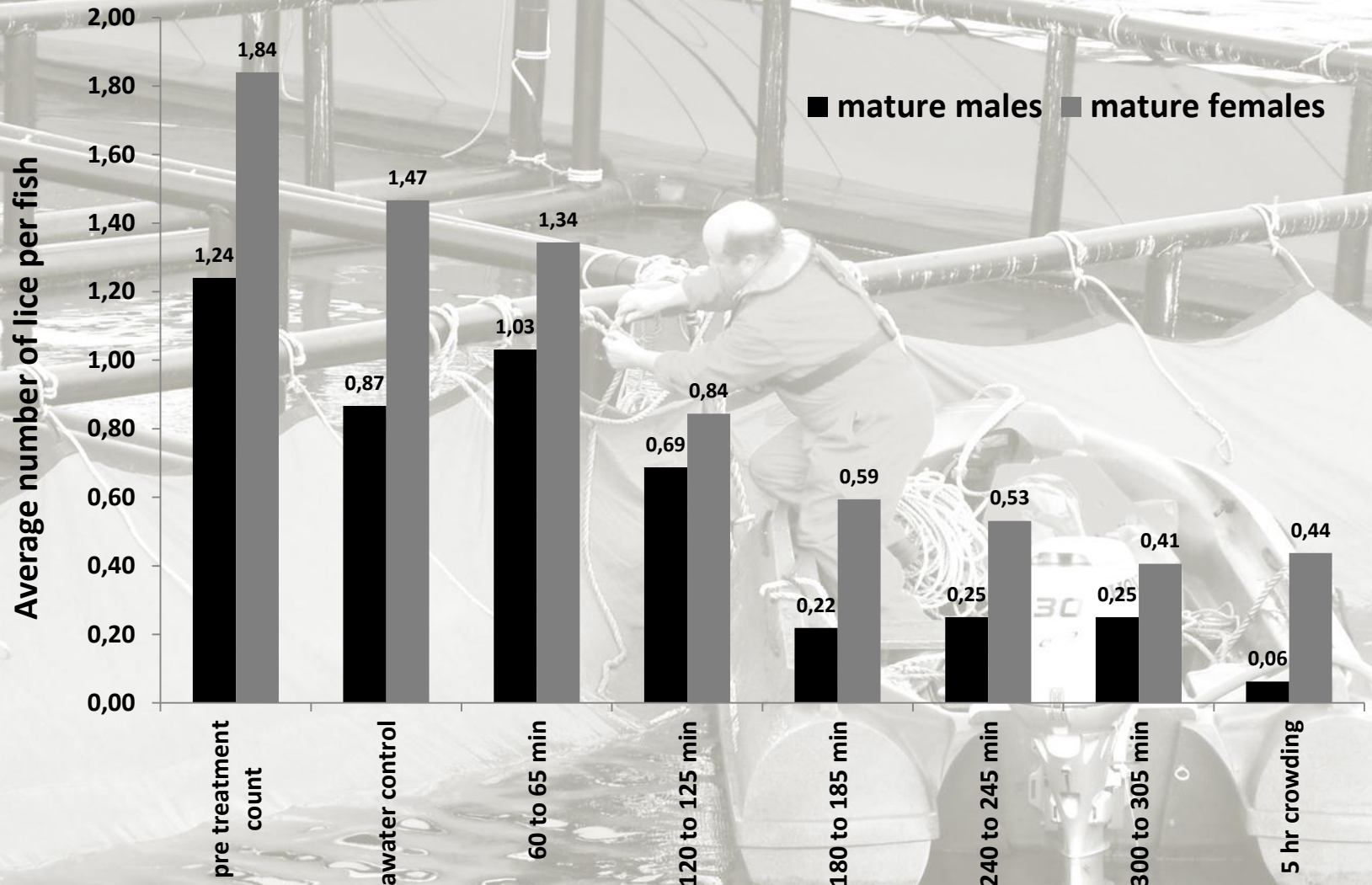


RESULTS

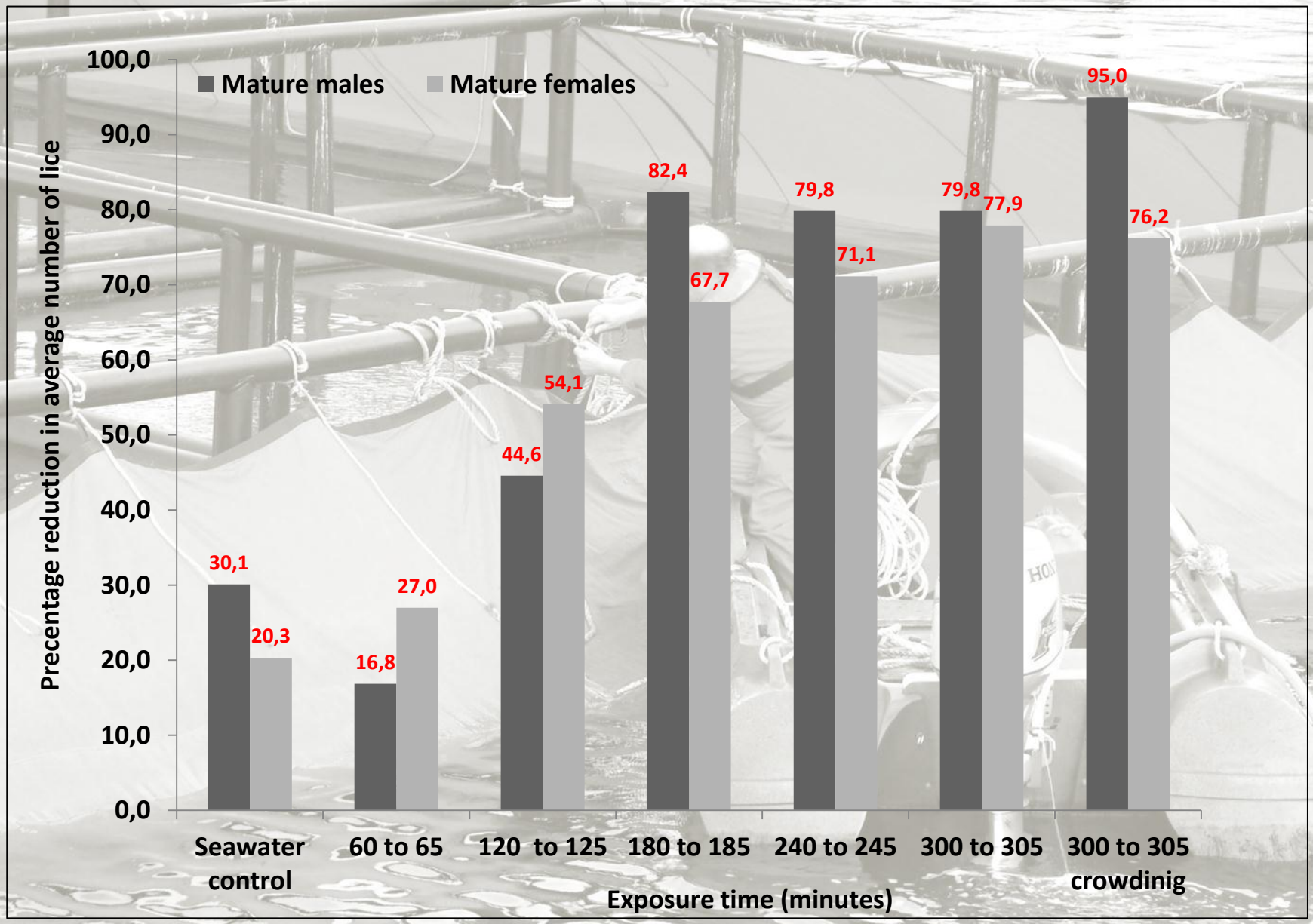
Bioassays: The potential effects of freshwater

	Mature females	total number of lice	live	Affected	Dead	% affected	% dead
BIOASSAY 1	1 hour exposure in freshwater	10	5	1	4	10,0	40,0
	1 hr revival in seawater		7	1	2	10,0	20,0
	2 hr revival in seawater		3	6	1	60,0	10,0
	Mature males	total number of lice	live	Affected	Dead	% affected	% dead
	1 hour exposure in freshwater	11	0	0	11	0,0	100,0
	1 hr revival in seawater		0	1	10	9,1	90,9
	2 hr revival in seawater		0	1	10	9,1	90,9
BIOASSAY 2	Mature females	total number of lice	live	Affected	Dead	% affected	% dead
	2 hour exposure in freshwater	14	2	0	12	0,0	85,7
	1 hr revival in seawater		2	1	11	7,1	78,6
	2 hr revival in seawater		0	1	13	7,1	92,9
	Mature males	total number of lice	live	Affected	Dead	% affected	% dead
	2 hour exposure in freshwater	14	0	0	14	0,0	100,0
	1 hr revival in seawater		0	0	14	0,0	100,0
	2 hr revival in seawater		0	0	14	0,0	100,0
BIOASSAY 3	Mature females	total number of lice	live	Affected	Dead	% affected	% dead
	3 hour exposure in freshwater	10	0	5	5	50,0	50,0
	1 hr revival in seawater		0	0	10	0,0	100,0
	2 hr revival in seawater		0	0	10	0,0	100,0
	Mature males	total number of lice	live	Affected	Dead	% affected	% dead
	3 hour exposure in freshwater	10	0	1	9	10,0	90,0
	1 hr revival in seawater		0	0	10	0,0	100,0
	2 hr revival in seawater		0	0	10	0,0	100,0
SW Control	Mature females	total number of lice	live	Affected	Dead	% affected	% dead
	4hr 30 min	10	10	0	0	0,0	0,0
	Mature males	total number of lice	live	Affected	Dead	% affected	% dead
	4hr 30 min	10	10	0	0	0,0	0,0

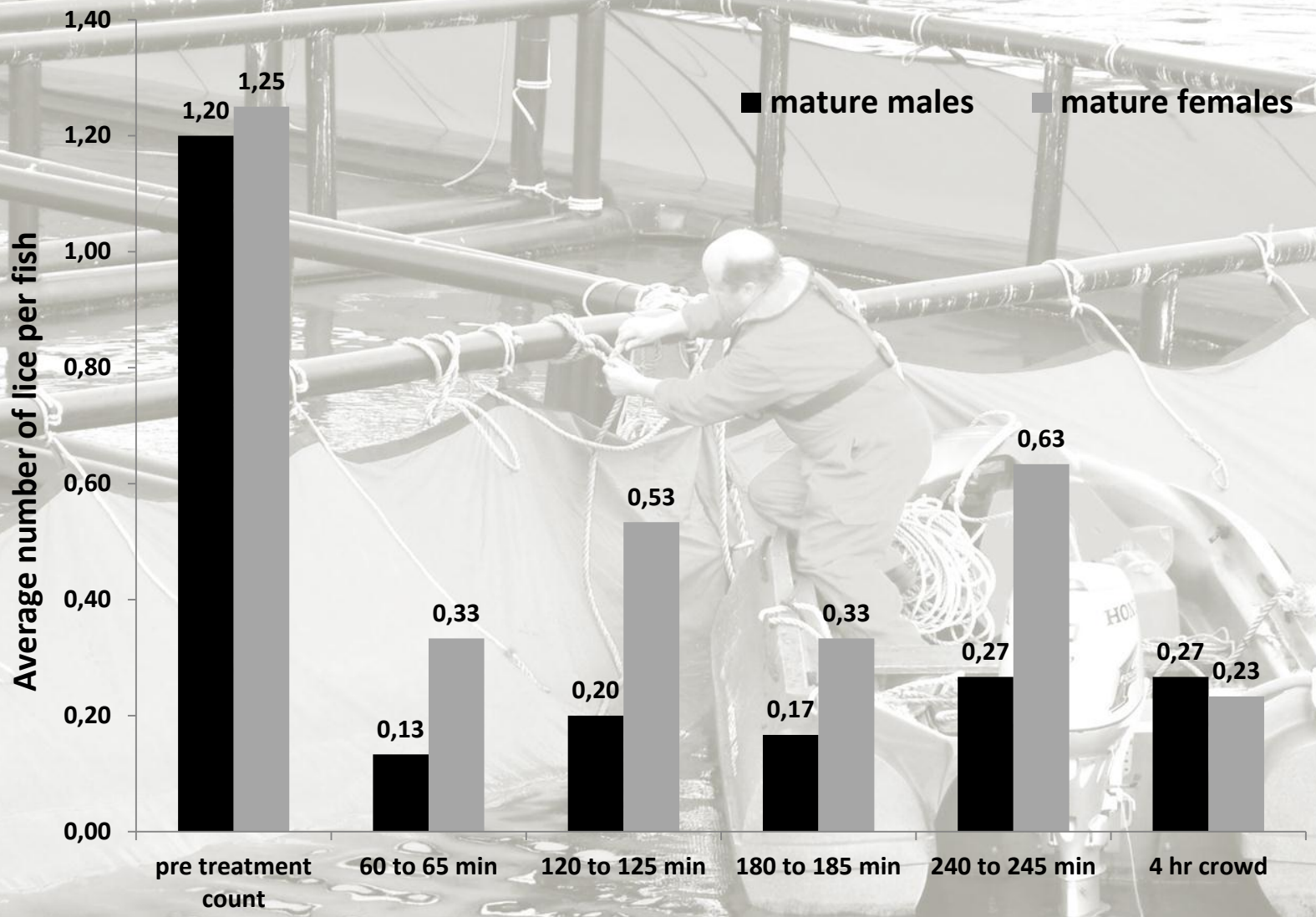
First study: reduction in average number of male and female lice after each exposure time



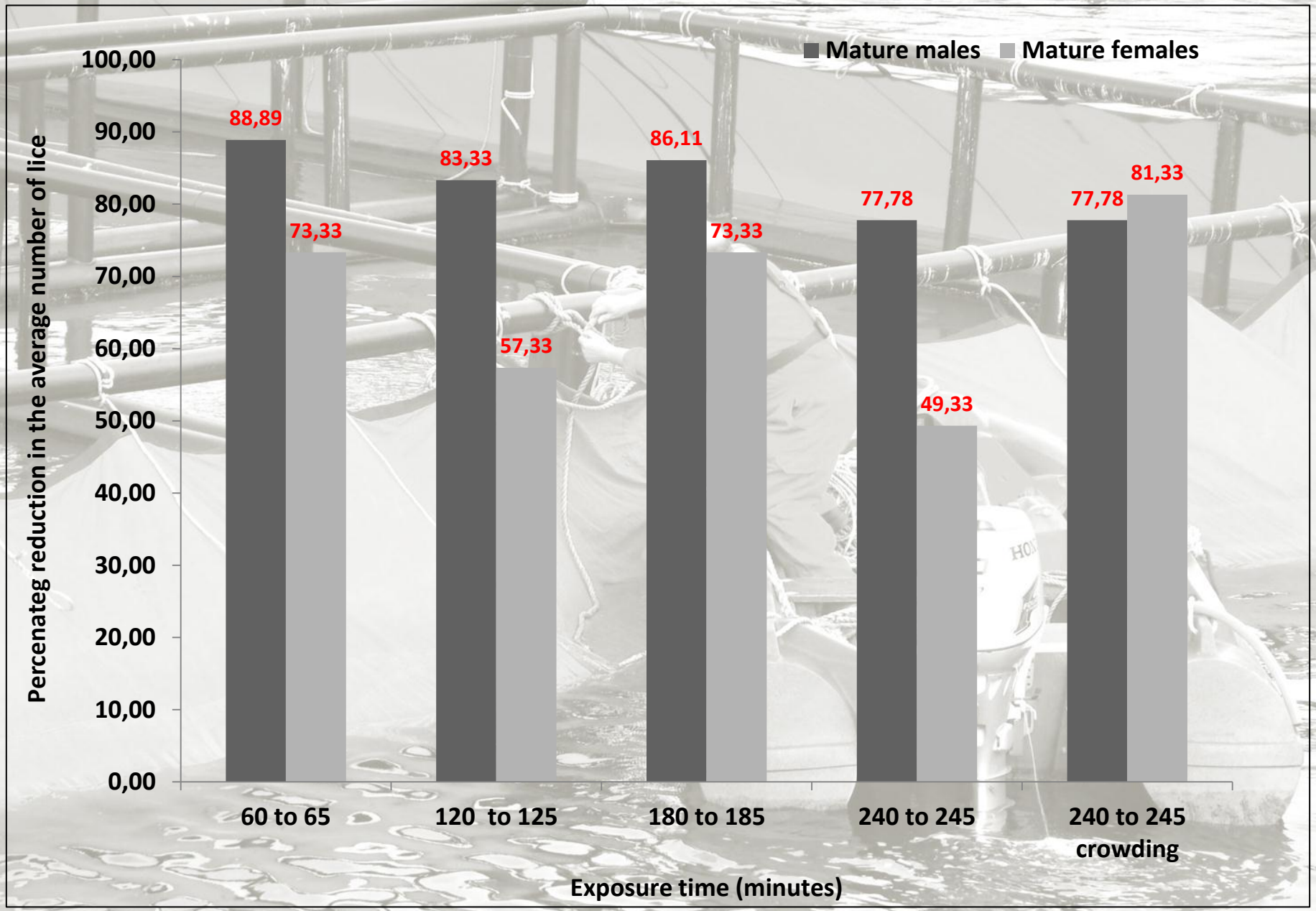
First study: Percentage reduction in mature male and female lice



Second study: The enhanced effects of crowding



Second study: Percentage reduction in mature male and female lice



Conclusions

- Very clear effect of freshwater in reducing both the number of adult male and female lice on infected salmon
- Significant reduction of both mature male and female lice by 82.4% and 67.7% respectively after three hours - FIRST STUDY
- Mature male lice seem to be more sensitive to freshwater compared to mature females
- Cessation in further reductions in attached lice after three hours.
- The lice that remained attached to the fish after 3 hours exposure may have a more secure attachment to the fish (particularly mature females).
- The lice that persist on the fish are more representative of the infectious population in terms of health status, reproductive capacity and/or age and are more resistant to the effects of freshwater than their conspecifics which were affected by exposure.

- No evidence of pre-adult lice on the fish from 60 to 65 minutes onwards during study 1 and a reduction from 0.5 to 0.03 pre-adults per fish after 4 hours exposure during study 2. This suggests that this stage of the sea lice life cycle is also sensitive to short term exposure to freshwater

The % enhanced effects of crowding

- First study: Mature male lice from group 5c (figure 4) which was subjected to an additional crowding of the fish prior to counting giving an enhanced percentage reduction of 95%.
- Second study: Percentage reduction of 89% in mature males and a 73% reduction in mature females - AFTER ONLY 1 HOUR EXPOSURE!
- Enhanced percentage reduction of 72% for males and 46% for females compared to the results after 1 hour exposure without crowding from study 1

This present study has clearly shown that in addition to copepodid susceptibility to low salinity conditions, adult lice are susceptible to low salinity levels under 1 ‰.

Conclusions - What next?

1. **The use of Well boats – commercial application.**

- Large-scale project
- More fish
- Technical challenges – water quality (filtering, buffering etc.)
- Freshwater intake
- Biomass
- Transfer of fish (in and out)
- Test regime

2. **Longer-term exposure**

- Establishment of freshwater layers in small-scale cages
- Use of desalination equipment/freshwater source
- Monitor sealice infestations, growth and behaviour of salmon
- Protocol established