Refinement and large-scale experiments

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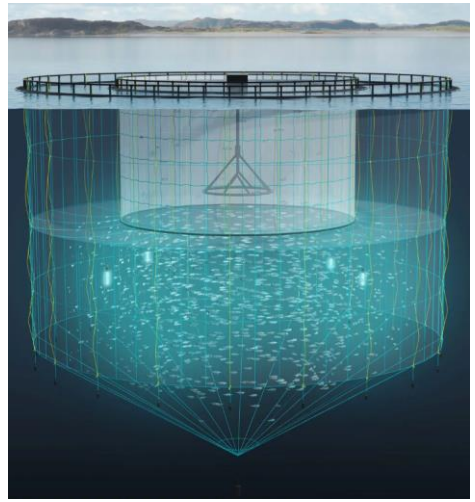
An optimal approach to develop applied concepts for fish

Pilot trial → Small-scale trial → Large/commercial-scale trial

Including

- new cage technologies,
- treatment methods,
- behavioural manipulations,
- welfare measures or scoring
- Autonomous monitoring or identification
- Etc… (from the experience of projects in my research group)

Biology/welfare-based concept development – Snorkel cages



A salmon louse prevention strategy…

Research-scale trials

- Stien et al., 2016
- Oppedal et al., 2017
- Oppedal et al., 2019
- Wright et al., 2018b
- Wright et al., 2019

Synergistic research

Samsing et al., 2016
Bui et al., 2013; 2016; 2020b
Geitung et al., 2020
Wright et al 2018a

Commercial-scale trials

Wright et al., 2017
Geitung et al., 2019
Bui et al., 2020
Oldham et al., 2023

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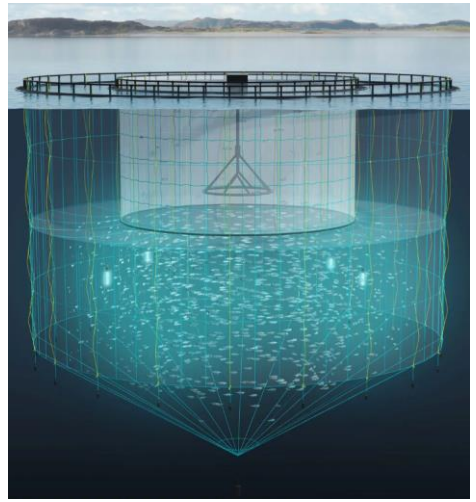


But.. Some things do not scale up the same

Biology/welfare-based concept development – Snorkel cages

Challenges at experienced at commercial cages:

- Structural integrity
- Oxygen levels and water quality
- Occasional increase in disease agents within snorkel area (e.g. algae, AGD)
- Exacerbation of deformities
- Water in the swim bladder





Refinement and applied research

Two examples –
research scale (and challenge with the results being 'relevant')
commercial scale (and challenges with enforcing 3 R's)

Aim: to investigate delousing methods and their effect on a) fish welfare, and b) effective removal of lice

Thermal treatments
Mechanical treatments
Freshwater baths

For new methods, MT require documentation that the method is suitable for fish welfare. Vague what this means – are results from small scale trials that show good welfare adequate for a commercial use?

A small-scale delousing experiment

The difference in the ‘experience’ a fish is exposed to in:

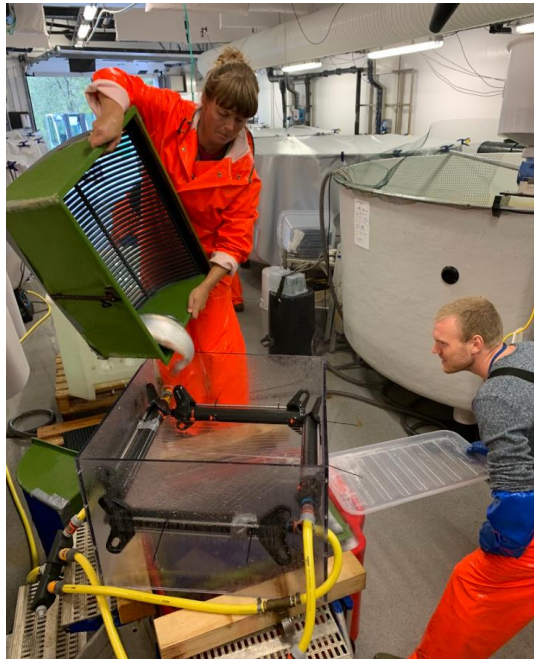
Research scale

- Lowering water level in tank
- Netting with dip net
- Mechanical treatment and/or
- Thermal treatment and/or
- Freshwater bath
- Netting/transfer to run, back into tank

Commercial scale

- Crowding
- Pumping
- Mechanical treatment or
- Thermal treatment or
- Freshwater bath
- Pumping back into net pen

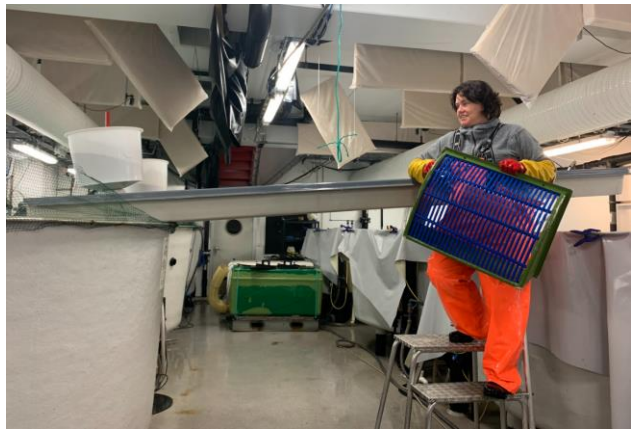
Research scale







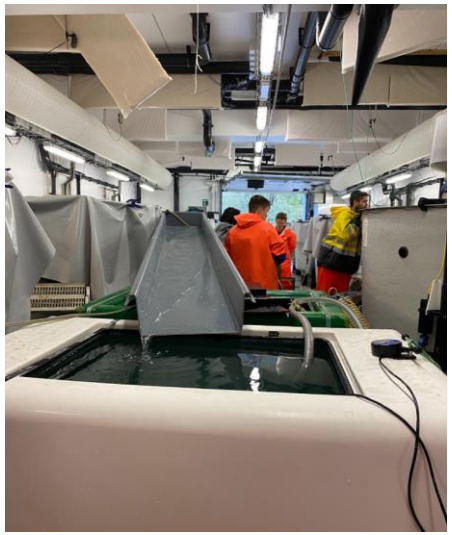


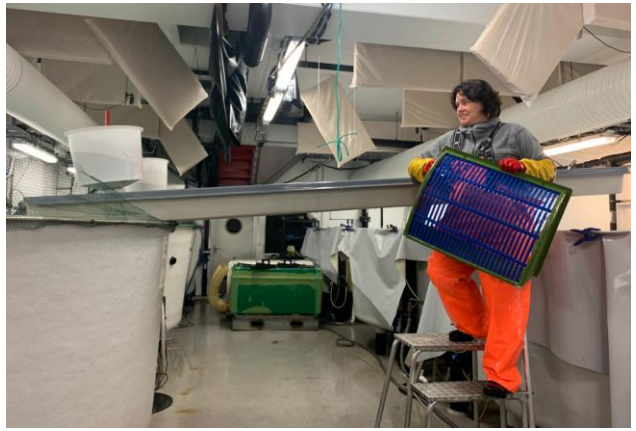


Refinement in the experiment

Measures to optimise the welfare experience of the fish

- Reduce netting
  - By using runs
  - Releasing fish from the holding tank
  - Thermal exposure in ‘baskets’ instead of nets or re-netting
- Light sedation prior to moving, or prior to catching for samples
- Treating single fish at a time for better control (mechanical and thermal)





BUT the more we do to Refine the experiment, the more it moves away from what fish experience in a commercial setting.

Commercial practice







Realistic conditions?

Often, we are criticised for doing small-scale experiments where the results will not be translatable to commercial practices (especially with relation to delousing)

Delousing of rainbow trout

Case study:
Does a ‘new’ combination delousing method (shower with warm water) have a negative welfare effect on trout?

- Ideally, one should run a pilot test first. Small scale, in tank facilities.
- OR test only the ‘new’ part of the method?
- Include different factors – ambient temperature, fish size, etc.
- Gradually scale up (tanks – small sea cages – commercial cage)

➢ Does what you see at small scale, actually scale up when applied to commercial? Will the same challenges be present in both scales?

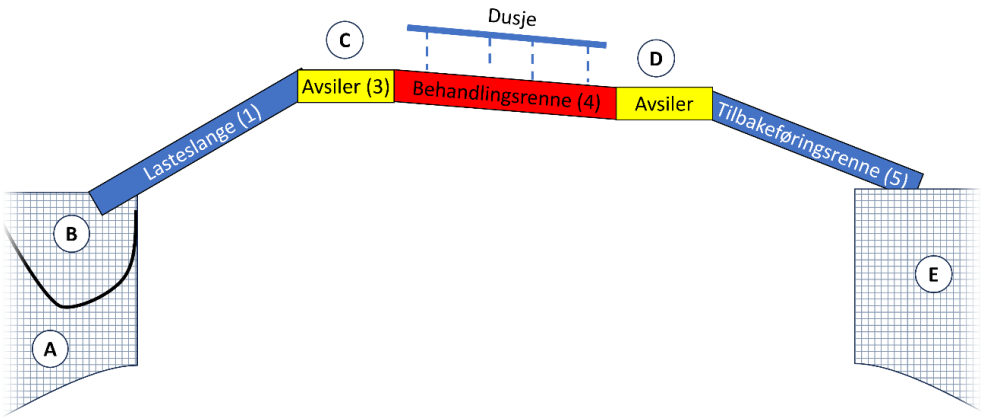
Limitations of a small-scale study

- Slow development process compared to the demand of the industry. They may seek alternative ways to get a method approved faster
- Does isolating the effect of the one treatment (i.e. warm water shower) and other aspects of the process, separately, reflect the accumulative burden of the «whole experience» (i.e. crowding, pumping, warm water shower, draining, pumping back into a new cage). How confident would we be in the results to then make a recommendation that would possibly allow industry to use the method?

Delousing of rainbow trout

Table B1. Experimental set-up for testing the method (number of cages). The numbers in the table indicate the number of replicate cages to be included in the experiment. Each replicate cage will treat ~1000 fish (of which 80 will be sampled for lice counts and welfare), therefore total N fish = 48 000.

Sea Temperature	>0.5 - 1.5 kg	>1.5 - 3 kg	>3 kg
7-11°C	4	4	4
>11 - 16 °C	4	4	4
< 7 °C	4	4	4
> 16 °C	4	4	4



Planned method:

- Fish crowded, only 1000 pumped
- Temperature gradually increased (Refinement)
- Sampled at multiple points along the run
- Held in a ‘recovery net’ for 1 hour
- Monitor mortality

- Compare different stages of the treatment
  - Status prior to treatment (A)
  - Effect of crowding and pumping (B vs C)
  - Delousing effect (C vs D)
  - Procedural impact on welfare (B vs D)

Refinement in the experiment

During the treatment:

- Pumping speed
- Gradual increase in treatment temperature
- Experienced assessors (to make sampling rapid)

Risk Assessment

We outlined criteria that

a) Had to be satisfied before the treatment could begin
b) Would initiate a change in operations
c) Had to not be exceeded during treatment (otherwise treatment should cease immediately)

How much control does a researcher actually have over these criteria in reality?

Challenge: Three parties are involved here

1) farmers that are actually operating the equipment etc, and monitoring water quality or fish behaviour

2) third party service (i.e. fish vets) that are doing the welfare assessments, lice counts, and risk assessments, and making decisions

3) Us, the researchers, that are planning the experimental design and analysing the data

(one of) our solutions: ensure there was a legal contract in place between the parties that everyone had to stick to the methods and criteria defined in the FOTs application. We hoped that would enforce adherence from each party (but again, it was managers that were signing the contract, not participants)

Practical issues with commercial scale trials

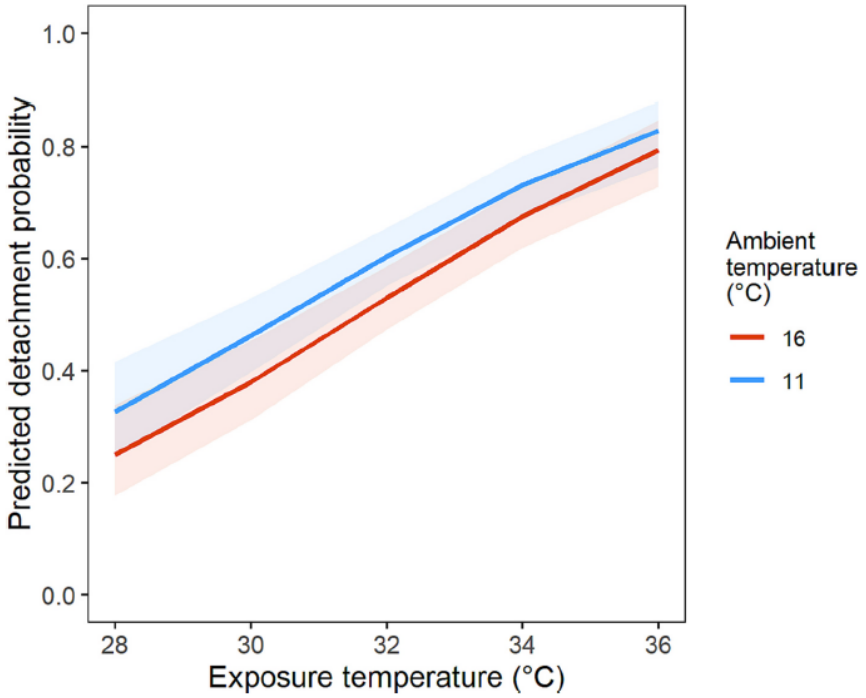
- Care of animals by research facilities vs large scale operations
  - Discrete isolation of when fish become experimental animals (and thus, our responsibility)
  - Individual observations of 1000 fish during crowding or treatment?
- Challenge with ‘experimental design’. For this study, we would have wanted to closely observe fish afterwards to assess welfare/health status and mortality. Impossible to distinguish treated individuals; normal options (pit tagging, external tags, camera observations to count dead bodies) not viable for a commercial cage. Best option would be to apply the treatment to the whole cage and record mortality before and after, but that increases N dramatically. We decided to analyse any increase (in the hour or days after treatment) in mortality from pre-treatment levels – not accurate but a good trade off.
  - Mortality levels of 0.2% increase is considered severe. But what is the basis of this value, how biologically significant is it?
- How to apply humane endpoints to fish in the system, or in a sea cage with 10000’s of other individuals? How would we even identify them?
  - Here, we ‘end’ the experiment after the fish are released from the recovery net. So monitoring of individuals in the larger cage was not necessary

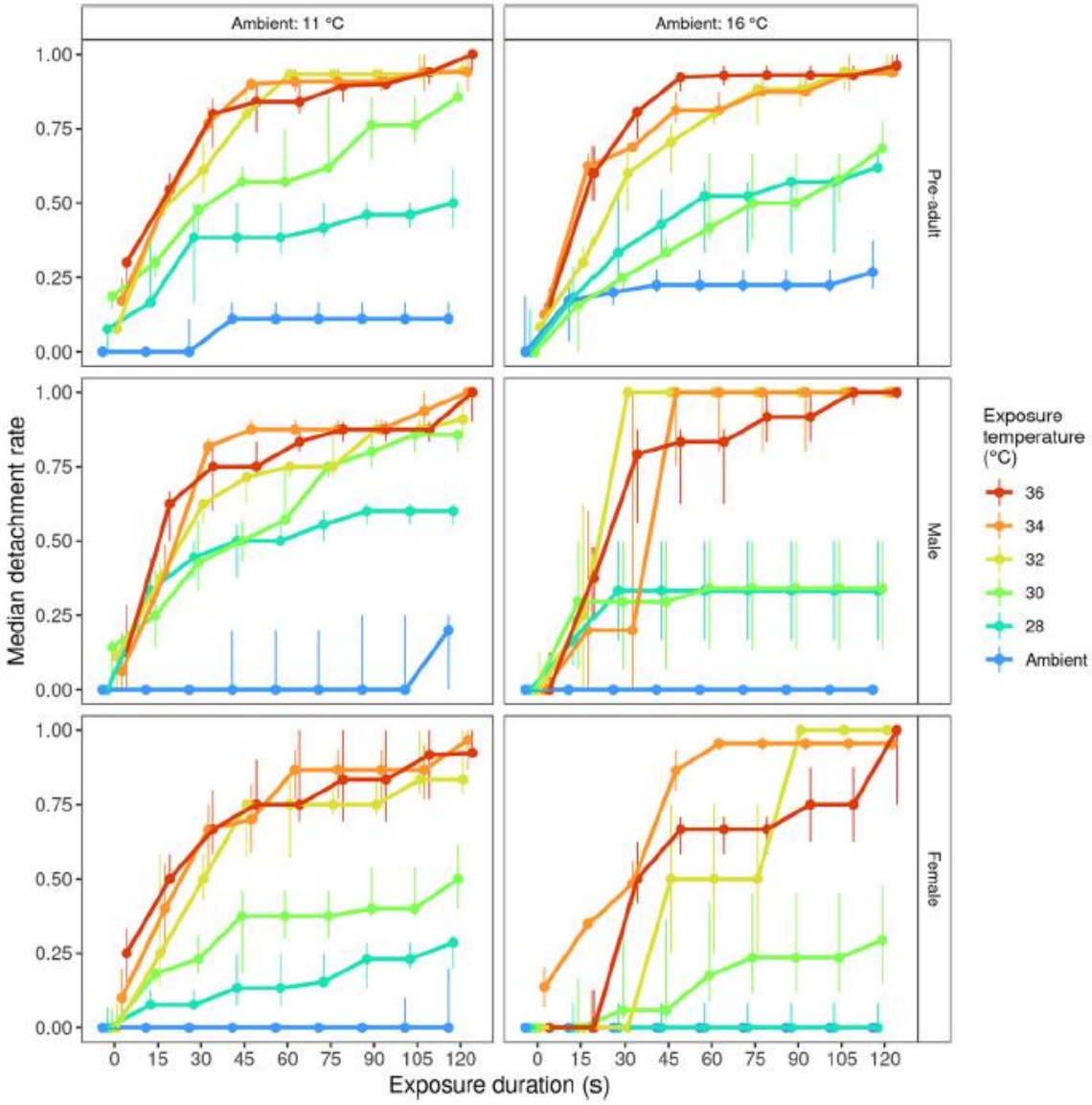
Any thoughts about this would be appreciated!!

Food for thought

Could use dead fish (Replacement) to test concepts first.

- E.g. Nilsson et al. used dead fish to specifically test the thermal thresholds of sea lice. Looked at survival of lice with exposure to extreme temperatures, and also how long does it take before removal





Nilsson et al. 2022

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Replacement: Atlantic salmon vs rainbow trout?



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Replacement: Atlantic salmon vs rainbow trout?

We potentially need lots of animals in our experiments, so it is important that data (reports, theses, etc) from commercial trials (many privately operated) should be made publicly available so that our requirement for the number of animals could potentially be reduced

- Stronger evidence base for experiment planning
- **Reduction**

Food for thought

What is the balance between

Refinement and Reduction in experiments (few individuals)

versus

The impact of the applied procedure on fish in production systems (>10000 individuals)?

