Final report on sampling and analyses of sea lice larvae in the Shuna Sound region(I),and sampling of sea lice on wild fish in the Shuna Sound region (II)

January 2023

Scottish Government
Riaghaltas na h-Alba

Final report on sampling and analyses of sea lice larvae in the Shuna Sound region (I), and sampling of sea lice on wild fish in the Shuna Sound region (II)

CES SPILLS: WP3

Report to satisfy requirements of SPILLS Deliverables D07 and D08.

Authors and Contributors (I): Helena Reinardy, Kim Last, Lars Brunner, Gail Twigg, Eilidh MacLeod, Anthea Ofori, Ilaria Stollberg, and Sarah Reed

Scottish Association for Marine Science (SAMS)

Author (II): Philip Gillibrand, MOWI Scotland Ltd.

Contents

Summary and key lessons learned

This report covers the methodology and results on the field sampling campaign from April – October 2021 for collection, identification, and quantification of counts of nauplii and copepodids from *Lepeophtheirus salmonis* and *Caligus elongatus* sea lice larvae.

KEY POINTS

- Detection of sea lice larvae was 4.8 % out of 372 total samples collected. Given the low capture success, it is unsurprising that no spatial or temporal patterns in sea lice larvae abundance were found.
- Low capture success is in agreement with other zooplankton sampling methods for sea lice larvae capture and is likely due to patchiness and temporal movement and low overall abundance in the water column.
- Failure to capture sea lice larvae does not equate to their absence, and care must be taken to avoid conclusions of no sea lice larvae present at the place and time sampled.
- Zooplankton sampling methods could be improved with smaller mesh sizes of nets and increased volumes sampled; however, these improvements would be accompanied with much increased effort for manual sorting and sample analyses and may not result in a net increase in efficiency of the method.
- Sea lice larvae identification by light microscopic analyses by trained zooplankton taxonomists is insufficient for 100% confidence of differentiation between *L. salmonis* and *C. elongatus* as well as differentiation between nauplii I and nauplii II stages.
- Alternative more precise methods for identification of sea lice species and stages are required.

- In the absence of fully-efficient methods for sea lice larvae capture and identification (species and stages), alternative methods for sea lice larvae dispersal model validation are required.
- Although the low number of wild fish samples analysed in 2021 made firm conclusions difficult to draw, the limited data suggested that there was a lice-related risk to sea trout in the Sound of Shuna Management Area.

1. Introduction

To date, research on the ectoparasitic copepod sea lice has primarily focused on the fish-attached stages. Sampling, quantifying, and analysing the zooplanktonic larval stages (two nauplii moults and the infective copepodid stage, Figure 1) has proven technically challenging. In the absence of validated methods for capturing and quantifying larval dispersal, computational models have been developed to predict movement of larval particles from the aquaculture farm source into the wider water currents. The purpose of this work package is to: Test methods for capturing and quantifying planktonic stages of sea lice larvae; Quantify sea lice with an environmental sampling campaign designed and informed from sea lice dispersal modelling; Link results to modelled predications, and; Collect data of attached stages on both wild and farmed fish.

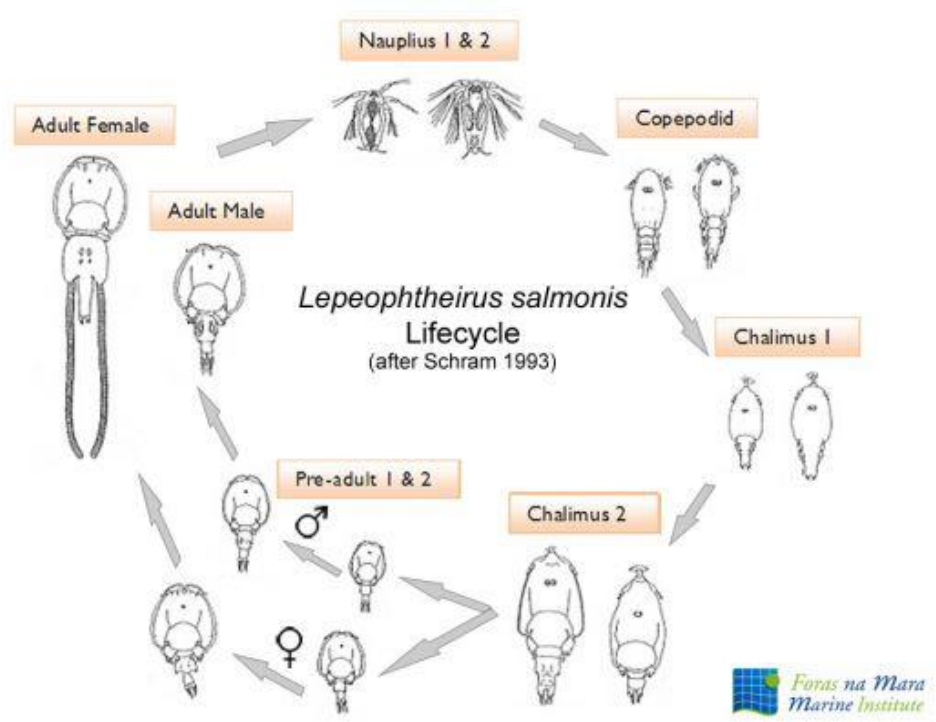
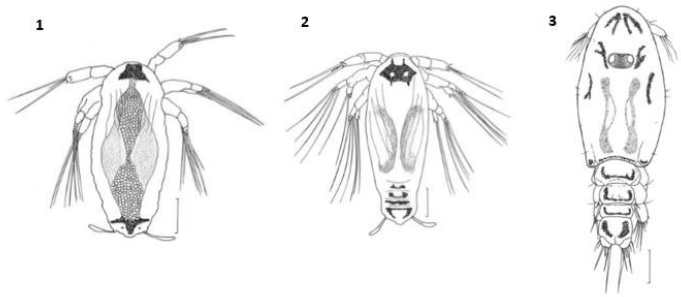


Figure 1: The life cycle of the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837). From Marine Institute (https://www.marine.ie/site-area/areas-activity/aquaculture/sea-lice/life-cycle-salmon-louse). Adapted from Hamre *et al.,* 2013.

Both *Caligus elongatus* and *Lepeophtheirus salmonis* sea lice are found in Scottish waters. The former a more generalist parasite infecting many fish species with the latter limited to salmonid hosts (salmon, trout, char). Both have similar life cycles, with two nauplii stages followed by an infectious copepodid stage (Figure 2).

A



B

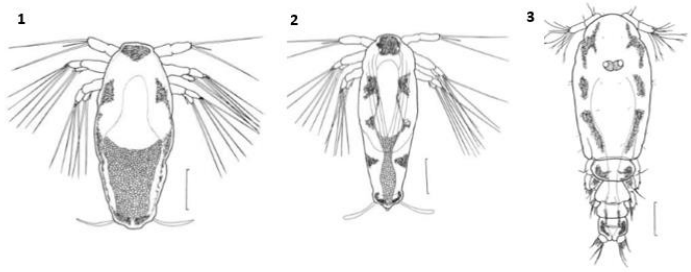


Figure 2: Pelagic larval stages of *Lepeophtheirus salmonis* (A) and *Caligus elongatus* (B), including newly hatched first nauplius (1), free-swimming second nauplius (2), and free-swimming infectious copepodid (3). Scale bar 0.1 mm. Adapted from Schram, 2004.

While much of the natural history and ecology of the pelagic stages of sea lice larvae are still unknown, some aspects have been investigated and are directly relevant to the design of a sampling strategy. Pelagic larval stages are highly seasonal, with a spring peak in release into the water column when adults are present on fish in coastal waters and when phytoplankton abundance is high. A second much smaller peak in

abundance is also often seen in the autumn. Typically, fewer than 300 nauplii are hatched from a single pair of egg strings on gravid adult females. Densities in the water are therefore typically low, although peaks can occur with rapidly growing on-farm infestation outbreaks. Adult female sea lice may extrude egg strings which contain anywhere between 150-900 eggs. Many different variables interact to influence egg numbers, such as age of the female louse (the first egg string pair is the shortest), fish health and physiology, anti-lice treatments on farms, seasonality, and local adaptations of lice. However, environmental conditions are possibly the most important in controlling hatching and are certainly the most studied. For example, egg hatching success strongly correlates with high salinity (34 PSU) and temperatures between 15-20°C under which conditions all eggs hatch (Samsing *et al.*, 2016). In brackish waters of 20 PSU, 78% of eggs hatch, with less than 20% of nauplii viable (Brooker *et al.*, 2018). In *L. salmonis* eggs hatching and interval between larval moults is highly dependent on temperature i.e. embryo developmental time ranges from 5.5 –17.5 days, 5-15°C, nauplius 1 stage duration ranges from 9 hours (15°C) to 2.5 days (5°C), and nauplius 2 stage lasts between 36 hours (15°C) and 1 week (5°C) (Marine Institute, https://www.marine.ie/Home/site-area/areas-activity/aquaculture/sea-lice/life-cycle-salmon-louse).

The concentration of larvae is predicted to be highest in shallow near-surface coastal water and lowest in areas of freshwater river input as the larvae do not tolerate salinities below 20 PSU (Bricknell *et al.,* 2006). Habitat preferences change as larval development advances with nauplii typically clustering at and below the halocline (the interface between fresh surface waters and deeper saline waters). Copepodids, which unlike nauplii are present at salinities as low as 16 PSU, tend to spread evenly and vertically in the water column even in the presence of strong salinity gradients (Crosbie *et al.*, 2019). Temperature also influences the distribution of sea lice larval stages. Whilst the naupliar stages avoid waters >10°C,

copepodids display only a weak negative response to water temperatures >12˚C (Crosbie *et al.,* 2020). While sea lice larvae are thought to favour shallow, saline waters, their behavioural traits are flexible. Coates *et al.*, (2020) proposed that tolerance to high pressure may be a heritable trait in sea lice, with depth-resistant families distributing lower in the water column (Coates *et al.*, 2021).

Sea lice larvae are considered positively phototactic and undergo reverse diel vertical migration (rDVM) with movement towards the water surfaces during the day and downwards at night (Heuch *et al.*, 1996). rDVM is especially pronounced in the copepodid stage. This view has been recently challenged by a study by Szetey *et al.*, (2021), suggesting that light does not influence the position of copepodids in the water column. Copepodids tend to aggregate at the surface regardless of light conditions, unlike earlier naupliar stages which undergo rDVM. Furthermore, they show a strong positive phototaxis to point light sources, such as those present on fish farms at night (Nordtug *et al.*, 2021). In summary, there is much uncertainty on the progression of behaviour with sea lice larval stage; these information gaps negatively impact models for sea lice larval dispersal and hinder field sampling.

A few studies have investigated larval sea lice sampling strategies particularly: Depths for sampling; Net mesh size and; various methodological techniques, (Nicholas and Thompson, 1991; Nelson *et al.*, 2018; Nilsen, 2016; Skarðhamar *et al.*, 2019). A significant review of sampling methods has recently highlighted the key aspects of sampling (Fernandez-Gonzalez *et al.*, 2022), but was unavailable at the time of the SPILLS sampling design.

The objectives of the field and laboratory analyses in WP3 were to:

1. Test out multiple field sampling methods for capturing sea lice nauplii and copepodid larvae and undertake a field sampling campaign.
2. Establish a process for subsampling, processing, and visual identification of sea lice larvae from the field sampling campaign.
3. Quantify sea lice larvae from a field sampling campaign designed and informed from sea lice larvae dispersal modelling.

2. Methods

2.1 Sampling locations

Six sites around the Shuna Sound area were selected as locations for sampling of pelagic stages of sea lice (nauplii and copepodids). These sites constituted locations which were selected on the basis that models:

1) Agreed and show accumulation at sites or;
2) Disagreed on the sites of accumulation or;
3) Agreed there is no accumulation at sites.

The sites, as shown in Figure 3, were 1: Eilean Arsa, 2: Northeast Shuna, 3: Loch Melfort, 4: Southern Approaches to the sound of Shuna, 5: Asknish Bay, and 6: Musgan, shallow southern shoreline of Asknish Bay. The sites were also selected to include areas close to Eilean Arsa and Asknish Bay where wild fish sampling and lice counting was planned, to optimise boat travel time, and to provide convenient access through Craobh Haven marina for mobilization. Sites 1 and 2 had good model agreement as lice hotspots, sites 3 and 5 showed discrepancies between the modelling on presence of lice. The shallow site 6 was selected to test the modelled indications that sea lice larvae can be washed against the shore, and site 4 was consistently modelled to have low sea lice larvae abundance.

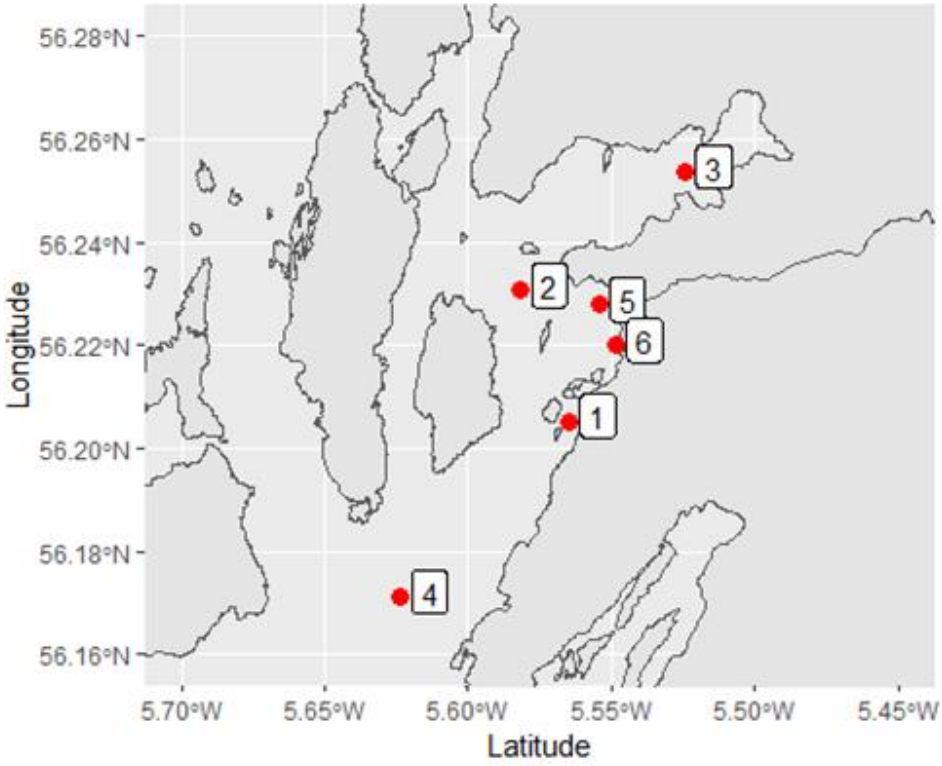


Figure 3. Locations of pelagic sea lice larvae sampling. Red circles indicate the locations of sampling: 1: Eilean Arsa; 2: Northeast Shuna; 3: Loch Melfort; 4: Southern Approaches to the Sound of Shuna; 5: Asknish Bay; and 6: Musgan, shallow southern shore of Asknish Bay.

2.2 Oceanographic parameters

Conductivity as a proxy for salinity, as well as temperature, and depth data were collected using a CastAway-CTD designed for in-shore use. The instrument was hand-deployed to ≤18 m at each station depending on water depth. Deployment was made by hand on a lanyard in free fall at a rate of ~1 m/s. Accuracy of recording for salinity was ±0.1 Practical Salinity Units (PSU) and for temperature ±0.05°C. Data was downloaded post-deployment and processed according to manufacturer's guidelines (see https://www.ysi.com).

2.3 Field sampling trials

Sampling trials were conducted in March 2021 and involved testing of submersible pump from R/V Seòl Mara, kayak and canoe tow of small net (diameter 45 cm; 250 µm mesh size), and shore-based plankton and

submersible pump collection. These trials concluded that kayak or canoe tows were not ideal due to: Net drag making it very hard to tow; Inconsistent tow speeds and; Time required for multiple tows of sufficient volumes. The plankton pump system was also not ideal due to the low volume of pumping, but the pump worked well from the shore. The trials resulted in the purchasing of an additional WP2 net, a Bongo system of paired 100/200 µm nets, and additional pieces for the submersible pump system to be workable from a boat deck. Due to field sampling time constraint the Bongo net was only used on one occasion.

2.4 Field sampling main campaign

Two approaches to sampling sea lice larvae in the wild were employed: Submersible pump using the R/V Seòl Mara and, surface net trawl using the R/V Uisge (Figure 4).

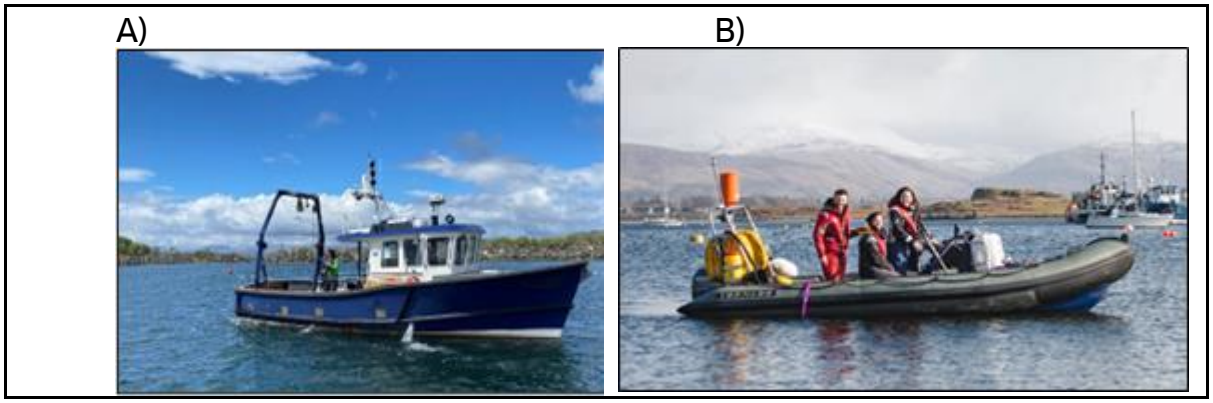


Figure 4: SAMS vessels used in SPILLS sea lice larvae surveys A) 10.4m Seòl Mara and B) 5.8m RIB Uisge. R/V Seòl Mara provided the necessary platform to power a submersible pump and A-frame to suspend the WP2 net whilst the R/V Uisge provided access to the very shallow near shore sampling site (Musgan) for surface trawl sampling. Image credits: A) Kim Last B) SAMS.

Sampling using the R/V Seòl Mara involved pumping seawater using a heavy-duty impeller driven submersible waste-water pump (Tsurumi 50PU2.75S) at 3 depths (0.5 m, 6 m and 12 m) into a WP2 net (mesh size 200 µm, mouth opening 0.75 cm) which was half-submerged off the stern of the vessel with weighted cod end (mesh size, 50 µm). This was carried out

over a period of 15 minutes with water flow rate recorded (FLOMEC TM, Great Plaine Industries) in US gallons with subsequent conversion to litres. The deck configuration for the submersible pump method is shown in Figure 5. After 15 minutes of pumping water into the WP2 net, the net was raised onto the deck and rinsed from the outside with seawater from the submersible pump. The contents of the net cod end were poured through a 125 µm sieve and stored in a 500 ml screw top sample container with 10% buffered formaldehyde solution until later analysis in the laboratory.

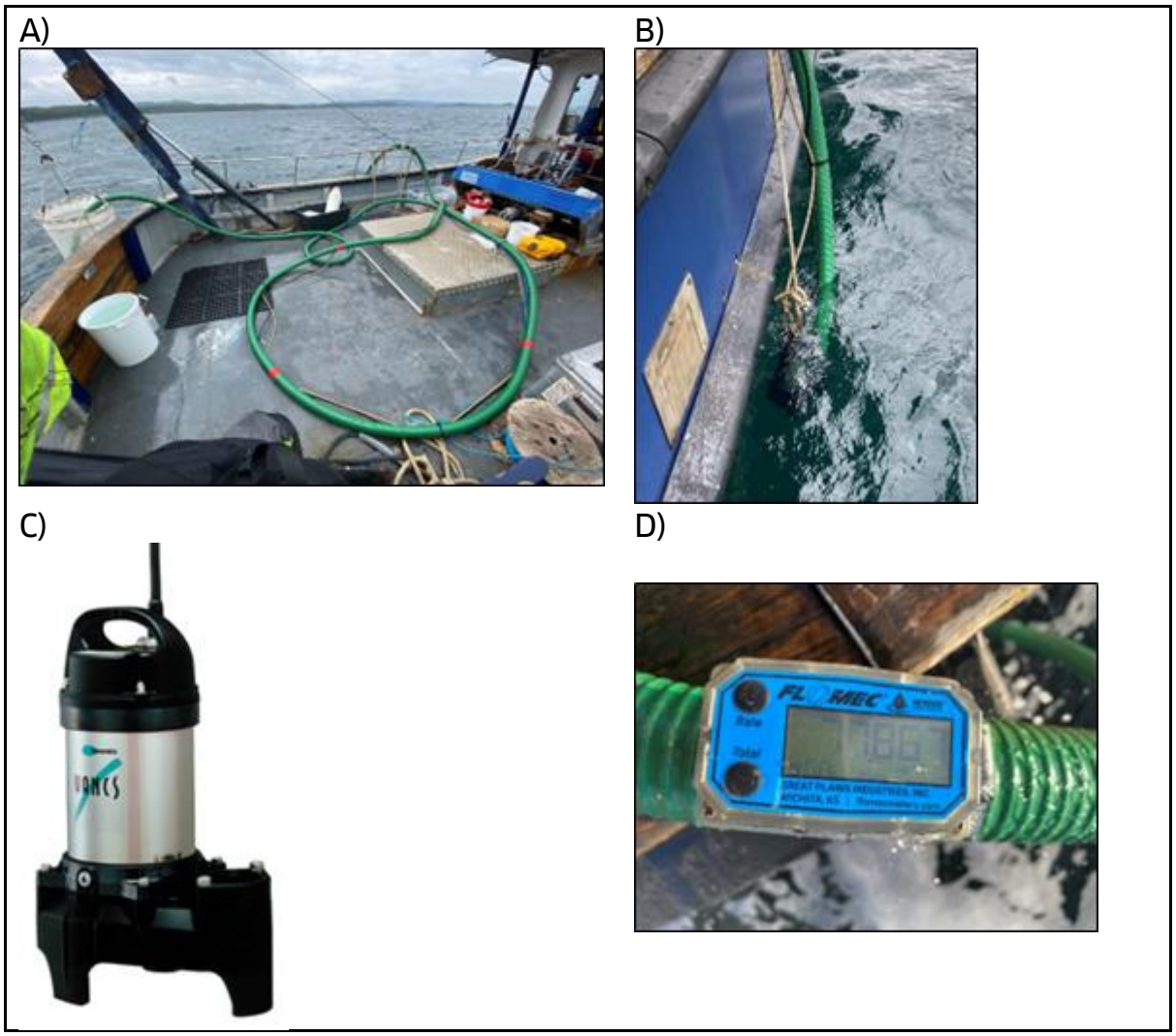


Figure 5: Submersible pump methodology on the R/V Seòl Mara: A WP2 net was suspended from the A-frame off the stern of the vessel (A) and supplied by a hose over the port side (B). The hose was attached to a submersible pump (C) which was tied off on the guard rail and lowered to specific sampling depths. Pump rate to the net was recorded using an in-line flow meter (D). Image credits Kim Last (A, B, D) and https://www.prestigepumps.co.uk (C).

Sampling on the R/V Uisge involved trawling a modified net at the water surface (mesh size 233 µm, mouth opening 0.57 cm) with 'sock' cod end (mesh size, 100 µm) behind the vessel for 5 minutes (Figure 6A). A standard boat fender (diameter 24 cm) was attached to the top of the ring net so that the net was always maintained at the waters' surface. Trawl rate was determined using a flow meter (KC Denmark, model 23.090) which was tied into the mouth of the net (Figure 6B). Pre- and post- recording of impeller rotation counts on the flow meter provided the necessary data to determine subsequent flow rate calculations to determine volume fished as exemplified in section 2.6.

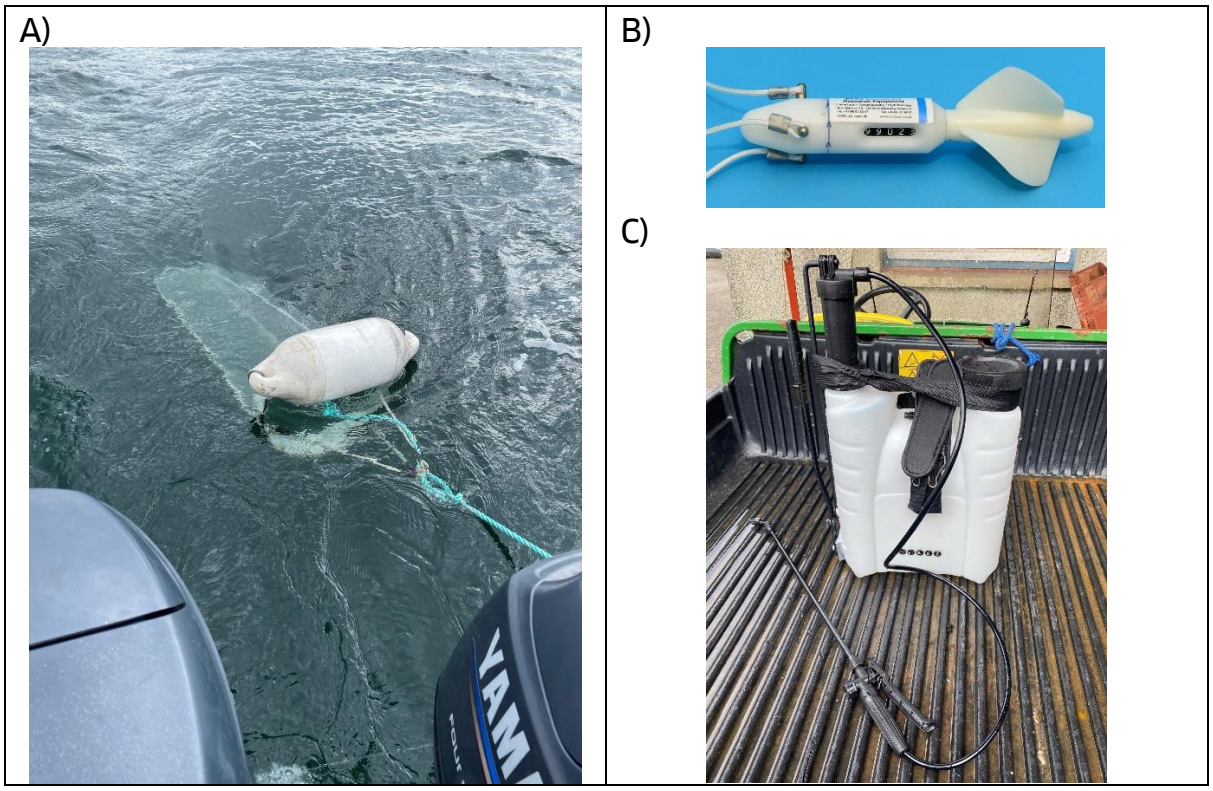


Figure 6: Surface net trawl configuration on the R/V Uisge: the net was towed behind the RIB at the water surface A) where flow rate through the net was determined using a flow meter B). Once the net was recovered it was rinsed down on deck using a garden sprayer C). The plankton sample was preserved for later analysis. Image credits: Kim Last (A, C) and KC Denmark A/S (B).

Once underway, 20 m of warp was streamed to reduce turbulence from the outboard propellers and wake of vessel whilst the net was fishing. At the end of the trawl the engine was disengaged, and the net recovered by hand. This was rinsed with seawater into the 'sock' cod end using an 'off-the-shelf' 12 l garden sprayer (Figure 6C). Sample sieving and preservation was then carried out as for the submersible pump method described above.

Sampling campaigns were undertaken over 2-5 days, every month (April, May, June, July, August, September, and October). An overview of the field sampling is outline in Table 1.

Table 1: Sampling overview and summary April-October 2021. All samples in triplicate.

Sampling	April	May	June	July	August	September	October
Method development	Site 1						
Surface, 255 µm, 5 min. trawls	All sites	All sites	All sites	All sites	All sites	All sites	
Submersible pump (Depth stratified) 200 µm, 15 min		0.5, 12 m	0.5, 12 m	0.5, 12 m (Site 4) 0.5, 6, 12 m	0.5, 6, 12 m (not Site 4)	0.5, 12 m (Site 4) 0.5, 6, 12 m	0.5, 12 m (Site 4) 0.5, 6, 12 m
Twin bongo, 100/200 µm, surface			All sites				
Notes					MOWI sites fallow	MOWI BDNC restocked	

2.5 Sample processing and microscopic identification of sea lice larvae

Zooplankton samples from the field sampling which had been fixed in 10% buffered formalin solution (4% formaldehyde), were transferred to the microscopy laboratory at SAMS for processing and identification, following

the chemical safe system of work (SSW) internal protocol. All handling of formalin solution was carried out in a designated formalin fume food and handled with personal protective equipment (PPE: nitrile gloves, laboratory coat, safety glasses). The formalin fixed samples were first rinsed through 150 µm sieve with tap water and transferred to Stempel pipette in 250 ml tap water. The full sample was gently mixed by figure-of-eight movement (avoiding vortex) for equal distribution in volume before removal of a 2 ml subsample into a viewing chamber. In total, 50 ml of subsample (20% of total sample) was analysed. After subsampling, the remaining sample was transferred into storage pot in a final 70% EtOH solution for long-term storage.

Sub samples were viewed under light microscope (Zeiss Stemi 2000-C or Zeiss AxioVert 200). All individual zooplankton were observed and any sea lice nauplii or copepodids found were imaged (Zeiss AxioCam MRC), removed, and stored separately. Sea lice species and larval stages were established for comparison against laboratory-reared type specimens and identification keys (Conway, 2012; Schram *et al*., 2004). Copepodids were confirmed to the species level. A final confirmation step in the identification process was achieved by sending all imaged specimens to a taxonomic expert (David Conway, Marine Biological Association, Plymouth, UK).

To verify and correctly identify sea lice larvae, type specimens of gravid female *L. salmonis* and *C. elongatus* were collected, hatched in larval aquariums, and stages preserved for microscopic identification and training. This was done as part of a separate study (Ofori, 2022). Images of nauplii I, nauplii II, and copepodids from each species were taken under light microscopy and scanning electron microscopy. Analysis using the SEM was performed using the JEOL JSM-6390LV microscope with a tungsten filament to produce an electron beam. Sea lice larvae were filtered unto the nucleopore track-etch membrane filter and mounted onto the aluminium

stubs (approximately 2.5 cm in diameter) with double sided tape. Uncoated samples were directly imaged in Low Vacuum (LV) and variable pressure. The pressure in the LV-SEM was set at 49 Pa and captured at 17 kV. Images from type specimens were used for stage and species differentiation, and consequently each specimen collected and imaged from the field samples were double-checked and confirmed to stage (nauplii I, nauplii II, or copepodids) or species (copepodids).

2.6 Data Analysis

Larvae counts were calculated as a concentration within a volume of sampled water. Volumetric calculations for the trawl sampling were made using two flow meters: GO Environmental (No #B 25298) and KC Denmark A/S (model: 23.090). Flow meter calculations for the GO Environmental were based on rotor rotations using the following equation:

Eq. 1 DISTANCE in meters = Difference in COUNTS (X) 26,873/ 999999

Eq. 2 VOLUME cubic meters = 3.14 (X) (net diameter)^2 (/) 4 (X) Distance

Where 10 counts are equal to 1 rotor revolution and the standard speed rotor constant = 26,873

For the KC Denmark A/S (model: 23.090) calculations were as follows:

Eq. 3 DISTANCE in meters = COUNTS x 0.3

Eq. 4 VOLUME in cubic meters = 3.14 (X) (net diameter) ^{2} (/) 4 (X) Distance

The GO flowmeter was used between April – July at which point it failed. The replacement KC flow meter was used for the remainder of the surveys, August – October. For the submersible pump method volumes of sea water were directly measured using a flow meter supplied by Great Plains Industries TM150 Flowmeter/Totalizer, 10 to 100 GPM.

For microscopic identification, 20% of the field sample was processed with the final larvae concentration value calculated as follows:

Eq. 5 Larvae number in cubic meter = (count x 5) / volume seawater sampled (m^3)

3. Results and Discussion

3.1 Oceanographic conditions

3.1.1 Temperature

Sea temperature at water depths ≥4 m for all sites from April to October ranged between 9.2°C – 16.3°C with the minimum recorded in April and maximum in September (Figure 7). Temperatures were highest at the surface for all stations in all months. Change in temperature with depth within each month was minimal (range ≤2°C) except for Loch Melfort in July (Figure 7C; range 2.9°C) and Musgan in August (Figure 7F; range 2.1°C) showing weak stratification but without an overt thermocline. Overall temperature profiles reveal well-mixed water masses as expected which are the result of a highly dynamic environment influenced by the strong diurnal tidal flows of the Sound of Luing and the Gulf of Corryvreckan close to sampling locations. The only stations to reveal weak thermal stratification (thermoclines) were Loch Melford with more restricted tidal exchange than the other sites and Musgan, which is a very shallow near-shore station where the cause of stratification was probably the results of solar gain at the time of sampling on a warmer than average day. We conclude that temperature cues for sea lice larvae dispersal are probably weak, at least in the surface waters (≤18 m), most relevant to salmon aquaculture enclosures.

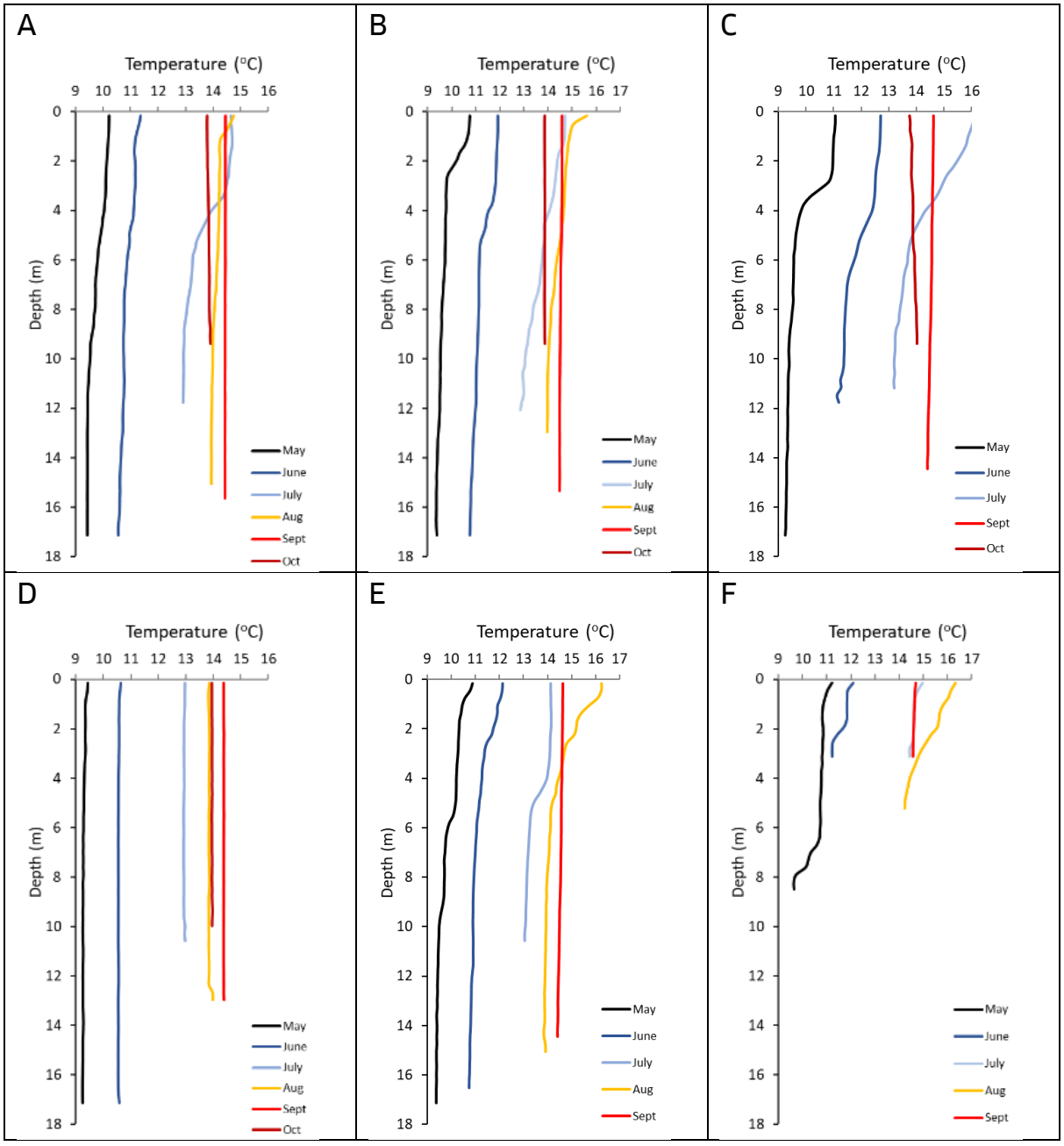


Figure 7: Monthly vertical temperature profiles for stations A) Eilean Arsa B) NE Shuna C) Loch Melfort D) S. Approaches E) Asknish Bay F) Musgan. Note: Missing data for Loch Melfort in August and Asknish Bay and Musgan in October.

3.1.2 Salinity

Salinity for all sites from April to October ranged between 30.6 – 33.8 PSU (Figure 8). As a general trend maximum salinities occurred in July and August with the minimum in September and October (except for Musgan where this was in June) (Figure 8F). This may be explained by increased precipitation at the end of the summer and into the autumn when compared to the height of the summer. Salinities changed little with depth but where there were differences, fresher more buoyant water, was found in the surface. Overall salinity, as with temperature profiles, reveal well-mixed water masses which are the result of a highly dynamic tidal environment. The only stations to reveal weak density stratification (pycnoclines) exceeding 1 PSU over the sampled depth, were Loch Melfort (October, Figure 8C) and Musgan (June, Figure 8F). By way of explanation Loch Melfort is more restricted in its tidal exchange than the other sites and Musgan is a very shallow near-shore station influenced by river input with high temporal variability. We conclude that salinity cues for sea lice larvae dispersal are probably weak in this area other than in Loch Melfort or at very near shore sites in the vicinity of estuaries.

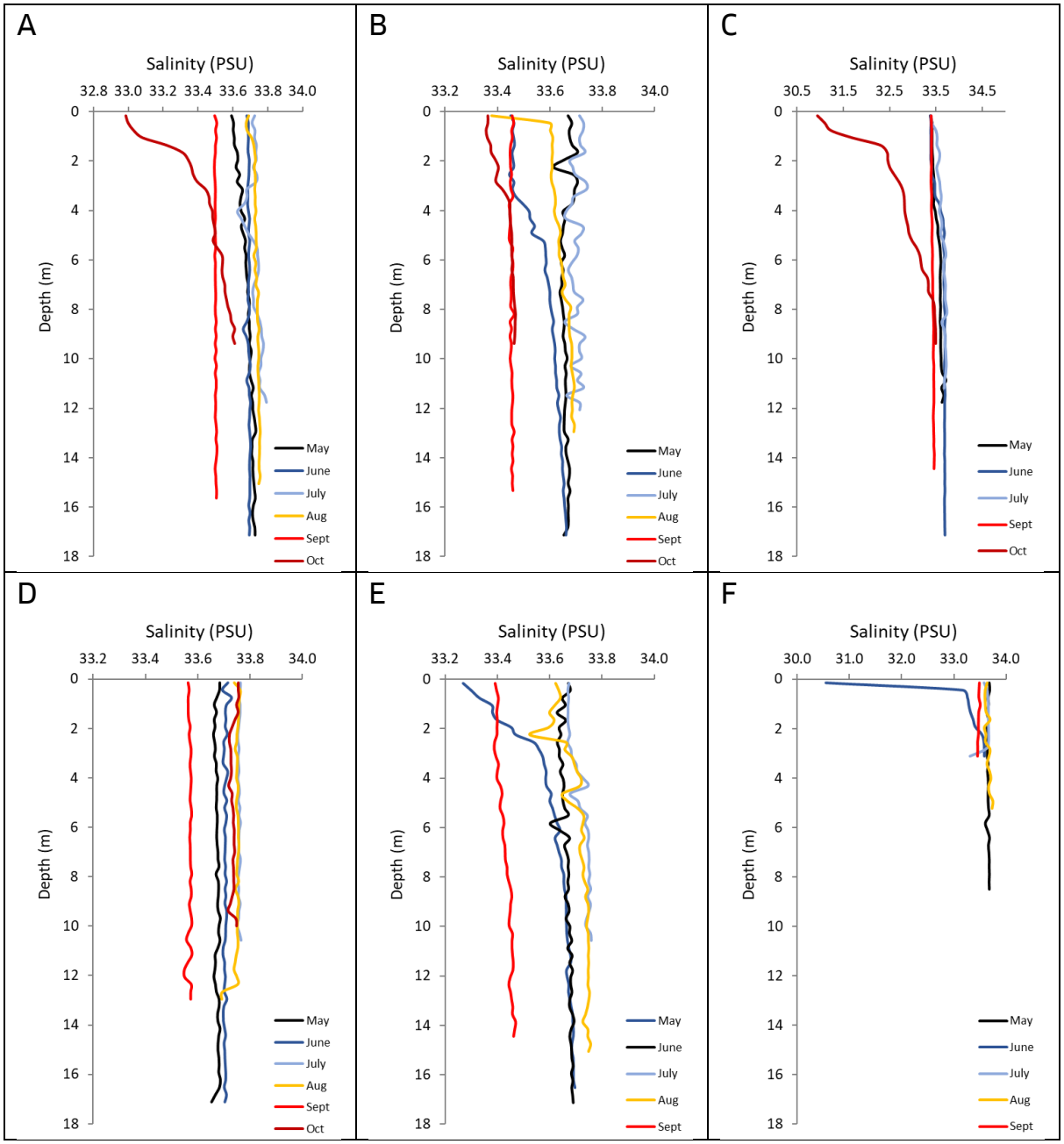


Figure 8: Monthly vertical salinity profiles for stations A) Eilean Arsa B) NE Shuna C) Loch Melfort D) S. Approaches E) Asknish Bay F) Musgan. Note: Missing data for Loch Melfort in August and Asknish Bay and Musgan in October.

3.2 Microscopic determination of stage and species

Laboratory-reared type specimens produced useful light images for differentiation between stages and species (Figure 9,10). Light microscopy identified a more pronounced posterior point between the balancers of *C. elongatus* nauplii (Figure 9E) which became a useful feature to differentiate between the two species. Additionally, SEM identified species differences in the maxillipeds of the copepodids, with *C. elongatus* having two additional bifurcated barbs compared with the corresponding smooth maxilliped of *L. salmonis* (Figure 10). Pigmentation was a key identification feature that was useful and characteristic in freshly fixed samples; however, pigmentation becomes undefined after long-term fixation (Schram *et al.*, 2004, Figure 11) and was no longer identifiable in the field samples.

Despite extensive training, scrutiny of type specimens and keys, and consultation with an external taxonomy expert, definitive species-level identification was not achieved to 100% confidence. Copepodid identification relied on visual confirmation of presence/absence of barbs on maxillipeds; however, maxillipeds were not always clear enough to identify presence or absence of barbs. A higher level of confidence was achieved at the sea lice level to report that all nauplii and copepodids were either *C. elongatus* or *L. salmonis* and were not other copepods or zooplankton species. Differentiation between nauplii I and nauplii II was also not achieved to 100% confidence level, due to the variation in length and width.

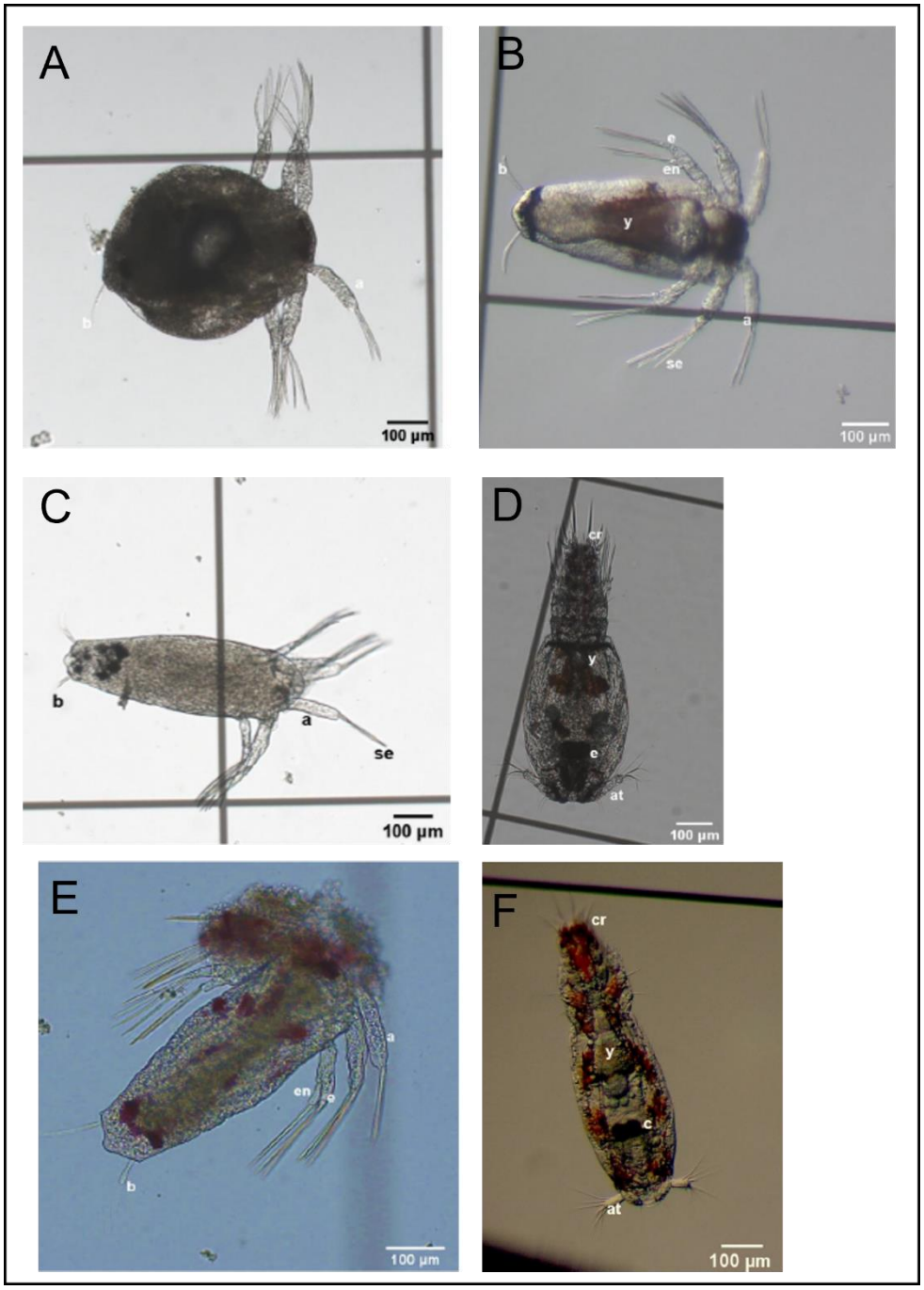


Figure 9. Larval stages of *L. salmonis* (A-D) and *C. elongatus* (E-F) from dorsal views under light microscopy. Stages include newly hatched nauplii I (A), nauplii I (B), nauplii II (C and E), and copepodids (D and F). Abbreviations: a, appendages; at, antennule; b, balancer; cr, caudal rami; e, exopod (nauplii) or eye (copepodid); en, endopod; se, setae; y, yolk reserves. Images: A. Ofori.

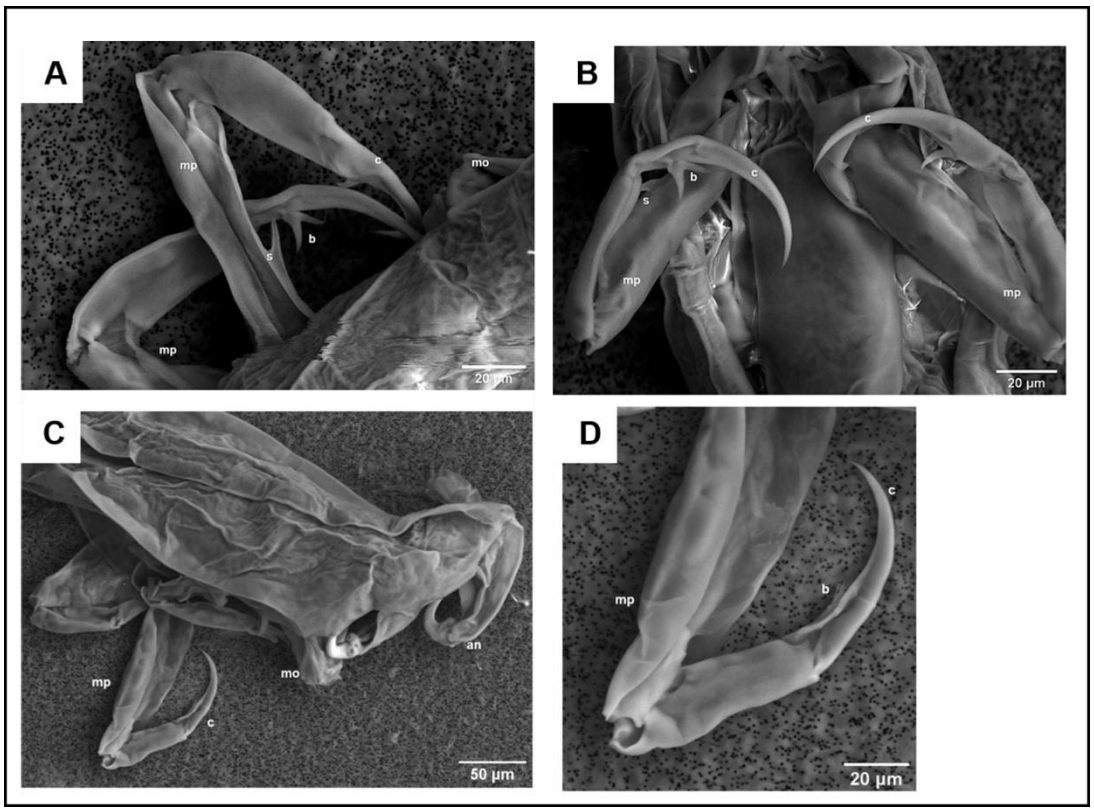


Figure 10. Anterior-lateral scanning electron micrographs of copepodid larvae stages of *C. elongatus* (A-B) and *L. salmonis* (C-D). Abbreviations: an, antenna; b, barb; c, chelae; mo, mouth, mp, maxilliped; s, spine. Images: A. Ofori.

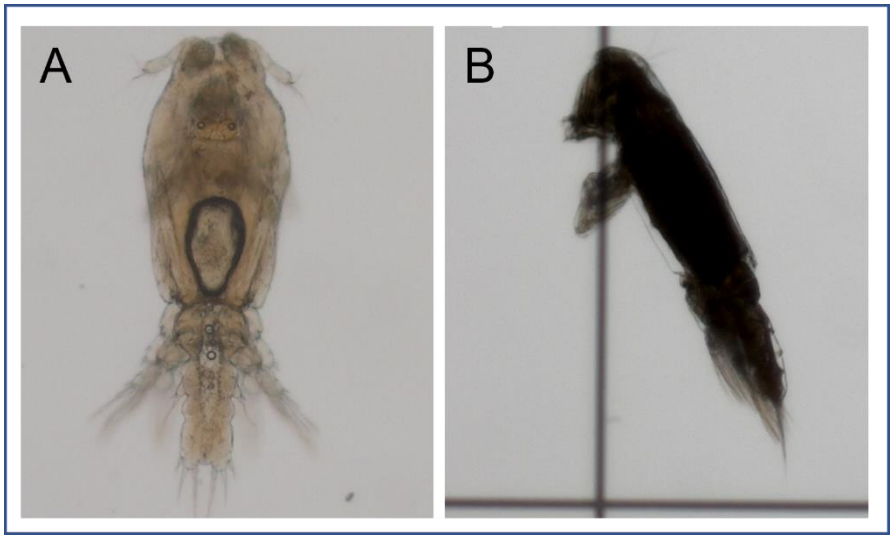


Figure 11: Deterioration of pigmentation in copepodids fixed in formalin for 7 days (A, laboratory reared) or after 120 days (B, samples collected from the field sampling campaign). Images: A. Ofori.

3.3 Sea lice larvae in the wild

3.3.1 Abundance and morphometrics

Out of a total of 372 field samples collected monthly from April – October 2021, 19 samples (5.1 %) contained identifiable sea lice larvae and 18 of which (4.8%) contained sea lice larvae (1 sample contained a pre-adult *L. salmonis*) (Table 2). Most samples did not contain sea lice larvae in the volume analysed (20% of total captured water sample). It may therefore be concluded that more of the sample should be analysed, however the time consideration of scaling up to full sample processing and analysis is considerable. On average, 1 sample took between 0.5 – 1.5 days to process. The variation in time required to fully look through the subsample was due to overall zooplankton abundance, with abundant samples corresponding to zooplankton blooms in early summer and again in late summer (June and September). Therefore, the effort of trained taxonomic experts per unit time for processing the samples was extensive, and an estimated 348 days of two people's time was spent processing the samples.

Care must be taken to understand the implications of these low capture rates and low rates of identification, with the key point of absence of capture or identification not equating to absence in the water at the time of sampling. The issues around capturing and quantifying concentrations of sea lice larvae to accurately understand concentrations in the water column at the time of sampling are abundant and complex (Fernandez-Gonzalez e*t al.,* 2022).

Length and width measurements of laboratory reared and field-sampled sea lice larvae are listed in Table 3. Overall, the measurements were consistent to those reported in other studies, and the distribution of sizes overlapped in stages and species and therefore being an unreliable differentiating metric (Schram *et al.*, 2004).

Table 2. Quantified and identified sea lice from field sampling

Tow no.	Month	Site	Method	Depth (m)	Nauplii count	Copepodid count	Preadult count	Nauplii (no./m^3)	Copepodid (no./m^3)
13	April	5	Trawl	1	0	1	0	0	0.2
15	April	5	Trawl	1	0	1	0	0	0.15
31	May	1	Pump	1	0	1	0	0	1.13
51	May	4	Pump	1	1	0	0	1.13	0
57	May	5	Pump	12	0	1	0	0	1.13
134	June	1	Pump	12	0	1	0	0	1.13
137	June	1	Pump	6	0	1	0	0	1.13
148	July	4	Pump	12	0	2	0	0	2.26
151	July	4	Pump	1	1	0	0	1.13	0
210	Aug	3	Pump	6	1	0	0	1.13	0
232	Aug	1	Pump	1	0	0	1	0	0
239	Aug	5	Pump	1	0	1	0	0	1.13
241	Aug	4	Pump	1	0	1	0	0	1.13
300	Sep	4	Pump	1	0	1	0	0	1.13
325	Oct	2	Trawl	1	0	1	0	0	0.16
338	Oct	4	Pump	12	0	1	0	0	1.13
345	Oct	1	Pump	12	0	1	0	0	1.13
359	Oct	3	Pump	1	0	1	0	0	1.13
371	Oct	5	Pump	1	0	2	0	0	2.24

Locations: 1, Eilean Arsa; 2, Northeast Shuna; 3, Loch Melfort; 4, Southern Approaches; 5, Asknish Bay; 6, Musgan.

Table 3. Length and width measurements from sea lice larvae collected from the field, compared against data from type specimens hatched from gravid females in the laboratory and from selected studies. Length/width measurements in µm with format of data presented: Mean ± SD, range, N.

	Nauplii I	Nauplii II	Copepodid
SPILLS samples (spp. undetermined)			
Length	561 ± 1, 561 – 562, 2		704 ± 31, 660 – 758, 8
Width	180 ± 8, 175 – 186, 2		228 ± 33, 155 – 263, 8
Caligus elongatus			
SPILLS samples			
Length			662 ± 38, 631-704,3
Width			193 ± 48, 154-246, 3
Piasecki, 1996 (Canada)			
Length	448 ± 5, 10	487 ± 20, N?	661 ± 30, 308
Schram, 2004 (Norway)			
Length	461 ± 11, 39	514 ± 11, 33	637 ± 12, 18
Width	197 ± 8, 39	190 ± 6, 33	216 ± 7, 19
Laboratory-reared (2021, Scotland)			
Length	465 ± 15, 19	606 ± 10, 22	684 ± 16, 15
Width	188 ± 8, 30	205 ± 10, 22	229 ± 7, 15
Lepeophtheirus salmonis			
SPILLS samples			
Length			706 ± 15, 695-716, 2
Width			231 ± 4, 228-234, 2
Johnson and Albright, 1991 (Canada)			
Length	540 ± 40, 25	560 ± 10, 16	700 ± 10, 25
Width	220 ± 10, 25	200 ± 10, 16	280 ± 10, 25
Schram, 1993 (Western Norway)			
Length	511 ± 24, 30	606 ± 10, 22	684 ± 16, 15
Width	188 ± 8, 30	205 ± 10, 22	229 ± 7, 15
Laboratory-reared (2021, Scotland)			
Length	514 ± 34, 3	592 ± 19, 34	829 ± 26, 33
Width	197 ± 12, 3	196 ± 5, 38	273 ± 12, 33

3.3.2 Methodological effectiveness

Two approaches were employed in sampling for sea lice larvae: Submersible pump and surface trawl. Other than Musgan, which was too shallow to sample with the R/V Seòl Mara, all sites were sampled using both methods. The aim of this was to determine relative effectiveness of the methods in catching sea lice larvae. Before this is discussed however, important differences between the fishing methods are highlighted.

The submersible pump method employed a 200 µm mesh size whilst the trawled net was 255 µm. The initial reason for choosing different mesh sizes was a trade-off between volume fished and the catch efficiency of the net. Whilst 15 minutes plankton pump sampled 4.43 m^3, the trawled net sampled mean 29.0 m^3 (±4.8 S.D.) in only 5 minutes. Initial trials in April/May using an even smaller mesh net of 100 µm for surface tows resulted complete clogging from phytoplankton. For this reason, a larger mesh size was ultimately chosen for the main trial.

The relative effectiveness of the two methods is marked. The plankton pump method sampled far more sea lice larvae (n=16, all stages) over the course of all the surveys than did the surface trawl (n=3, all stages). This is despite the latter sampling >18 times the volume of seawater for a given period when compared to the former. We suggest that the plankton pump method is more effective at catching sea lice larvae than the surface trawl method, even though the latter has, to date, been the preferred sampling approach in the literature (see references in Fernandez-Gonzalex *et al.*, 2022). It is likely that the limited success of the surface trawl was the choice of mesh size. Our data reveal that for laboratory reared lice larvae the carapace width ranges between 188 - 229 µm (Table 3). It has been experimentally determined that a 190 µm mesh would have ~40% capture efficiency dropping to near zero for a mesh size of 270 µm

(Nicholas and Thompson, 1991). Recent work has shown that a minimum mesh size of 150 µm is required to successfully capture sea lice larvae with 6-10 m^3 filtered to obtain reliable and representative estimates of lice abundance (Fernandez-Gonzalex *et al.*, 2022). Skarðhamar *et al.,* (2019) recommended a mesh size of 180 µm and suggested vertical plankton tows as an additional effective capture methodology. The advantage of this approach over the submersible pump method is the speed of sampling. However, this approach does not allow for depth discrete sampling. Direct comparisons of methodologies are often impossible since the environments i.e., plankton communities between different habitats vary (i.e., between a Norwegian Fjord (Skarðhamar *et al.*, 2019) and the work for this report).

Initial concerns that the plankton pump method may damage sampled zooplankton were unfounded since microscopic examination found no evidence of this. Indeed, on several occasions juvenile fish (lumpsuckers), were entrained into the pump impeller, captured in the net where they were then released unharmed. Furthermore, we established that the plankton pump method allows very precise sampling of a known volume of water at a specific depth, which contrasts with the variability in volume estimations for the surface trawl or vertical haul methods (Skarðhamar *et al.*, 2019). Here, depth stratified sampling would require complex, expensive net opening/closing mechanisms which would probably be impractical for near-shore, shallow environments. The disadvantage of the plankton pump method is that smaller volumes of water are sampled for a given period when compared to trawling, sampling is spatially constrained and finally, a source of power is required to run the pump which would be impractical in a small vessel.

3.3.3 Interpretation of sea lice larvae abundance data

The number of captured larvae were too low to draw major conclusions on their abundance in the wild. However, some useful trends and patterns have emerged. No larvae were captured at the Musgan shallow site (Figure 12B), despite initial dispersal modelling suggesting the bay may collect and trap larvae close to the shore. The pump method was not deployed at this site due to the shallow water, so it is possible that there are underestimations of lice number for this site. Seasonal trends in their distribution were also hard to identify (Figure 12C) due to low numbers captured. However, there is some indication of the expected autumn bloom with the highest number of copepodids captured in October. The prevalence of copepodids across sampling methods might be partly ascribed to differences in behaviour and activity between early and later larval stages. Copepodids aggregate in surface waters, environmental conditions notwithstanding, and only migrate downwards when conditions are unfavourable (Crosbie *et al.*, 2020, 2019) and may explain the high frequency of capture at the surface (Figure 12D). Nauplii, on the other hand, have been found to concentrate at the pycnocline (Crosbie *et al.*, 2020, 2019), a layer of seasonally-variable depth which preserves lice-attracting olfactory cues produced by fish. However, since only three depths were sampled other, potentially relevant depths, might have been missed. While nauplii seek colder water in laboratory conditions, other studies have shown that nauplii might actively swim to the warmest layers in the water column, likely preferred by their target fish hosts (á Norði *et al.*, 2015).

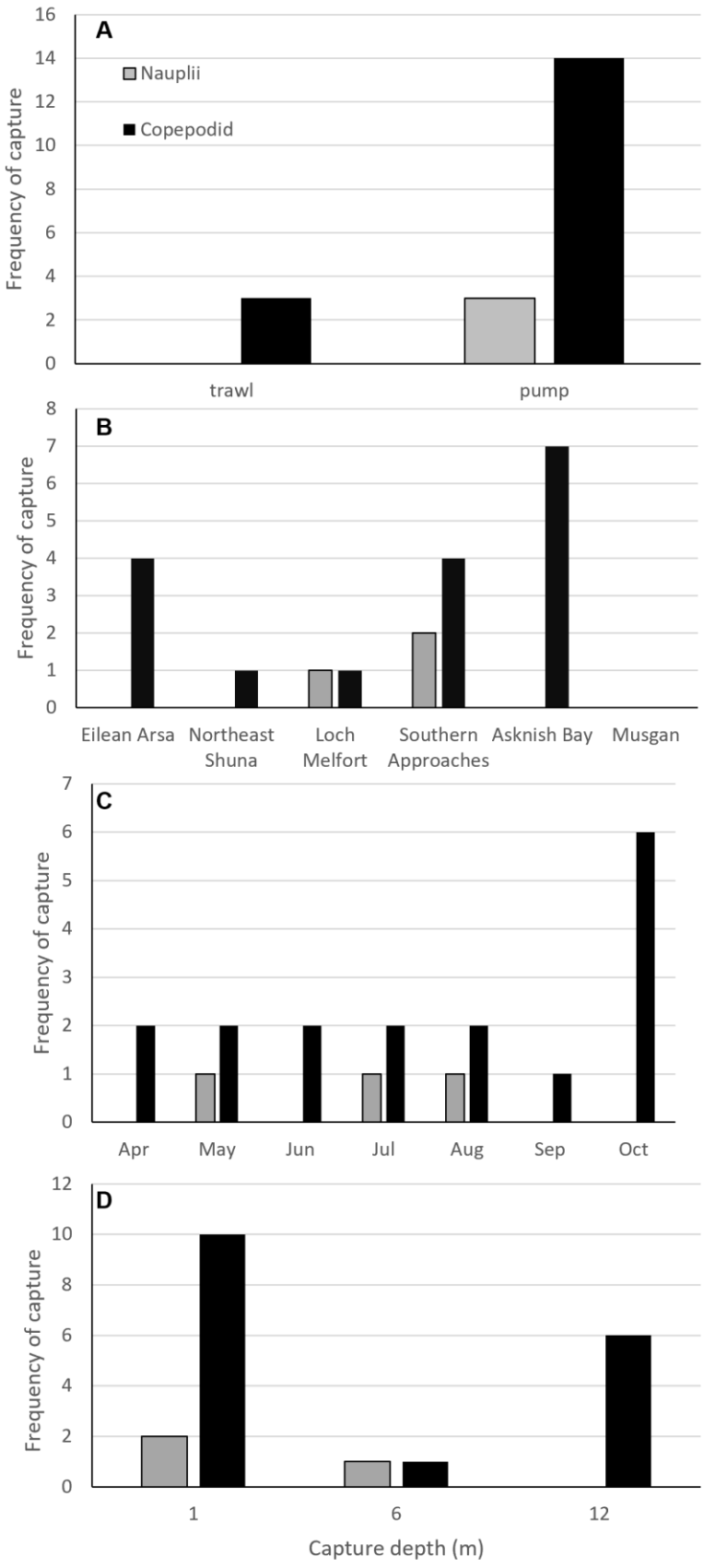


Figure 12: Sea lice larvae capture frequency across the 2021 field season, compared against capture method (trawl or pump, A), location (B), month (C), and depth of capture (D).

Hydrographic and particle tracking models, validated by quantified sea lice larvae concentrations by e.g., plankton sampling within the water column, could be valuable for predicting short-term risks of sea lice infestations between farm sites. However, plankton trawls and sea lice sampling from farm sites can also provide information on the genetic makeup of local populations, which can then be used to track long-term connectivity between sites (Jacobs *et al.*, 2018). Although population tracking may be achieved between existing sites, the dispersal of sea lice to new systems is harder to predict and requires a greater understanding of the behaviour of the planktonic stages of sea lice larvae. Sea lice larvae behaviour cannot be described by a single set of parameters: Different stages display different habitat preferences, flexibility, and activity patterns, as well as a host of adaptations driven by anthropogenic activity. As a result, plankton trawling and other methods of investigation of larval dispersal must account for these variations and plan accordingly, for example by sampling wider swaths of the water column rather than focusing exclusively on surface waters.

4. Sea lice sampling on wild fish in the Shuna Sound Area

As part of an Environmental Management Plan (EMP) for the Sound of Shuna agreed between Mowi Scotland Ltd, Kames Fish Farming Ltd and the local fisheries stakeholders Argyll Fisheries Trust (AFT) and the Argyll District Salmon Fishery Board (ADSFB), monitoring of lice burdens on wild fish began during spring and summer 2021. Whilst not strictly part of the SPILLS project, the results were obviously of interest and relevant to the project and are summarised here. A report for the 2021 sampling season was compiled by AFT and is included as a supplement to this report.

Within the area covered by the Sound of Shuna EMP, a coordinated sampling of wild salmonids was undertaken in order to: (1) monitor the

abundance of salmonid (primarily juvenile sea trout) populations; (2) record the infestation pressure of sea lice on juvenile salmonid populations and; (3) provide data to validate connectivity modelling predictions and to better understand the relationship between modelled and realised lice pressure on wild salmonids.

The experimental design for component (2) of the data collection included seine netting for juvenile sea trout populations in near-shore waters and deployment of a Fyke net at selected locations for a number of periods. Sampling began in June 2021 and continued through until late September. Sampling locations were guided in part by preliminary modelling results of lice dispersal, but ultimately were driven by practical considerations.

The fyke net was deployed successively at three locations near Craobh Haven and in Loch Melfort between June and September 2021. A total of 9 sea trout were caught, although other species were also trapped (including wrasse, sand smelts, ling, conger eel, mackerel and a wild salmon). Weekly seine netting was undertaken from June through August in both Loch Melfort and Loch Craignish. A total of 4 small sea trout (in Loch Melfort) were caught. Several sites were scoped at low and high tide but a combination of heavy weed growth and either too shallow or too deep shoreline was problematic. Lice counts on all captured fish were made and are reported as part of the local Environmental Monitoring Plan (Argyll Fisheries Trust, 2021). The work was very useful for establishing methods that were successfully deployed to capture a much larger sample of trout in 2022. However, the sample of trout collected in 2021 was too small to provide useful information to compare with outcomes of the lice dispersal models tested in the SPILLS project.

5. References

Argyll Fisheries Trust (2021). Sea Lice Burdens of Sea Trout at Sound of Shuna, Argyll, 2021. http://argyllfisheriestrust.co.uk/application/files/2616/7103/2121/Sound_of_Shuna_Sea_Lice_Monitoring_Report_2021_Final.pdf

á Norði, G., Simonsen, K., Danielsen, E., Eliasen, K., Mols-Mortensen, A., Christiansen, D.H., Steingrund, P., Galbraith, M., Patursson, Ø., (2015). Abundance and distribution of planktonic *Lepeophtheirus salmonis* and *Caligus elongatus* in a fish farming region in the Faroe Islands. Aquaculture Environment Interactions 7, 15–27.

Bricknell, I. R., Dalesman, S. J., O Shea, B., Pert, C. C., & Mordue Luntz, A. J. (2006). Effect of environmental salinity on sea lice *Lepeophtheirus salmonis* settlement success. *Diseases of Aquatic Organisms*, *71*(3), 201.

Brooker, A.J., Skern-Mauritzen, R., and Bron, J.E. (2018). Production, mortality, and infectivity of planktonic larval sea lice, *Lepeophtheirus salmonis* (Krøyer, 1837): current knowledge and implications for epidemiological modelling. ICEA Journal of Marine Science, 75(4):1214-1234.

Coates, A., Johnsen, I.A., Dempster, T., and Phillips, B.L. (2021) Parasite management in aquaculture exerts selection on salmon louse behaviour. Evolutionary Applications, 14:2025-2038.

Conway, D.V.P. (2012). Marine zoplankton of southern Britain. Part 2: Arachnida, Pycnogonida, Cladocera, Facetotecta, Cirripedia and Copepoda (ed A.W.G. John). Occasional Publications. Marine Bioogical Association of the United Kingdom, No 26 Plymouth, United Kingdom 163pp.

Crosbie, T., Wright, D.W., Oppedal, F., Dalvin, S., Myksvoll, M.S,, and Dempster, T. (2020). Impact of thermoclines on the vertical distribution of salmon lice larvae. Aquaculture Environment Interactions, 12:1-10.

Crosbie, T., Wright, D.W., Oppedal, F., Johnsen, I.A., Samsing, R., and Dempster, T. (2019) Effects of step salinity gradients on salmon lice larvae behaviour and dispersal. Aquaculture Environment Interactions, 11:181-190.

Fernandez-Gonzalez, V., Ulvan, E.M., Sanchez-Jerez, P., Diserud, O.H., Toledo-Guedes, K., Casado-Coy, N., Klebert, P., and Uglem, I. (2022) Abundance of sea lice larvae in plankton samples: determination of optimal sample sizes. Aquaculture, 551:737919.

Hamre, L.A., Eichner, C., Caipang, C.M., Dalvin, S.T., Bron, J.E., Nilsen, F., Boxshall, G., and Skern-Mauritzen, R. (2013). The salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) life cycle has only two chalimus stages. PLoS ONE 8(9):e73539.

Heuch, P.A., Parsons, A. and Boxaspen, K., (1996). Diel vertical migration: a possible host-finding mechanism in salmon louse (*Lepeophtheirus salmonis*) copepodids?. Oceanographic Literature Review, 4(43), p.401.

Jacobs, A., De Noia, M., Praebel, K., Kanstad-Hanssen, Ø., Paterno, M., Jackson, D., McGinnity, P., Sturm, A., Elmer, K.R., Llewellyn, M.S., (2018). Genetic fingerprinting of salmon louse (*Lepeophtheirus salmonis*) populations in the North-East Atlantic using a random forest classification approach. Scientific Reports 8, 1203.

Nelson, E. J., Robinson, S. M. C., Feindel, N., Sterling, A., Byrne, A., and Ang, K. P. (2018). Horizontal and vertical distribution of sea lice larvae (*Lepeophtheirus salmonis*) in and around salmon farms in the Bay of Fundy, Canada. Journal of Fish Diseases, 41: 885–899.

Nichols, J.H. and Thompson, A.B., (1991). Mesh selection of copepodite and nauplius stages of four calanoid copepod species. Journal of Plankton Research, 13(3), pp.661-671.

Nilsen, M. K. (2016). Sampling strategies, distribution and concentration of planktonic salmon lice copepods in the Outer Hardangerfjord and the Altafjord. The Arctic University of Norway. 66 pp. https://munin.uit.no/bitstream/handle/10037/10003/thesis.pdf?sequence=2&isAllowed=y.

Nordtug, T., Kvæstad, B., and Hagemann, A. (2021). Responses and preferences of salmon louse (*Lepeophtheirus salmonis* Krøyer, 1836) coppodids to underwater artificial light sources. Aquaculture, 532:736036.

Ofori, A. (2022). Identification and enumeration of sea lice larvae in zooplankton assemblages. MSc Thesis in fulfilment of the Erasmus Mundus Joint Masters Degree in Aquaculture, Environment and Society Plus.

Schram, T. A. (2004). Practical identification of pelagic sea lice larvae. Journal of the Marine Biological Association of the UK, 84(01), 103-110.

Samsing, F., Oppedal, F., Dalvin, S., Johnson, I., Vågseth, T., and Dempster, T. (2016). Salmon lice (*Lepeophtheirus salmonis*) development times, body size, and reproductive outputs follow universal models of temperature dependence. Canadian Journal of Fish and Aquatic Sciences, 73:1841-1851.

Skarðhamar, J., Nilsen Fagerli, M., Reigstad, M., Sandvik, A., and Bjørn, P. (2019). Sampling planktonic salmon lice in Norwegian fjords. Aquaculture Environment Interactions, 11: 701–715.

Szetey, A., Wright, D.W., Oppedal, F., and Dempster, T. (2021). Salmon lice nauplii and copepodids display different vertical migration patterns in response to light. Aquaculture Environment Interactions, 13:121-131.