Aquaculture 422–423 (2014) 54–62

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Assessment of sea lice (*Lepeophtheirus salmonis*) management in New Brunswick, Canada using deltamethrin (AlphaMax®) through clinical field treatment and laboratory bioassay responses

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ARTICLE INFO

Article history:
Received 1 October 2013
Received in revised form 22 November 2013
Accepted 25 November 2013
Available online 4 December 2013

Keywords:
Sea lice
Lepeophtheirus salmonis
Deltamethrin
Atlantic salmon
Bioassay
Efficacy

ABSTRACT

The control of the ectoparasite, sea lice (*Lepeophtheirus salmonis*), on farmed Atlantic salmon in Eastern Canada was complicated by the development of resistance to emamectin benzoate, the primary in-feed medication used since 2000. Field efficacy and bioassay assessments were initiated to address the emergency authorization of deltamethrin (Alpha Max®) used in limited circumstances in 2009 and again in 2010. Under farming conditions present in the Bay of Fundy, deltamethrin consistently reduced pre-adult (male and female) and male adult lice stages in the range of 88–98% compared to pre-treatment levels. Cage-level reductions for both adult female and chalimus lice stages varied considerably with median reductions of around 50% or less commonly observed for either stage. *In vitro* bioassays using field collected mobile stages of sea lice generated average effective concentration (EC_{50}) values that were lower for combined stages of pre-adult and adult male lice compared to either pre-adult female or adult female lice stages. Stage ($p < 0.001$) and temporal ($p < 0.001$) differences were observed for EC_{50} values. Both field treatment observations and *in vitro* assessments of sea lice responses reflected greater reductions after deltamethrin exposure for pre-adult and adult male lice compared to adult female lice stages. Variable response occurring in different lice categories is likely to affect the successful field application of this treatment and is an important factor to consider when deciding how best to report efficacy.

1. Introduction

Sea lice (*Lepeophtheirus salmonis* and *Caligus* sp.) infestation of farmed salmonids, particularly Atlantic salmon (*Salmo salar*), represents a significant economic and health burden in regions where salmon are intensively cultured (Costello, 2006; Heuch et al., 2005). Infestation of cultured Atlantic salmon by *L. salmonis* has been reported in the North Atlantic and Pacific (Pike and Wadsworth, 1999; Revie et al., 2009). The more host indiscriminant *Caligus rogercresseyi*, remains the predominant threat to the Chilean industry (Bravo et al., 2008) with the less well documented, but still economically impactful, *Caligus elongatus* and *Caligus orientalis* also reported in the North Atlantic (Hogans and Trudeau, 1989; Revie et al., 2002; Schram et al., 1998; Tully, 1989) and Japan, respectively (Nagasawa, 2004).

The use of long-term integrated biological and chemical strategies for managing sea lice is an important tool in the sustainability of salmonid aquaculture industries in many countries and the environment in which they operate. Generally, integrated pest management strategies use a combination of best management practices and treatment measures in an attempt to effectively and economically control sea lice infestations in an environmentally sustainable manner. Chemical treatments are used as a key component of these management plans and include a variety of agents (Boxaspen, 2006; Brooks, 2009; Pike and Wadsworth, 1999). Decisions as to the timing and choice of treatment should be based on a program of regular monitoring of lice species, stages and numbers, as well as previous medicine used (rotation of different modes of action necessary) and assessment of fish health. Monitoring the abundance of sea lice on farms and the development of resistance to chemotherapeutants are important factors in the successful management of this parasite (Denholm et al., 2002; Heuch et al., 2005; Sevatdal et al., 2005; Treasurer and Pope, 2000; Westcott et al., 2004) providing decision support tools for delivery of evidence-based outcomes to all levels of policy makers. Unfortunately, reduced sensitivity in *L. salmonis* to a range of chemotherapeutants, from different geographical areas where salmonids are intensively cultured in sea water, have been reported (Bravo et al., 2008; Denholm et al., 2002; Fallang et al., 2004; Jones et al., 1992, 2002; Lees et al., 2008a; Sevatdal and Horsberg, 2003; Treasurer et al., 2000; Tully and McFadden, 2000).

Deltamethrin [(S)-α-cyano-3-phenoxybenzyl,(1R)-*cis*-3-(2,2-dibromovinyl)-2,2 dimethyl-cyclopropane carboxylate] is a synthetic type II pyrethroid insecticide and acaricide used for the topical control

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http://dx.doi.org/10.1016/j.aquaculture.2013.11.027

of ectoparasites in cattle, sheep and poultry. It is also available as Alpha Max® (Pharmaq) for use in Atlantic salmon aquaculture to treat sea lice infestations (Bravo, 2013; Fisheries and Oceans Canada, 2012; SEPA, 2008; Sevatdal and Horsberg, 2003). Several clinical treatment failures and reduced sensitivity to deltamethrin have been reported from Norway (Sevatdal and Horsberg, 2000, 2003) with unverified anecdotal reports of reduced sensitivity from Scotland and Ireland (Sevatdal et al., 2005). Reduced sensitivity has also been documented *in vitro* using bioassays (Sevatdal and Horsberg, 2003). Over the past decade, bioassay protocols have been adapted for use as a common tool to monitor sea lice resistance to chemotherapeutants globally (Bravo et al., 2008; Helgesen and Horsberg, 2013; Saksida et al., 2013; SEARCH, 2004; Sevatdal and Horsberg, 2003; Sevatdal et al., 2005; Westcott et al., 2008; Whyte et al., 2013).

In July, 2009, a one-year emergency authorization, for the application of Alpha Max® (deltamethrin), was issued by the Pesticide Management Regulatory Agency, a branch of Health Canada, under the Pest Control Products Act (Department of Fisheries and Oceans, 2010). The limited use of this product required documented evidence of efficacy in the field, together with in vitro bioassay data to identify trends in sea lice tolerance to this chemotherapeutant (Department of Fisheries and Oceans, 2010). A key component of an integrated pest management plan is monitoring treatment efficacy for each product to detect early indications of tolerance development. Field treatment efficacy is evaluated by comparing sea lice counts (by stage) pre- and post-treatment of a sample of fish from treated cages. Accurate measures of the clinical responses for different stages of sea lice performed in multiple cages at multiple sites, before and after field treatments, are required for optimization of control measures by farms and, to further justify continued access to therapeutic products. The lack of effective chemotherapeutic control options for sea lice infestations significantly impacted the New Brunswick salmon farming industry in 2010, in terms of financial losses.

The objective of this project was to provide critical evidence for sea lice control decisions at the government policy and farm management levels for the use of deltamethrin under the unique salmon culture conditions of the Bay of Fundy, New Brunswick, Canada. The information provided was primarily based on treatment efficacy as measured by the direct measurement of different stages of sea lice reduction post-treatment and through *in vitro* bioassays to inform the description of trends in tolerance patterns for deltamethrin in New Brunswick.

2. Materials and methods

2.1. Sea lice categorization

The sea lice monitoring program in New Brunswick requires lice to be categorized by species (*Lepeophtheirus* sp. and *Caligus* sp.), stage (larval, pre-adult and adult) and sex (male and female). Sea lice categories for field counts included: [1] chalimus (Chalimus 1-IV); [2] pre-adult (stages I and II) (male and female) and adult male lice (PAAM); [3] adult female lice (gravid and non-gravid) (AF). Pre-adult (stages I and II) (male and female) and adult male lice stages were combined into one category to facilitate ease of counting as separating them by sex and stage is time-consuming, labor-intensive and potentially leads to unhealthy consequences for fish due to excessive handling when lice numbers are high. Categorization differed for the bioassay procedure whereby pre-adult lice were further separated by stage (stage II) and sex; pre-adult male (PAM) and pre-adult female (PAF). Differences in sex and stage responses to treatment have been observed in bioassays (Sevatdal et al., 2005; Westcott et al., 2008; Whyte et al., 2013) which suggest that mixing different sexes and stages of lice within a single bioassay may result in pertinent information being overlooked. Immature male lice are distinguished from mature male lice by the surfaces of the second antennae and the presence of a rough-surfaced pad (post-oral adhesions pad) located near the base of the first maxilla (Johnson and Albright, 1991), a process that is facilitated by magnified inspection. To efficiently assign hundreds of field-collected lice of mixed ages to multiple bioassay containers with as little impact on their survival as possible, PAM lice were further combined with adult male lice to create a PAM-AM category.

2.2. Sea lice monitoring and treatment efficacy

Sea lice abundance and response to deltamethrin (AlphaMax®) treatment under field conditions of use were examined by performing pre-treatment and post-treatment counts, at least weekly. These counts were reproducible (similar counts by different trained personnel at the same site or the same trained personnel at different sites) and were applied in a similar manner across the industry (Elmoslemany et al., 2013). *In vitro* bioassays contributed descriptive information on the trend in tolerance patterns for deltamethrin in New Brunswick, Canada. Marine Atlantic salmon (*S. salar*) aquaculture sites in the Bay of Fundy (Fig. 1), which received treatments with deltamethrin during the period July to September 2009, were identified and assessed for sea lice numbers during focused monitoring, as part of the integrated sea lice monitoring program coordinated by the New Brunswick Department of Agriculture, Aquaculture and Fisheries (NBDAAF). Recent development of resistance to emamectin benzoate (SLICE®) (Jones et al., 2013) necessitated rapid action and, at the time, no alternative treatments were permitted in the region. Sites receiving treatment were chosen based on conditions related to emergency permissions arranged by NBDAAF and as a result, no randomized comparisons or untreated control cages/sites were feasible.

Mean cage and treatment event levels of site-level sea lice abundance were estimated based on samples of between 10 and 15 fish per cage and from all cages at each site (3 to 9 cages per site). Counts were performed on each cage, as close to the treatment day as possible, at a point no more than 4 days prior to treatment (pre-treatment count) and multiple times within 14 days following each treatment (post-treatment counts). Pre and post count data were available for 6 treatment events involving a total of 41 cages from 4 sites within the same management area of the Bay of Fundy, New Brunswick (Fig. 1). Counting of all cages would occur on the same day but treatment days differed slightly, thus cages were often measured at different days post-treatment based on the day of treatment.

All treatments were performed on-site using skirt-style enclosures. Curtains of tarpaulin material were deployed to fully surround the cage to an appropriate depth (generally the lower end of the tarpaulin should be at least 2 m deeper than the lowest depth of the net holding fish to be treated, as dictated by emergency permits), but without an enclosed bottom, and were open to limited water exchange. The prescribed treatment dose was 3 parts per billion (ppb) deltamethrin and an exposure duration of 40 min. Supplemental aeration was added during the exposure period.

Fish sampled for sea lice abundance counts were collected using a sample procedure involving capture (with a dip-net) of fish attracted to the surface with feed, followed by anesthesia using tricaine methane sulphonate (TMS; Syndel Laboratories), at a dose of approximately 100 mg L^{-1}. Each stage of lice was counted and recorded on a per-fish basis. The percentage knock-down values for sea lice were estimated based on the number of lice recorded during the pre-treatment count conducted closest to the time of deltamethrin treatment (when there was more than one count) and the average count in the first 7 days post-treatment period.

2.3. Sea lice collections for bioassay

Sea lice were collected from fish originating at Atlantic salmon marine cage sites located in the Bay of Fundy, New Brunswick, during routine sea lice counting on sites which had received treatments