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Baseline

# Occurrence of antiparasitic pesticides in sediments near salmon farms in the northern Chilean Patagonia

Felipe Tucca [a,*], Heriberto Moya [a], Karla Pozo [b,c,d], Francesca Borghini [d], Silvano Focardi [d], Ricardo Barra [a]

[a] Facultad de Ciencias Ambientales y Centro EULA-Chile, Universidad de Concepción, Barrio Universitario s/n, P.O. Box 160-C, Concepción, Chile
[b] Research Center for Toxic Compounds in the Environment (RECETOX), Faculty of Science, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic
[c] Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Alonso de Ribera 2850, P.C. 407 01 29 Concepción, Chile
[d] Department of Physical, Earth and Environmental Sciences, University of Siena, Via Mattioli 4, 53100 Siena, Italy

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ABSTRACT

Growth of the aquaculture industry has triggered the need for research into the potential environmental impact of chemicals used by salmon farms to control diseases. In this study, the antiparasitic pesticides emamectin benzoate (EB), diflubenzuron (DI), teflubenzuron (TE), and cypermethrin (CP) were measured in sediments near salmon cages in southern Chile. Concentrations for EB were between 2.2 and 14.6 ng $g^{-1}$, while the benzoylphenyl ureas DI and TE were detected in the ranges of 0.1 to 1.2 ng $g^{-1}$ and 0.8 to 123.3 ng $g^{-1}$, respectively. These results were similar to data reported for the Northern Hemisphere. On the other hand, the pyrethroid CP was detected in higher concentrations, ranging from 18.0 to 1323.7 ng $g^{-1}$. According to reported toxicity data, this range represents a potential risk for benthic invertebrates. This report is the first baseline attempt at assessing antiparasitic pesticide levels in the Chilean Patagonia.



Aquaculture, and its role as a human food source, has increased globally in recent decades. However, concerns have emerged about its adverse impacts on marine ecosystems (Naylor et al., 2000; Buschmann et al., 2009). Studies have documented the increased presence of organic matter in sediments and consequent changes in sediment composition, the increased use of chemicals in salmon farming, and the impact of escaped salmon from farms, among other issues (Volpe et al., 2000; Soto and Norambuena, 2004; Burridge et al., 2010).

Chilean salmon farms have been severely impacted by several fish pathologies (Bravo et al., 2011), with the copepod *Caligus rogercresseyi* (Boxshall and Bravo, 2000) having the greatest impact in southern Chile (Hamilton-West et al., 2012). Various antiparasitic pesticides have been used to treat, prevent, and mitigate parasitic infestations. Currently, pesticides such as emamectin benzoate (EB), teflubenzuron (TE), diflubenzuron (DI), deltamethrin (DE), and cypermethrin (CP) are used to combat parasitic diseases. These antiparasitic pesticides are either applied using bath treatments (CP and DE) or are orally administered via fish feed (EB, DI, and TE). The main characteristics of these pesticides are low solubility in water, a high octanol-water partitioning coefficient (log $K_{ow} > 5$), and a high capacity for absorption by suspended matter, thereby reaching bottom sediments. Therefore, the potential exposure and bioavailability of pesticides to sediment-associated organisms, such as benthic invertebrates, may have deleterious effects (Ernst et al., 2001).

The Los Lagos Region (41° 28′ 18″ S; 72° 56′ 18″ W) of the northern Chilean Patagonia (Fig. 1) has a history of high-density salmon farming. Further expansions of salmon aquaculture southwards have raised concerns about the release of pesticides in marine ecosystems. The aim of this study was to report on concentrations of antiparasitic pesticides in sediments near salmon farms in the northern Chilean Patagonia. The results of this study provide new information about the potential risks of pesticides to non-target organisms in northern Patagonia, such as marine benthic invertebrates.

During December 2010, December 2012, and February 2013 (i.e., austral summer), surface sediment samples were collected at four salmon farms near the city of Puerto Montt and near the northern coast of Chiloe Island, both sites within the northern Chilean Patagonia (Fig. 1). All four salmon farms (S1, S2, S3, and S4) carried out specific antiparasitic pesticide treatments against sea lice. The avermectin EB and the benzoylphenyl ureas TE and DI were used by farm S1, while farms S2, S3, and S4 used the pyrethroids CP and DE to treat salmon. The dates of pesticide applications for each farm are shown in Table 1. At farm S1, EB and DI had been used in medicated feed for a long time prior to sampling, while TE was only applied from December 5–10 (2010) as part of antiparasitic accumulation testing in sediment. At farms S2, S3 and S4, pyrethroids had been used to treat fish before, however, the sampling was only performed after medication (Table 1).

* Corresponding author at: Faculty of Environmental Sciences and EULA-Chile Centre, University of Concepción, P.O. Box 160-C, Concepción, Chile.
*E-mail addresses:* ftuca@udec.cl, f.tucca@gmail.com (F. Tucca).

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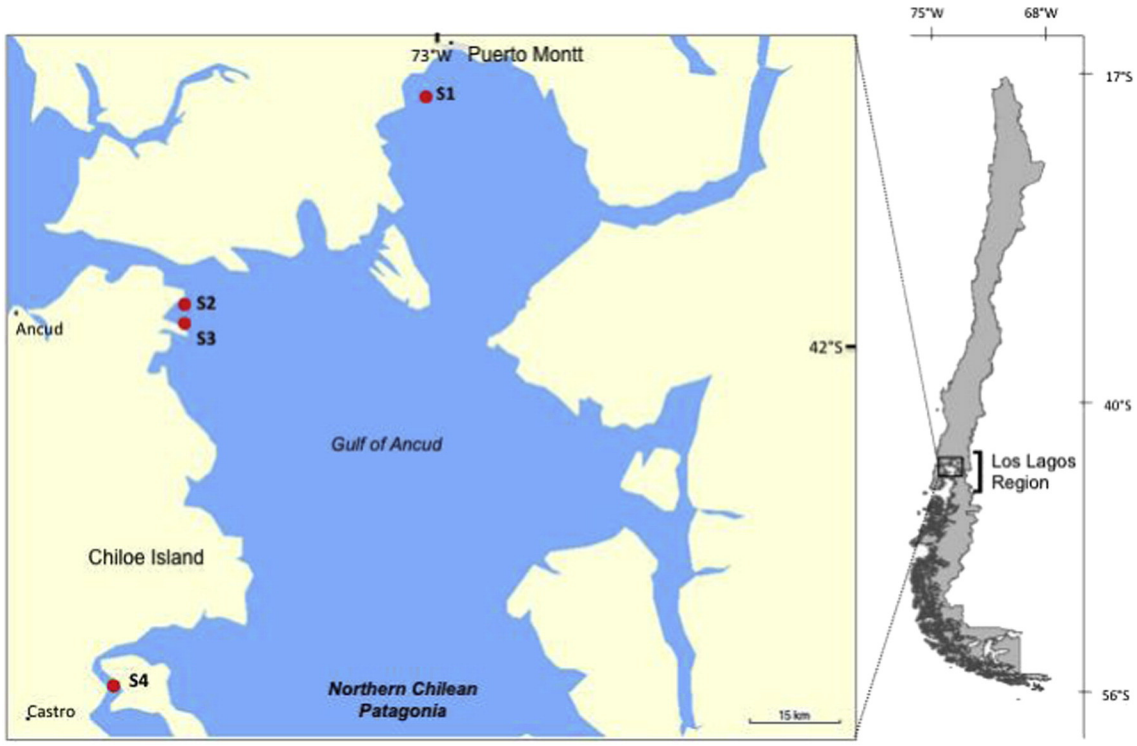


**Fig. 1.** Sampling locations for sediments around salmon farms in northern Chilean Patagonia.

Sediment samples were obtained using a Van Veen grab sampler (462 $cm^2$) near salmon cages. The sampling depths at the farm sites ranged from 40 to 60 m, with a mean current of <10 cm $s^{-1}$ (Table 1). Sampling was conducted at distances ranging from 0 to 100 m, according to the dominant current and tidal influence. A total of 60 samples were collected, considering at least 5 replicates for each sampling point at the farms. Samples were stored in darkness to prevent contamination, transported in an ice cooler to the laboratory, weighed, and freeze-dried at −50 °C and 0.2 mbar until further chemical analyses and organic content measurements. The organic matter in sediment samples (5–10 g, dry weight) was determined by weight loss on ignition at 360 °C for 16 h.

In the laboratory, the freeze-dried sediment samples (5–10 g, dry weight) from salmon farms treated with EB, DI, and TE (Table 1) were homogenized with anhydrous sodium sulfate and extracted three times with 50 mL of n-hexane in an ultrasonic bath for 20 min. Following this, samples were centrifuged at 1500 rpm to separate the sediment and solvent extract. Final solvent extracts (150 mL) were concentrated to 5 mL in a rotary evaporator and exchanged in 1 mL of methanol. The above procedure was checked for recovery and reproducibility. Procedural blanks and surrogate recovery tests were conducted for quality assurance and quality control (QA/QC). The recovery method consisted of analyzing 5 g of sodium sulfate (n = 3 replicates) treated as field samples and with a known spiked concentration (500 ng $\mu L^{-1}$) of the target compounds. Recovery standards were satisfactory, with averages of ~70% for DI, 75% for TE, and ~72% for EB.

The final extracts were analyzed using liquid chromatography-mass spectrometry with an electrospray ionization source (ESI LC-MS/MS) to determine DI and TE concentrations. In turn, EB concentrations were determined using atmospheric pressure chemical ionization. For DI, TE, and EB, a reversed phase column (Spherisorb ODS2 Hypersil, 150 × 2.1 mm ID, 5 mm particle size) was used. The solvent flow rates were applied according to Barnes et al. (1995) with modifications according to Krogh et al. (2008), including a flow rate of 300 μL $min^{-1}$ and 5 μM ammonium acetate in methanol. LC-MS analysis was performed using a thermal system with a Finnigan surveyor autosampler, an MS pump, and a Finnigan LTQ. Following preliminary evaluation by direct infusion of standard solutions, ESI LC-MS analysis was performed in the negative ion mode for DI and TE. EB was determined in the positive atmospheric pressure chemical ionization mode. Absorption and characteristic $MH^+$ and $MS^2$ spectra were used to identify peaks. Concentrations were determined by comparing peak areas with those of standard solutions prepared with commercially available, purified pesticides from Sigma-Aldrich (Pestanal, St Louis, MO, USA). Data quality was checked every six samples by analyzing blanks (i.e., acetonitrile and methanol), which were always below the detection limit (0.1 ng $g^{-1}$). The analysis of replicate samples showed relative standard deviations of <10%.

For analysis of the pyrethroids CP and DE, sediment samples (1 g, dry weight) were extracted with a mixture of powdered copper (0.5 g) and *n*-hexane:dichloromethane (2:1, 20 mL) in an ultrasonic bath for 15 min at room temperature. Clean-ups with florisil

**Table 1**
Details of antiparasitic pesticide application at each salmon farm.

| Farms | Medication date | Treatment | Sampling | Depth (m) | Current speeds (cm $s^{-1}$) |
|---|---|---|---|---|---|
| S1 | December 5–10, 2010 | EB, DI, TE | December 15, 2010 | 60 | 6.2 |
| S2 | December 16–20, 2012 | CP | December 23, 2012 | 50–60 | 6.0 |
| S3 | December 16–20, 2012 | CP | December 23, 2012 | 50–60 | 6.0 |
| S4 | February 1–5, 2013 | DE | February 10, 2013 | 40 | 7.6 |

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