Vol. 123: 181–192, 2017
https://doi.org/10.3354/dao03095

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published March 21

Factors influencing the long-term dynamics of larval sea lice density at east and west coast locations in Scotland

Anna J. Harte[1,2], Alan S. Bowman[1], Nabeil K. G. Salama[2], Campbell C. Pert[2,*]

[1]Institute of Biological and Environmental Sciences, School of Biological Sciences, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK

[2]Marine Scotland Science, Marine Laboratory Aberdeen, 375 Victoria Road, Aberdeen AB11 9DB, UK

ABSTRACT: Sea lice (Copepoda: Caligidae) are marine copepods that parasitize finfish, and in cases of high infestation can result in severe epithelial damage and mortality. In Scotland, 2 species of sea louse, *Lepeophtheirus salmonis* and *Caligus elongatus*, pose a significant economic burden to the marine Atlantic salmon aquaculture industry and potentially impact wild salmonids. The purpose of this study was to determine how the density of pelagic sea lice is affected by external variables, in order to improve our understanding of sea lice dynamics. Long-term data from 2 sampling sites on the east and west coasts of Scotland were modelled independently in conjunction with environmental and anthropogenic variables. Statistical analysis identified that at the east coast site, the most influential factor affecting lice density was salinity. On the west coast, salinity, rainfall and farmed salmon production year were most influential. Molecular and morphological techniques also showed that the individuals recorded on the east coast were *C. elongatus,* a generalist copepod parasite, whereas only the salmonid-specific *L. salmonis* were found on the west. These results reiterate the role of environmental factors in influencing sea lice dynamics, and that salmonids are the primary hosts of sea lice on the west coast, but there could be non-salmonid host species as well as salmonid species influencing east coast sea lice densities.

KEY WORDS: Caligidae · Scotland · Environmental parameters · *Caligus elongatus* · *Lepeophtheirus salmonis*

INTRODUCTION

Sea lice (Copepoda: Caligidae) are ectoparasitic marine copepods that feed upon the skin, mucus and blood of finfish, resulting in severe epithelial damage and possible mortality with high infestation levels (Costello 2006). In Scotland, 2 particular species of sea louse, *Lepeophtheirus salmonis,* Krøyer, 1837 and *Caligus elongatus,* Nordmann, 1832, are considered of significant interest, as *L. salmonis* is found in high numbers on farmed Atlantic salmon *Salmo salar* L., and *C. elongatus* is found both on salmonids and on other wild species (Kabata 1979), but to a lesser extent (Revie et al. 2002). The overall expense of sea lice treatment has been estimated to cost the industry around €0.25 kg^{-1} of salmon produced (Costello 2009a). Potential interactions with wild salmonid populations are also an ecological concern, as results from some studies have suggested that sea lice on farmed salmon could result in an increase in the sea lice infestation pressure along wild salmon migratory routes (Krkošek et al. 2006, Serra-Llinares et al. 2014).

While some studies have shown correlations between fish farms and population declines in adjacent wild salmonid populations (e.g. Butler 2002, Ford & Myers 2008, Middlemas et al. 2013), in others there is little evidence of sea lice-induced population regulation (Marty et al. 2010, Jackson et al. 2013). One of

*Corresponding author: campbell.pert@gov.scot

the difficulties of relating the sea lice burdens on farms to those on wild fish is the influence the environment can have on disease agents (Salama & Rabe 2013) and also the variability in environmental transport between farm and wild populations (Amundrud & Murray 2009, Salama et al. 2013, Johnsen et al. 2014). Factors such as temperature, wind direction, and salinity are significant (Boxaspen & Næss 2000, Bricknell et al. 2006, Amundrud & Murray 2009), with numerous other variables cited as having the potential to affect sea lice density (Heuch et al. 2000, Revie et al. 2003, Gillibrand & Willis 2007).

For example, the number of eggs produced by settled adult female *L. salmonis* is highly dependent upon the species, host, temperature, salinity and light (Johnson & Albright 1991, Boxaspen & Næss 2000, Heuch et al. 2002, Morton et al. 2004, Pert et al. 2012). In addition, the responses of planktonic and settled sea lice to some environmental variables differ quite substantially, an important factor considering that infestations of wild salmonids are likely to occur through encounters with the larval stages of sea lice that are transported out of the cages and into the wider environment (Costello 2009b). For example, attached adult *L. salmonis* are able to withstand lower salinity for longer than free-living stages, potentially due to the ability of settled sea lice to osmoregulate using the host's body fluids and tissues (Hahnenkamp & Fyhn 1985). Bricknell et al. (2006) demonstrated that free-swimming *L. salmonis* copepodids experienced significantly reduced survival and lower rates of successful settlement when salinity was below 29. Temperature has also been shown to influence copepodid sea lice (Costello 2006), with warmer sea temperatures reducing development times, thus resulting in a higher number of generations produced by adult females. Environmental variables that affect the physical aggregation of larvae, such as local wind conditions, are also important in determining larval distribution in small systems, with wind direction influencing surface currents and thus the direction and speed of larval transport (Amundrud & Murray 2009).

Penston et al. (2002) found that the number of gravid sea lice on farmed fish correlated closely with the number of larval sea lice in plankton samples collected at the head of Loch Shieldaig, Scotland. Further studies (Penston et al. 2004, 2008a,b, Penston & Davies 2009) provided increasing levels of evidence that planktonic sea lice are highly dependent upon the number of farmed fish that are present in the sea loch system. However, Penston et al. (2008a) also demonstrated that there were high levels of temporal variability of sea lice abundances, and Penston et al. (2008b) showed that the larvae could be transported several kilometres away from the point source. This would indicate that even if fish farms were the dominant source of sea lice larvae, environmental variables strongly influence their spatial and temporal aggregation both close to and distant from the farms.

The majority of sea lice plankton studies have been conducted over relatively short time periods (e.g. Penston et al. 2008a,b), and were undertaken in salmon farming regions; however, there is little indication of the environmental and anthropogenic influence on longer-term sea lice population dynamics. Therefore the aims of this study were to use data obtained from 2 unique time series of plankton sampling undertaken over 10 yr to develop statistical models to ascertain the relative influence of key variables on planktonic sea lice densities at 2 independent sites in Scotland, representing an area of intensive aquaculture and an area remote from fish farming activity.

MATERIALS AND METHODS

Study sites

Plankton samples were collected from 2 locations in the Scottish coastal zone, with one located 3 km off the coast of Stonehaven (56° 57.80′ N, 2° 06.20′ W) on the northeast coast of Scotland and the other at Loch Shieldaig (57° 30.90′ N, 05° 39.19′ W) on the west coast of Scotland (Fig. 1A). At both sites, Marine Scotland Science (MSS) have been collecting plankton samples for over 10 yr, providing a reliable and long-term data series with consistent methodologies within each site. The Shieldaig sampling site is located in an area containing 5 marine Atlantic salmon aquaculture sites, with Loch Shieldaig located between 2 larger lochs, which collectively make up the Loch Torridon system (Fig. 1B). The Loch Shieldaig site has a maximum tidal range of 4 m, with circulating currents that can be significantly influenced by strong winds (Murray & Gillibrand 2006). The Stonehaven site on the east coast has a maximum tidal range of 2.23 m, and is highly unlikely to be affected by any marine aquaculture units, as there are no active open water fish farms within ~200 km of the Stonehaven sampling site (Fig. 1C).

Plankton sampling

The weekly plankton samples and hydrographic data from the east coast site have been collected from

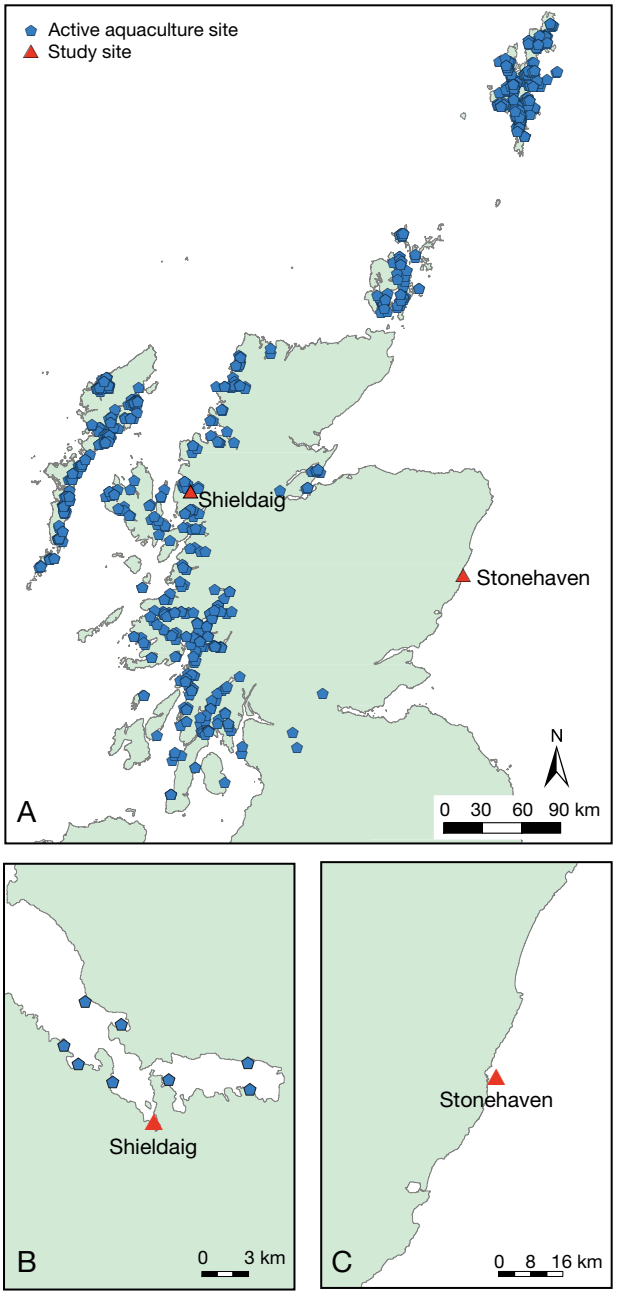

Fig. 1. (A) Scotland, showing both study sites in relation to their proximity to active seawater finfish farms (National Marine Planning Interactive Map, 2015). (B) The west coast study site located in Loch Shieldaig, which is surrounded by 6 active marine fish farms and (C) the east coast sampling site, located 3 km off the coast of Stonehaven, a site at least ~200 km away from the closest marine fish farm

a sampling point located 3 km off the coast of Stonehaven since 2003. Bresnan et al. (2015) used this dataset and another from the west coast to investigate and describe the comparative physics, nutrients and plankton communities of the east and west coast sampling sites, finding significant differences between the hydrodynamics, source of nutrients and zooplankton biomass, along with high inter-annual variability of these factors within both sites.

The archived Stonehaven samples for the years 2006 to 2013 were collected weekly using a double bongo net with 68 µm mesh, winched vertically through the water column, from a depth of 40 m to the surface. The cod end filter was immediately placed into 4% formalin and transported back to the MSS Laboratory in Aberdeen. Biological material was separated from the filter and transferred into 70% ethanol. One sample for every month between 2006 and 2013 was selected for screening and analysis at random using the RAND function in Excel. Additional samples were also collected for this trial from Stonehaven during 2014 and 2015 using a 150 µm mesh filter net towed horizontally through the water column at 1 m depth for 5 min at an average speed of 1 to 2 knots. Once the tow had been completed, the cod end was removed, inverted, and the biological material was washed using filtered seawater into a container of 4% formalin.

At Loch Shieldaig, continuous weekly samples for the entire study period were collected consistently at high tide, by hand-towing a 68 µm mesh filter sized net along a 50 m transect through the intertidal waters at 1 m depth near the mouth of the River Shieldaig. The sample was then washed down with fresh filtered seawater and stored in 4% formalin. Sub-sample sea lice counts and species identification from weekly samples collected before 2015 from the Loch Shieldaig site were provided by J. Raffell (MSS). For the statistical analysis, 1 sample mo^{-1} was selected at random using the RAND function in Excel. Samples from 2015, from both sites, were also sieved through a 500 µm mesh to remove large detritus prior to analysis.

Sample analysis and sea lice identification

Each plankton sample was sorted using an Olympus SZ-CTV microscope. Potential sea lice were removed from the samples and stored in 70% ethanol. Individual sea lice were identified using a Nikon CFW15X Optiphot compound microscope using anatomical characteristics detailed in Schram (2004). The keys in Kabata (2003) were used to identify the 3 adult sea lice isolated from plankton samples.

Sub-sampling was necessary on 3 occasions when whole samples contained more than 40+ sea lice. Sub-samples of 25 individuals were taken for analy-

sis, by randomly selecting individual lice from a well-mixed sample. In brief, individual sea lice were separated by date of collection, and each of these tubes containing more than 40 individual sea lice was agitated by hand, and a graduated 3 ml pipette was used to draw out an aliquot of the sample. The aliquot was then placed into a black viewing dish, and 25 sea lice from the aliquot were removed without using a microscope to ensure there was no possibility of species bias. A power analysis of the sample size estimated that there was a probability of 0.89, with 95% confidence, that the sub-sample accurately represented the entire array of species present in that sample. Real-time qPCR was used to confirm the species of the individual sea lice, following the protocol developed by McBeath et al. (2006).

Explanatory variable data

Five environmental variables were used as explanatory variables. These were the number of wild salmonids in the rivers of the surrounding fishery districts (District Salmon Fishery Board areas), wind speed, rainfall, seawater temperature and salinity. Wind speed and rainfall data were provided by the UK Meteorological Office using proxy data from the closest surface stations available, viz. Aultbea (35 km away from Shieldaig) and Inverbervie/Dyce (13 and 27 km from Stonehaven, respectively). The remaining datasets were sourced from MSS Marine and Freshwater Laboratories, with the exception of water temperature data for Loch Shieldaig, which was provided by Marine Harvest (Scotland) Limited. Datasets were averaged over a monthly period before being used for statistical analysis.

Monthly indices of relative abundance of wild trout and salmon were derived from rod catch numbers in the Dee and Don Districts for the east coast, and the Applecross, Balgay and Torridon districts for the west coast, provided by MSS (Marine Scotland Science 2014; see Table 2). No catch data are available to give an estimate of abundance of salmonids in the rivers during the winter months (closed season). For the dataset, these months were therefore given a value of 0 for number of fish.

Additionally, the 2015 wild salmonid dataset is not complete, as the surveys are collated in April of the following year, and therefore it was not possible to ascertain any monthly estimates of salmonid numbers at the time of writing. In order to keep wild salmonids as an explanatory variable in the model, estimates of monthly salmonid numbers for 2015 were calculated by taking the mean and standard deviation for each of the months over the previous 9 yr, and using the rnorm function in R (version 3.2.1) (R Core Team 2014) to select 50 random values within the normal distribution. These values were then averaged to provide a single value per month for 2015.

An additional binomial variable included in the Loch Shieldaig dataset was the aquaculture production year. Farmed fish spend up to 2 yr in sea cages, and there is often a significantly higher number of sea lice attached to fish in the second year of the cycle (McKibben & Hay 2004). We therefore considered it prudent to include this binomial variable in the Loch Shieldaig model. It was also included in the Stonehaven model to account for the possibility of any 2-yearly cycles that may be naturally present.

The sampled volume of water was calculated for each methodology by multiplying the area of the mouth of the plankton net by the length of each tow. The vertical sample volume for the 2006 to 2013 Stonehaven samples was 5.02 m^3, the horizontal sample volume for the Stonehaven samples from 2013 to 2015 was 29.1 m^3, and the horizontal hand tow volume for all the Shieldaig samples was 3.53 m^3. The differences in sample volumes between the methodologies was accounted for using the offset function in R (R Core Team 2014) to create equivalent sea lice densities, as suggested by Zuur et al. (2009). A flow meter was not used for the Loch Shieldaig site, as the abundant seaweed at this intertidal, shallow sampling site would have made using a flow meter futile. In addition to this, a study on sea lice density in the Loch Torridon system, but at greater depths, found that when using flow meters, the filtering efficiency maintained a relatively constant 70% efficiency over numerous sampling times (Penston et al. 2004). Thus to retain consistency, flow speed was not included in the analyses for either site, and all estimates of density are assumed to represent the minimum values, following the same protocol used by McKibben & Hay (2004).

Statistical analysis

The influence of both the environmental and anthropogenic factors on sea lice density were explored using the methods outlined for plankton datasets by Zuur et al. (2009), Poisson datasets in Zuur et al. (2012) and as employed by Penston et al. (2008a,b). In brief, count data with offset volume and rainfall as explanatory variables were used as a test dataset to

explore several different model types, including general linear models (GLMs), general additive models (GAMs) and then finally GAMs using the negative binomial family function. An analysis of the Pearson's residuals and degrees of freedom residuals were investigated, and further used to determine the dispersion values. A dispersion value close to 1 indicated that the model was a suitable fit for the data, and the plot function in R (R Core Team 2014) allowed the visualisation of the data which enabled the residuals to be checked for normality. The Poisson function was not used, as the data were too overdispersed to meet the underlying assumptions of Poisson distributed data of equal variance, and data were not transformed due to the high proportion of zero counts.

Fixed effects models were used, as all variables in the model were of significant interest and had the potential to be biologically relevant in predicting sea lice density. The data collection was undertaken following consistent methodology, sampling location, time of collection and number of sampling points throughout the study. Temporal variance may diminish the biological relevance of the factors being highlighted in the models. While seasonal factors that are known to be important in determining sea lice have been included in the models as separate variables (e.g. temperature, salinity, production year), there may be other unrecorded factors that change over time, such as production cycles (Revie et al. 2002). The selected models were then used to test the full datasets for each site, starting with all the variables and any biologically relevant interactions (e.g. salinity and temperature), and then using a backwards-stepwise approach to remove the least significant variables one at a time, until the model contained only significant terms. A multi-model inference approach was used, using the MuMIn package in R (Barton 2015). Both Akaike's information criterion (AIC) value and the Bayesian information criterion (BIC) model selection methods were used to determine which model best fit the data, alongside the percentage of residual deviance explained. All statistical analyses were performed using the statistical computing software R (R Core Team 2014).

RESULTS

Species identification

For both sites, samples from every month between 2006 and 2015 were screened for sea lice copepodids. In total, 151 individual sea lice were identified to species and stage using both morphological and molecular techniques. Of these, 3 were adult *Caligus elongatus*, and were not included in the statistical analysis. Two sea lice were damaged during the morphological identification process from samples taken on 27 January and 5 February 2015, and 3 specimens could not be identified using either technique. No *Lepeophtheirus salmonis* were found in the Stonehaven samples. Initial identification using morphological techniques suggested that 7 *C. elongatus* copepodids were isolated from the Shieldaig samples, but these were not consistent with the molecular results, which indicated that all the Shieldaig samples were *L. salmonis*. Previous identification work by J. Raffell using the key from Schram (2004) confirmed that all previous Loch Shieldaig samples from 2006 also only contained *L. salmonis*, although there could be a degree of misidentification in these samples, as several authors (Schram 2004, McBeath et al. 2006) have identified errors of around 25% when larval sea lice are characterized based on morphology.

The total number of sea lice copepodids collected from the representative monthly Stonehaven samples between 2006 and 2014, and the weekly Stonehaven samples in 2015, was 48. The highest density of sea lice found in any Stonehaven sample was 1.1 louse m^{-3}; however, the most common value was 0, and there were very low values overall. There may be suggestions of peaks in February, June and August/September (Fig. 2), but month was not found to be a significant factor in the model.

The total number of sea lice copepodids collected from the weekly Loch Shieldaig samples between 2006 and 2015 was 434. Of these, 90 were randomly selected to be included in the monthly analysis. Fig. 2 shows the density of sea lice plotted against month, with 1 very high-density value of 14.2 m^{-3} in March 2015, and a smaller peak of 3.4 m^{-3} in August 2011.

Salinity at Stonehaven varied seasonally from the lowest value of 33.9 in April 2008 to the highest of 34.8 in October of 2008, 2011 and 2012 (Fig. 3). In contrast, salinity was highly variable in Loch Shieldaig (Fig. 3). There was no correlation between salinity and rainfall at either site. Wind speed and rainfall did correlate in Loch Shieldaig (r = 0.73; Fig. 4).

Statistical analysis

Graphical data exploration highlighted a strong correlation (r = 0.94) between methodology and site,

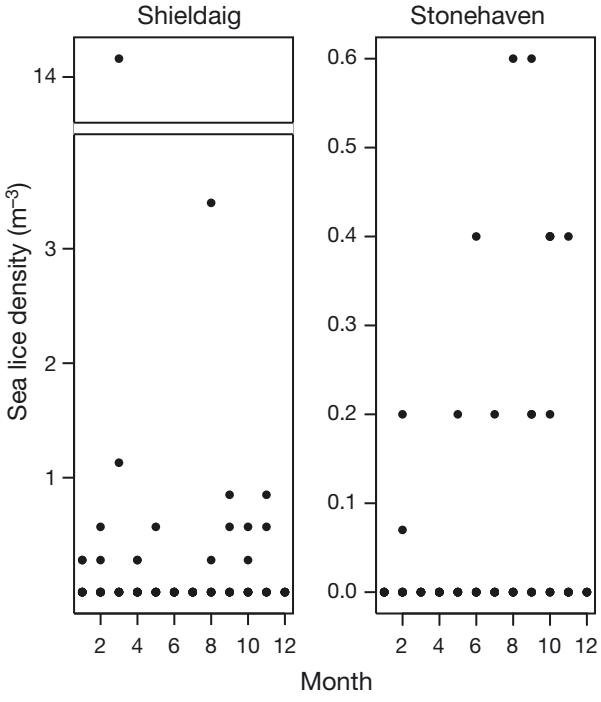

Fig. 2. Monthly distributions of *Lepeophtheirus salmonis* (Shieldaig), and *Caligus elongatus* (Stonehaven) densities from Loch Shieldaig and Stonehaven samples collected using hand and boat tows, respectively, from the years 2006 to 2015. There is an obvious discrepancy between the 2 sites in terms of the magnitude of the densities, with the maximum recorded density value in Shieldaig at 14.2 sea lice m^{-3}, while the Stonehaven site had density values of <1 louse m^{-3}

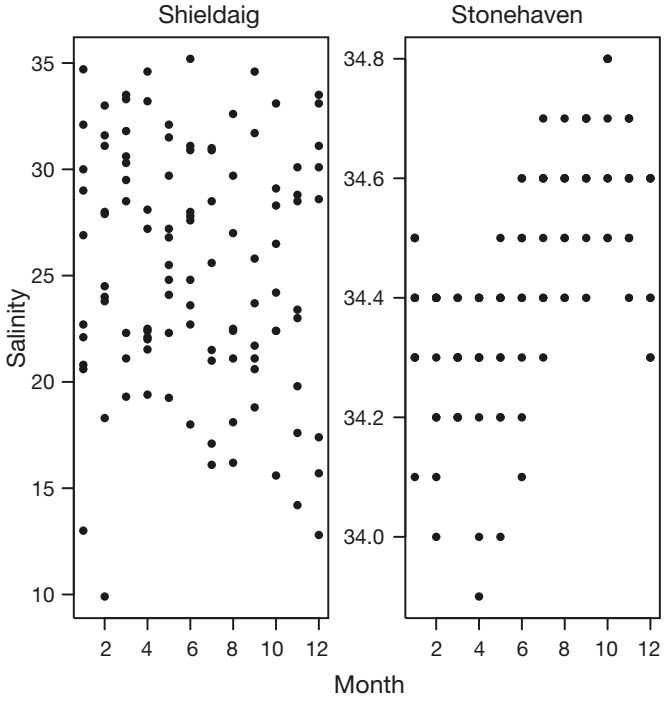

Fig. 3. Salinities recorded in each month from 2006 to 2015 at the 2 sample sites Loch Shieldaig and Stonehaven. The variation between the 2 sites is evident, with the much shallower Loch Shieldaig site having a salinity range of ~25 with apparently random fluctuations, whereas the deeper Stonehaven site range was <1, and followed a clear pattern of peaks and troughs throughout the years

as although different methodologies were used, they were almost completely consistent within each site, with the exception of the change in sampling regime from vertical to horizontal towing at Stonehaven from 2013 onwards. Due to this high correlation, methodology was removed from the model. Analysis of the dataset in its entirety revealed that site was a very influential factor ($p < 0.01$) in terms of predicting sea lice abundance. Site also correlated with rainfall, wind speed and salinity, so we decided that the 2 sites would be modelled separately, as there were very different levels of variability within each factor depending on the site. Statistical models were created separately for each site following the same process as detailed in the statistical methodology. Initial testing of the simplest model types showed that GLMs and GAMs were not suitable fits for either dataset due to high overdispersion and inappropriate residuals. A negative binomial GAM was the final model chosen, as it had dispersion values close to 1 and acceptable residuals. Both AIC and BIC model selection methods showed the same results in terms

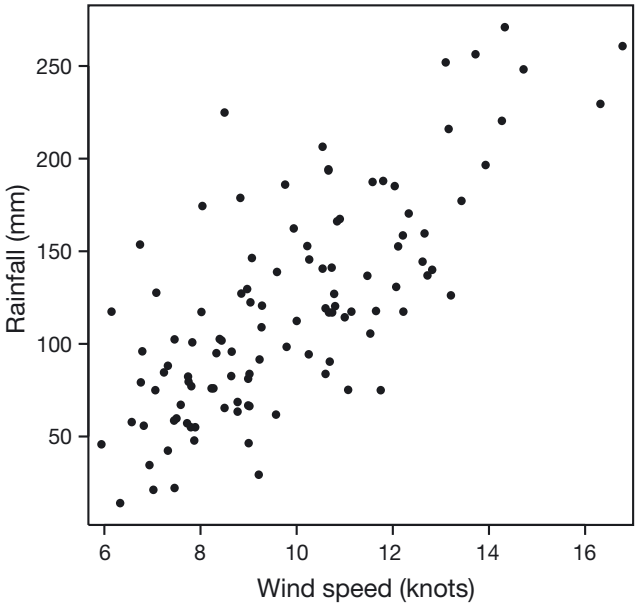

Fig. 4. Each sample point of the Loch Shieldaig dataset, showing a positive correlation between average monthly wind speed and total monthly rainfall

of model ranking, and thus only AIC values are reported for simplicity.

Shieldaig

The simplest model of sea lice density at the Loch Shieldaig site that had the lowest AIC/BIC value and included only significant terms was model C6 (Table 1A), which highlighted the variables rainfall ($p < 0.01$), salinity ($p < 0.05$) and aquaculture production year ($p < 0.001$) as the influential factors. The smoother (*s*) of rainfall (Fig. 5) was important, because for the same model without the smoother, the AIC value was higher by more than 2 points, and the percentage of deviance explained was much lower.

The final model for predicting sea lice density at the Loch Shieldaig site was:

$$\text{Sea lice density} = -9.0 + s(\text{Rainfall}) + 0.1(\text{Salinity}) + 2.5(2^{\text{nd}}\ \text{Production Year})$$

Stonehaven

The best fitting and least complex model of sea lice density at the Stonehaven site that included influential terms and had a low AIC value was model B8 (Table 1B), which highlighted the variable salinity ($p < 0.05$) as an influential factor, and also suggested that the number of wild salmonids ($p = 0.05$) may be relevant.

The optimum model for predicting sea lice density in coastal waters off Stonehaven was:

$$\text{Sea lice density} = -188.1 + 5.219(\text{Salinity}) + 0.009(\text{Number of wild salmonids})$$

DISCUSSION

We found that the density of sea lice larvae in Scottish coastal waters can differ greatly depending on the location, with the west coast site influenced by rainfall and aquaculture, and the east coast site influenced predominantly by salinity, but also very slightly by wild salmonid numbers. Identification work also revealed a divide in species composition between the 2 sites, with Stonehaven samples identified as *Caligus elongatus,* and those from Loch Shieldaig identified as *Lepeophtheirus salmonis.* This is the first study to investigate long-term trends of sea lice densities in Scottish waters, and provides a comparison between farming and non-farming regions.

Table 1. Individual negative binomial general additive models (NB GAMs) that were run on each dataset, with each variable included and their relevant interactions if present. The Akaike's information criterion (AIC) value and the percentage of deviance explained are listed for each. C: number of sea lice; R: rainfall (*s*: smoother function); W: wind speed; Sa: salinity; T: temperature; Y: year; M: month; P: production year; N: number of wild salmonids. (A) NB GAM models that were run for the Shieldaig data. (B) NB GAM models that were run for the Stonehaven data

Model	Variables	AIC	% deviance
(A)			
C1	C ~ *s*(R)+W+T×Sa×N+P+Y+M	155.9	65.8
C2	C ~ *s*(R)+W+T×Sa+N+P+Y+M	151.2	64.3
C3	C ~ *s*(R)+W+T×Sa+P+Y	148.6	62.4
C4	C ~ *s*(R)+W+T×Sa+P	148.0	60.7
C5	C ~ *s*(R)+ T×Sa+P	148.4	58.1
C6	C ~ *s*(R)+Sa+P	151.9	55.3
C7	C ~ R+Sa+P	154.5	48.5
(B)			
B1	C ~ *s*(R)+W+T×Sa×N+P+Y+M	163.0	43.9
B2	C ~ *s*(R)+W+Sa×N+P+Y	160.7	32.9
B3	C ~ *s*(R)+W+Sa×N+P	153.2	18.1
B4	C ~ *s*(R)+W+Sa×N	151.5	18.0
B5	C ~ *s*(R)+W+Sa+N	151.4	14.5
B6	C ~ *s*(R)+Sa+N	158.7	23.5
B7	C ~ R+Sa+N	151.5	14.7
B8	C ~ Sa+N	149.7	15.2

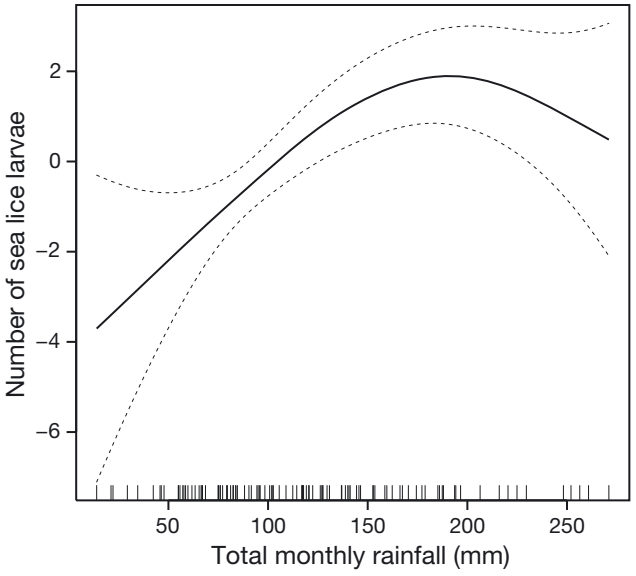

Fig. 5. Negative binomial general additive model smoother with confidence intervals showing how total monthly rainfall influences the number of sea lice in the Loch Shieldaig dataset, with a positive correlation up to ~190 mm of rainfall. The vertical tags on the *x*-axis indicate the rainfall value of each individual sample. The analysis was performed in R, using the mgcv package (Wood 2006)

For the Stonehaven samples, where no offshore aquaculture farms are present, the 2 factors that were highlighted as being influential on planktonic sea lice levels were salinity and number of wild salmonids. We had expected that salinity would be an important factor in terms of sea lice survival, and therefore abundance, as sea lice have lower rates of successful settlement (Bricknell et al. 2006) and reduced population sizes at lower salinities (Heuch et al. 2002). Data from this study showed that despite the annual salinity range at the Stonehaven site varying by just 1 (Fig. 3), this was found to be an important influence on density. One possible explanation for this result is that the salinity dataset is actually acting as a proxy for a different variable. When the salinity data for Stonehaven from the years 2006 to 2015 are plotted, there is an obvious annual pattern, with maximum values in October and minimum values in April (Fig. 3). The second influential variable, although not significant ($p = 0.05$) that was highlighted for Stonehaven was the number of wild salmonids which are hosts to *C. elongatus* (MacKenzie et al. 1998, Treasurer & Bravo 2011, O'Donohoe et al. 2015), and there were very low total numbers of sea lice found in Stonehaven, so it is possible that migrating wild salmonids are partially responsible for the *C. elongatus* found in the Stonehaven samples.

However, as previously mentioned, all of the sea lice found in the east coast samples were *C. elongatus,* a generalist parasite that has been observed infecting at least 80 species of fish (Kabata 1979). Previous studies performed on the east coast by Urquhart et al. (2008) showed no significant difference between the number of *C. elongatus* and *L. salmonis* present on wild sea trout *Salmo trutta*. No *L. salmonis* larvae were found on the east coast, however, and this could suggest that non-salmonid species, which do not act as hosts to the salmonid-specific *L. salmonis*, could also be affecting sea lice densities. Peaks of *C. elongatus*, including the presence of an adult female in the plankton sample, for which we could find no previous record in the literature, were observed in the raw data during some of the early autumn and late winter months. During this period there are considerable numbers of wild pelagic fish species present, such as herring *Clupea harengus*, L. and mackerel *Scomber scombrus*, L., and demersal species such as Atlantic cod *Gadhus morhua,* L., saithe *Pollachius virens,* L., and plaice *Pleuronectes platessa,* L., and these have been shown to be hosts of *C. elongatus* (Bruno & Stone 1990, Øines et al. 2006). Many of these species have spawning and migration routes close to the east coast of Scotland, with North Sea herring in particular spawning just off the coast of east Scotland in late summer to autumn months (Haegele & Schweigert 1985, Daan et al. 1990). It has been suggested that migrating wild Pacific herring *C. pallasii* are partially responsible for *Caligus clemensi* sea lice epizootics in British Columbia, Canada (Morton et al. 2008, Beamish et al. 2009), and the same situation could be occurring in Scotland with elevated levels of *C. elongatus* during periods when large numbers of *C. harengus* are present.

On the Scottish west coast at Loch Shieldaig, the significant factors for influencing planktonic sea lice levels were salinity, aquaculture production year and rainfall. Salinity in the Loch Shieldaig samples was highly variable, ranging from 5 to 34 (Fig. 3). Bricknell et al. (2006) demonstrated that when salinity drops below 29 for 1 h or more, sea lice survival becomes significantly compromised. Tucker et al. (2002) also showed that the development time of sea lice increases as salinity decreases, which would reduce population growth. Therefore, the considerable range in salinities in the very shallow sample site of Loch Shieldaig (Fig. 3) is likely to positively influence localised sea lice abundance. Although it appears there is a seasonal pattern in salinity, month was not a significant factor in the model descriptors. This is likely due to continual and gradual variation in salinity each month as opposed to discrete changes between months

The second factor included in the Loch Shieldaig model was the aquaculture production year, with the density of sea lice significantly higher in the second year of production. This has been demonstrated in numerous studies on the west coast of Scotland (McKibben & Hay 2004, Penston et al. 2008a,b, Middlemas et al. 2010), with the difference in sea lice levels between years several orders of magnitude higher for the second year of production in comparison to the first. This is likely due to the fact that at the beginning of the first year, as a result of the previous fallowing period, sea lice density will be at background levels with stocked smolts completely free from lice, and therefore it will take time for infestation levels to build up. The winter months will also reduce sea lice levels, as low temperature and light levels prolong hatching and development times (Boxaspen & Næss 2000). Once the second year of production is reached, however, there has been a static stock of salmonids that will have a degree of infestation, which, coupled with warming sea temperatures and longer days, provide optimum condi-

tions for rapid population growth in the second half of the year (Murray 2014). In addition to this, larger salmon provide more host surface area than smaller first-year stocks, with a study by Tucker et al. (2002) showing that size and number of sea lice parasites correlate closely.

The final factor that was important for Loch Shieldaig was rainfall, with increasing rainfall initially increasing the number of sea lice (Fig. 5). This is somewhat paradoxical, as it would be assumed that increasing rainfall would decrease salinity due to an influx of freshwater, and would reduce sea lice survival. Indeed, in a similar study, Costelloe et al. (1998) found that the density of larval sea lice in a loch on the west coast of Ireland negatively correlated with rainfall, which could indicate a relationship between the timing that salmonids return to freshwater and the seasonal climate in terms of temperature and salmonid behaviour. One possibility is that the rain is driven by landward winds, and it is the winds that force the larvae to aggregate in the shallow waters near the head of the River Shieldaig. Wind direction, which was not included in this analysis, is much more important in terms of physical sea lice aggregation (Amundrud & Murray 2009), as this influences surface currents, which are the primary mode of transport for positively phototaxic infective copepods (Heuch et al. 1995, Amundrud & Murray 2009). Interestingly, the relative AIC values of the models with and without a smoother on rainfall indicated that a model with a smoother was significantly better than one without. This points towards the non-linear relationship between sea lice and rainfall, as sea lice density increases with rainfall up until it appears to reach a threshold of around 200 mm month^{-1} (Fig. 5).

For the Loch Shieldaig dataset, the number of wild salmonids was not evident as a significant factor in the statistical model. This was not expected, as all of the fish farms in a farm management area will coordinate and synchronise their fallow periods with a minimum of 6 wk without stock, with the purpose of removing all sea lice from the system (Scottish Salmon Producers Organisation 2015). Subsequent smolts stocked from freshwater sites at the start of the first year of the production cycle will be completely free of sea lice, subsequently acquiring sea louse burdens from wild fish. The Stonehaven model suggested that wild salmonids were partially responsible for sea lice abundance fluctuations, and therefore it could be the case that wild salmonids are also influential in Loch Shieldaig, and this might be observed more clearly during or after fallowing periods, when it would be assumed that only wild salmon are present in the system, and no other outside influences such as sea lice from nearby lochs may be affecting sea lice abundance. The stock of farmed fish will provide a much larger host source for infective sea lice than motile wild salmonids, however, as numbers of wild salmonids in the northern hemisphere remain at low abundance levels (ICES 2015), while farmed Atlantic salmon biomass has been steadily increasing. In direct relation to this study, Table 2 provides data on the average numbers of wild salmonids caught on both the west and east coast sampling sites, and it is clear that the west coast Loch Shieldaig site has a much smaller wild salmonid population. However, it must be highlighted that the model that was suggested to be the most accurate for the Stonehaven system only explained around 15% of the deviance. This is a relatively low value and would suggest that there is a factor or factors missing from the model that would likely explain the observed variance in sea lice abundance on the east coast of Scotland.

This study demonstrates that sea lice densities in Scotland are influenced by site-specific factors, with salinity, rainfall and the aquaculture production year significant on the west coast site, and salinity and possibly wild salmonid numbers on the east coast site. Species identification suggests that salmonid-specific lice species dominate on the west coast, whereas generalist species are more common at the east coast site. To enhance the data and results of this study, further considerations are needed towards factors influencing environmental transmission such as currents and tides, as the relative hydrodynamics of the 2 sites are very different, and the sampling locations were also different in terms of distance from shore and sampling depth. Additional considerations are required for the influence of environmental factors on *C. elongatus* and *L. salmonis* independently, as well as the potential differences in sea louse numbers due to the variation in sampling methodology and location type at the 2 sites.

Acknowledgements. We thank the skipper and crew of the 'Temora' (Jim Brown and Paul MacDonald) as well as the willing volunteers who participate on the Stonehaven sampling rota as part of the Scottish Government project ST03p. We also thank Eileen Bresnan for the original idea of using the samples to analyse sea lice dynamics, Zoe Allcock for advice on the statistical analysis, Jim Raffell for assistance with the Shieldaig samples, Marine Harvest for providing west coast temperature data and Catherine Collins for guidance with the molecular identification. We recognise the valuable feedback provided by the 2 anonymous reviewers.

Table 2. Rod catch data for the east and west coast rivers used to estimate the number of wild salmonids present in the catchment area. Rod (released) catch data were collected from the Dee and Don districts for the east coast, and the Applecross, Balgay and Torridon districts for the west coast, and collated by Marine Scotland Science. To attain the monthly catch estimates, the number of fish caught each month was averaged between the number of districts. Data for 2015 were estimated by using the previous years' data to create 50 random values within the normal distribution, and taking the mean of these values

Month	Don	Dee	Average	Torridon	Applecross	Balgay	Average
2006							
2	1.3	58.3	29.8				0.0
3	8.5	176.5	92.5				0.0
4	27.8	197.3	112.5				0.0
5	20.3	262.8	141.5				0.0
6	23.5	321.5	172.5			15.0	15.0
7	41.0	131.8	86.4	8.0		2.0	5.0
8	56.8	258.8	157.8	8.0	6.0	58.0	24.0
9	150.0	494.0	322.0	2.0	6.0	14.0	8.0
10	246.8	2.0	124.4			8.0	8.0
2007							
2	1.3	59.3	30.3				0.0
3	8.8	122.8	65.8				0.0
4	9.3	113.8	61.5				0.0
5	16.0	214.5	115.3				0.0
6	19.5	222.5	121.0	3.0			3.0
7	17.3	233.5	125.4	6.0		5.0	6.0
8	41.8	379.0	210.4	8.0	8.0	29.0	15.0
9	106.8	439.5	273.1	12.0	12.0	3.0	9.0
10	92.0		92.0				
2008							
2	5.8	58.8	32.3				0.0
3	3.8	98.3	51.0			2.0	2.0
4	15.0	164.3	89.6				0.0
5	25.5	224.0	124.8				0.0
6	13.5	198.0	105.8				0.0
7	11.5	168.0	89.8				0.0
8	22.0	324.3	173.1		7.0	40.0	24.0
9	58.8	331.5	195.1		8.0	6.0	7.0
10	103.0	171.5	137.3			2.0	2.0
2009							
2	5.8	84.3	45.0				0.0
3	8.3	84.0	46.1				0.0
4	13.3	119.3	66.3				0.0
5	15.8	265.3	140.5				0.0
6	17.5	403.8	210.6				0.0
7	30.5	303.8	167.1			8.0	8.0
8	32.8	307.3	170.0		23.0	5.0	14.0
9	68.8	413.3	241.0		14.0	7.0	11.0
10	102.5	224.5	163.5			1.0	1.0
2010							
2	5.0	39.8	22.4				0.0
3	7.3	92.0	49.6				0.0
4	26.3	174.5	100.4				0.0
5	42.5	408.3	225.4				0.0
6	46.0	538.5	292.3			14.0	14.0
7	41.5	377.3	209.4			25.0	25.0
8	73.0	437.3	255.1		16.0	36.0	26.0
9	167.5	580.8	374.1		25.0	25.0	25.0
10	168.5	227.3	197.9			4.0	4.0

Month	Don	Dee	Average	Torridon	Applecross	Balgay	Average
2011							
2	4.3	92.8	48.5				0.0
3	15.3	111.3	63.3	41.0			41.0
4	11.5	241.8	126.6	43.0			43.0
5	16.0	326.3	171.1	165.0		7.0	85.0
6	24.8	375.5	200.1	63.0		18.0	46.0
7	32.5	316.5	174.5	34.0		16.0	25.0
8	64.3	377.5	220.9	51.0	10.0	15.0	25.0
9	85.8	391.0	238.4	226.0	8.0	6.0	80.0
10	111.8	211.5	161.6	62.0			62.0
2012							
2	4.5	75.0	39.8				0.0
3	5.3	62.3	33.8	42.0			42.0
4	4.3	134.8	69.5	12.0			12.0
5	40.3	234.5	137.4	13.0			13.0
6	35.0	361.3	198.1	70.0			70.0
7	69.5	452.5	261.0	31.0	5.0	7.0	14.0
8	66.0	349.3	207.6	40.0	2.0	35.0	25.0
9	86.8	373.5	230.1	50.0	8.0	14.0	25.0
10	150.0	182.0	166.0	37.0			37.0
2013							
2	4.3	47.8	26.0				2.0
3	8.8	70.8	39.8	32.0			32.0
4	32.8	128.3	80.5	77.0			77.0
5	26.8	181.8	104.3	38.0			38.0
6	11.3	244.0	127.6	68.0		1.0	23.0
7	11.3	112.8	62.0	96.0		2.0	33.0
8	19.8	187.0	103.4	358.0	8.0	2.0	120.0
9	40.5	263.3	151.9	308.0	7.0	18.0	111.0
10	140.0	217.3	178.6	372.0			372.0
2014							
2	2.5	56.8	29.6				0.0
3	9.3	75.8	42.5				0.0
4	15.8	83.0	49.4				0.0
5	19.0	180.8	99.9				0.0
6	23.8	245.0	134.4			8.0	8.0
7	8.0	92.5	50.3			7.0	7.0
8	26.8	240.3	133.5		12.0	49.0	31.0
9	41.8	170.8	106.3		3.0	6.0	5.0
10	76.8	97.3	87.0			34.0	34.0

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Editorial responsibility: Bernd Sures, Essen, Germany

Submitted: June 20, 2016; Accepted: December 12, 2016
Proofs received from author(s): March 10, 2017