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Prevalence and infection intensity of sea lice (*Lepeophtheirus salmonis*) on Atlantic salmon (*Salmo salar*) host is reduced by the non-host compound 2-aminoacetophenone

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ABSTRACT

Chemical cues, or semiochemicals, are used by parasitic sea lice (*Lepeophtheirus salmonis*) not only in the identification and location of host salmonid fish, but also in the avoidance of unsuitable fish hosts. In this study, an identified non-host semiochemical, 2-aminoacetophenone (2-AA), isolated from turbot (*Scophthalmus maximus*) was used in the field to reduce sea lice prevalence and infective capability. Field trials were conducted at an experimental fish farm in north-west Scotland, in order to determine the effect of 2-AA on *L. salmonis* abundance on Atlantic salmon (*Salmo salar*) hosts. Salmon smolts were maintained in small pens fitted with a polymer ribbon impregnated with 2-AA. The ribbon was designed to release 2-AA into seawater over an extended period. Three separate trials were undertaken in June–July and November–December 2011, over periods lasting 39, 11 and 11 days, when the fish were exposed to natural sea lice challenges. Prevalence and parasite loads observed were consistently lower on fish where the non-host cue was released. Prevalence ranged from 24–60% fish with lice in controls, to 12–35% fish in the 2-AA treatments, representing effective reductions of 43–62%. Mean parasite load ranged from 0.30–1.19 lice/fish in controls to 0.12–0.52 lice/fish in 2-AA treatments, representing effective reductions of 43–56%. The principle of interfering with ectoparasitism in fish by means of non-host semiochemical cues is demonstrated. Further investigations are required, in order to determine the potential value of semiochemical application as an effective pest management tool in the salmon farming industry.

1. Introduction

Ectoparasites that affect farmed livestock contribute directly to food insecurity on a global scale (FAO, 2005). At present, veterinary medicines are the main intervention against such ectoparasites, although alternative methods are under serious consideration. For example, the potential for manipulating ectoparasite behaviour via olfactory detection of semiochemicals (naturally-occurring behaviour and development-modifying chemical signals) presents opportunities for their control, via the development of novel repellents and attractants, provided that they can be deployed through an integrated management strategy, e.g., the push–pull strategy, which has been used highly successfully for pest management in some arable crop systems (Cook et al., 2007; Hassanali et al., 2009). The ability to manipulate ectoparasite behaviour can be exploited by developing repellents based on hypotheses that relate to the evolution of repellency, in particular the response of ectoparasites to semiochemicals emitted from related non-host species (Pickett et al., 2012).

Sea lice (Copepoda: Caligidae) commonly parasitise wild and farmed salmonids (Kabata, 1979; Pike and Wadsworth, 2000). The most important sea lice species in the North Atlantic region, *Lepeophtheirus salmonis* Krøyer, is a host-specialist that requires a suitable, salmonid host for settlement and survival (O'Shea, 2005). In seminal studies on the chemical ecology of salmonid–*L. salmonis* interactions, we identified two host-specific semiochemicals from the host, Atlantic salmon, *Salmo salar*, i.e. α-isophorone and 6-methyl-5-hepten-2-one (Ingvarsdottir et al., 2002), and demonstrated that attraction to salmonid semiochemicals could be removed by non-host species in the same class (Actinopterygii) e.g. the turbot, *Scophthalmus maximus*, with this removal being caused by turbot-specific compounds 2-aminoacetophenone (2-AA) and 4-methylquinazoline (Bailey et al., 2006). The removal of normal host location behaviour thus raised the prospect of developing a

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semiochemical-based strategy to control sea lice, as an integral part of pest management in salmonid aquaculture (Mordue, Luntz and Birkett, 2009). In this paper, we report on three field-based studies that investigate the potential to interfere with natural rates of lice infection in the marine environment, using slow-release formulations of 2-AA, identified as one of the non-host compounds from *S. maximus*.

2. Methods

2.1. Slow-release formulations

2-Aminoacetophenone (2-AA) was obtained from Sigma-Aldrich (>99% purity by GC) and formulated into PVC rope (5% w:w loading, Agrisense-BCS Ltd, Pontypridd, Wales, UK) that provided a slow and consistent approx. release rate of 0.48 mg/g/day.

2.2. Sea lice monitoring

Experiments to determine the effect of 2-AA on natural lice infestations were held at an experimental fish farm in north-west Scotland. Background lice numbers were checked routinely, by weekly examinations of captive fish ($n = 45$). Numbers of lice found on each fish are recorded, according to species (*L. salmonis*/*Caligus* sp. [typically *Caligus elongatus*]) and also sex/maturity stage for *L. salmonis* (chalimus, pre-adult, male, non-gravid/gravid female). Background lice data were therefore obtained for the period May–July 2011, before and during the trial period.

2.3. Fish infection trial

At the experimental site, trial fish were maintained in 12 cylindrical sentinel pens ($H = 1$ m, *dia.* $= 0.9$ m), fitted with 10 mm mesh netting and supported by stainless steel rings. The fish were exposed to challenges of sea lice that occurred naturally in the Loch Ailort system. Three separate trials were conducted:

Trial 1 (*long-line*) — each pen was stocked with 20 salmon smolts ($n = 12$ pens $\times$ 20 fish $= 240$ fish overall) and suspended on a long, floating line at 1–2 m depth. Alternating control ($n = 6$) and treatment ($n = 6$) pens were deployed along the line at 50 m intervals. The control pens were deployed on the line first (in order to avoid cross-contamination with 2-AA) and the treatment pens were then fitted with polymer ribbon and deployed later. Each treatment pen was fitted onshore with ribbon ($L = 22$ m/pen, $W = 660$ g/pen, loading = 5% 2-AA [i.e. 33 g/pen]). Deployed pens were soaked in position for 24 h in order to stabilise 2-AA release rates. The trial fish (mean wt = 80 g) were taken from a holding cage on site and anaesthetised prior to individual examinations for sea lice. The fish were then carefully introduced into the pens. During the trial, the fish were fed (standard feed pellets), using a plastic feeding tube and checked daily by eye/glass-bottomed viewer. Detailed checks of the pens and fish were made weekly, using a remote, underwater (Spyball) camera system. Trial 1 ran for 39 days (09 Jun–18 Jul, 2011).

Trial 2 (*cages*) — the pens used in trial 1 (6 controls + 6 treatments) were cleaned and re-used (the treatment pens were fitted with new polymer ribbon). Each pen was pre-soaked for 24 h and stocked with 20 salmon smolts, as before. The fish (mean wt = 65 g) were transported directly from a local freshwater hatchery (MH Glen Finnan) and so required no pre-trial examinations for lice. This time, the pens were stationed inside standard production cages (16 × 16 m sq.) stocked with large salmon ($n = 2000$ fish, mean wt = 3.5 kg). A pair of pens (1 control + 1 treatment) was deployed in each cage, located in opposite corners of the cage. All pens were located c. 20 m apart. The tops of the pens were set at 0.1 m above the water surface, in order to allow the fish access to the surface. The fish were fed and monitored daily as described previously. Trial 2 ran for 11 days (07–18 Nov, 2011).

Trial 3 (*cages*) — Trial 2 was repeated, with new fish (mean wt = 72 g) from MH Glen Finnan Hatchery. The pens were repaired, re-stationed (as before) and soaked for one week prior to re-stocking. However, the polymer ribbon was not changed for trial 3 (the ribbon used had by then been soaked in seawater for 3 weeks). The fish were fed and monitored daily as described previously. Trial 3 ran for 11 days (21 Nov–02 Dec, 2011).

At the end of each trial, the individual pens were taken aboard a workboat and immersed in a large tank of seawater and anaesthetic (tricaine mesylate [methanesulfonate]: TMS-MS-222, 100 ppm). The anaesthetised fish were then removed from the net and examined for sea lice. Numbers of lice found on each fish were recorded, according to species and sex/maturity stage (as described previously). The tanks were checked for dislodged lice and cleaned between sampling (pens). The emptied pens were then taken ashore and the examined fish were then killed humanely (by percussion) and discarded (fish farm biological waste is treated by ensilation).

2.3.1. Statistical analysis

Mean levels of mortality and prevalence of sea lice infection in each pen were compared using one-way ANOVA. Generalised linear mixed models (GLMM) with a Poisson distribution and log link function were used to determine the effect of treatment (2-AA ribbon) on lice count (n lice/fish). The GLMMs included treatment as a fixed effect and pen as a random effect. This allowed for the non-independence of individual fish within each pen when comparing mean numbers of lice/fish observed in treatment and control pens. Modelling assumptions were checked using standard diagnostic plots of residuals.

3. Results

3.1. Background sea lice numbers

In trial one, small numbers of sea lice were observed during May–July 2011 (Fig. 1a). Weekly mean loads ranged from 0 to 0.24 lice/fish (*Caligus* sp.) and 0 to 1.62 lice/fish (*L. salmonis*) during this period. Prevalence ranged from 0 to 76% fish/week infected. Lice numbers started to rise by late June, mainly due to the appearance of chalimus and pre-adult stages of *L. salmonis*. The former increased steadily between mid June and late July, from 0.02 to 0.18 lice/fish. Small numbers of *Caligus* sp. appeared briefly, on 08/07/11 (0.24 lice/fish) and then disappeared. Thus, few lice were present during much of the trial 1 period (Fig. 2a). In trial 2, numbers of sea lice rose steadily during November 2011 (Fig. 1b). Weekly mean loads ranged from 0.4 to 3.83 lice/fish (*L. salmonis*) during this period. Only one single *Caligus* sp. was recorded, on 18/11/11. Prevalence rose steadily during the trial two period, ranging from 40 to 97% fish/week infected. In trial 3, the steady increase in lice numbers observed by mid-November continued until early December 2011 (Fig. 1b). Weekly mean loads ranged from 3.83 to 6.00 lice/fish (*L. salmonis*) during this period. No *Caligus* sp. were found. Prevalence remained at high levels (>95%) during the trial 3 period.

3.2. Fish infection trials

For trial 1, as mentioned previously, background lice numbers were initially low and increased only gradually (Fig. 1a). Therefore, trial 1 was extended to 39 days in order to ensure a sufficient lice challenge. Fish mortality ranged from 2 to 8 fish/pen (10–40%) and 0 to 8 fish/pen (0–40%) in control and treatment pens, respectively. No significant difference in average mortality level between control and treatment pens was observed ($F_{1,10} = 0.04$, $p = 0.839$). Overall, 51/85 fish (60%) and 30/87 fish (35%) were infected with sea lice (all spp.), in the control and treatment pens, respectively (Table 1). This represents an effective 43% reduction in overall prevalence of lice-infected fish in the (2-AA) treatment pens. The observed prevalence