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APPLICATION OF A THIN-FILM PASSIVE SAMPLER TO MONITORING PYRETHROIDS USED BY SALMON FARMS: STUDY IN NORTHERN CHILEAN PATAGONIA

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Introduction

Passive sampler devices (PSD) in water may play a key role as monitoring tool to assess the exposure of contaminants. The spatial-temporal integrative information with PSD deployed in the field may give a useful method for the detection of bioavailable priority contaminants, as well as contaminants of emerging concern (Lohmann et al., 2012; Allan et al., 2013). In occasions some organic contaminants are hardly detected with conventional methods, as for example, grab sampling collection with bottles during a specific time and area, where typically lower concentrations from dissolved fraction (C_{free}) are not quantified.

During decades many PSD have been designed to detect hydrophobic organic contaminants (HOCs) in water (eg. Huckins et al., 1990; Allan et al., 2010; Choi et al., 2013; Booij et al., 2016), being most studies focused on polycyclic aromatic hydrocarbons (PAHs), and globally regulated contaminants such as organochlorine (i.e. PCBs, HCB) and polybrominated compounds (PBDEs). A number of hydrophobic compounds of emerging concern have also been sampled by PSDs (triclosan, pyrethroids, and organophosphates, among others) (Sacks & Lohmann, 2011; Harman et al., 2013; Moschet et al., 2014).

In theory, the passive samplers have been defined as devices that are based in an initial uptake of dissolved contaminants to PSD (kinetic phase) due to different concentrations between the two media (sampler-water). The net flow of contaminants from one medium to other continues until equilibrium is attained (Figure 1). However, PSD in kinetic phase is often affected for diverse environmental factors during its exposure in water, interrupting the uptake rate of contaminants on the sampler. Environment factors such as temperature, salinity, pH, hydrodynamic and biofouling could have a role that influences the uptake and equilibrium between the sampler and the aquatic surrounding medium (Vrana et al., 2002; Kaserzon et al., 2014; Jonker et al., 2015). The use of performance reference compounds (PRCs) for in situ calibration may result a feasible method to estimate sampling rates (R_s) in water, integrating the diverse factors mentioned above. These PCRs are released from the sampler following the same mechanisms as uptake of other analytes, which results in a similar curvature of the uptake and the release curves. Commonly, PCRs can be deuterated and ^{13}C-labelled compounds or other (non-labelled) analytes that do not occur at the exposure sites in significant amounts (e.g. PCB 4, 14, 29, among others) (Booij et al., 2007).

A good alternative of PSD in water is the ethylene vinyl acetate (EVA) copolymer. EVA has been identified as effective at measuring bioavailable contaminant fraction and it have been used as thin-film passive sampler for measuring pollutants dissolved in water (eg. St George et al., 2011; Tucca et al., 2014), as well as in sediment and biota (Wilcockson et al., 2001; Golding et al., 2008; Meloche et al., 2010). This copolymer is a flexible thin-film which can be easily processed in laboratory (low cost), being adapted to different substrates (eg. filters, glass, etc.). In addition, EVA is resistant to high pressures, temperatures, UV radiation and it is also water proof, making it an effective polymer for the purpose of capturing hydrophobic chemicals (Log K_{ow}>3) in the aquatic environment. This study attempts to assess the bioavailable fraction of pyrethroid pesticides (cypermethrin, CP; and deltamethrin, DE), widely used for the treatment of sea lice infections in salmon farming, using the EVA copolymer as passive sampler in different areas from southern Chile.

Materials and Methods

Study area. During years 2014-2015 different field campaigns between Puerto Montt city and Chiloe Island (41°S - 43°S, northern Chilean Patagonia) were conducted (Figure 2). Many salmon farms are

located in coastal areas; therefore, we monitored 6 salmon farms (S1-S6, Figure 2) during periods of treatments with antiparasitic pesticides such as synthetic pyrethroids (CP and DE). The use of pyrethroids has been widely required by the salmon industry to treat, control and mitigate parasitic diseases on fish. Direct emission of pesticides in the marine environment has produced uncertainty about the potential impact on non-target organisms living in the nearby area and the need to quantify them in the water phase.

Passive sampler preparations. Ethylene vinyl acetate pellets (EVA, Elvax 40W, DuPont Canada) were cleaned with methanol and stored in a glass jar at room temperature until be used. An EVA coating solution was prepared by dissolving 2 g of EVA pellets in 100 mL of dichloromethane (DCM). The solution was magnetically stirred for approximately 2 h until there were no visible signs of EVA pellets. Glass fiber filters (GFFs) (Diameter: 12.5 cm) were used as adhesion substrate of the copolymer. Each GFF was coated with EVA solution through of immersions into a glass precipitate. The GFF were dried under bell until complete evaporation of the DCM solvent, and then GFFs coated were weighed again to calculate EVA mass. This PSD were kept in darkness and room temperature to use in the field deployment.

In situ calibration. PSDs were deployed under environmental conditions for 30 days in seawater southern Chile (i.e. under conditions of flow, temperature, salinity and biofouling). This calibration method allowed to validating and interpreting data obtain under field conditions in comparison to laboratory conditions (Tucca et al., 2014; Booij & Tucca, 2015). During environmental exposure (30 days) three PSDs spiked with low concentrations of 10 organochlorine pesticides (plus a PSD blank) were recovered to measure the dissipation constant rate. These organochlorine pesticides were used as performance reference compounds (PCRs) in the experiment, due to its scarce environmental occurrence for the area studied. The estimation of the sampling rate (Rs) from samplers also was performed (Booij et al., 2007; Booij & Smedes, 2010). For in situ calibration was important to know the partitioning coefficients between the sampler and water (K_{sw}) of PRCs. Preferably, a large number of PRCs were used, covering a wide hydrophobicity range. In parallel, environmental physical-chemical properties (temperature, pH, salinity) were measured in the field during the exposure time.

Passive sampler deployment in field. PSDs were deployed around salmon cages with treatment (between 3 to 4 m approximately) during 7 days of exposure in seawater. Physical-chemical parameters were measured using a CTDO instrument as well. During the recovery, samplers were cleaned with distilled water (for eliminating debris and biofouling) and stored into glass containers and transported under dark conditions for chemical analysis. Some PSDs were deployed at long distance from salmon cages treated ("houseboat") to assess dispersion of pyrethroids.

Chemical analysis. Field calibration EVA samplers were extracted with ultrasound assisted extraction. The samplers were introduced into falcon tubes of 50 mL and extracted with acetonitrile for 30 min. After extraction, the collected extract was concentrated to 1.5 mL using a rotary evaporator (40°C; 175 mbar). 250 μL of the concentrated extract (1.5 mL) were introduced into a 20 mL vial and mixed with 8.75 mL of NaCl aqueous solution 2.5% w/v and the vial was shaken vigorously for 30 s. The extraction was carried out in HS-SPME mode with a PDMS fiber. The vial was heated at 80°C using the heating block on the CombiPal, and shaken for 15 min to 650 rpm. The extraction time was 89 min and after this time the fiber was introduced into the GC-MS/MS injection port for desorption of the pesticides. Pyrethroids were analyzed by GC-NCI-MSI (Feo et al., 2010).

Results and discussion

For northern Chilean Patagonia were detected high CP concentrations around treated salmon cages (Figure 2 and 3) in comparison to DE. The pyrethroid CP ranged between 3.4 - 87.2 ng L^{-1} in seawater, while DE were found between 1.1 - 3.2 ng L^{-1}. These levels were estimated assuming a sampling rate (R_s) of 0.72 L/d and an exposure time of 7 days in seawater (Tucca et al., 2014). The partition coefficient between EVA sampler-water estimated for both CP ($K_{sw\text{-}CP}$) and DE ($K_{sw\text{-}DE}$) were 6.2 and 5.8, respectively.

Sites S3 and S6 showed the highest concentrations of CP during the monitoring in treated salmon cages, with ~87 (±25, n=5) ng L^{-1}. Pyrethroids for sites S1 and S4 were not detected (Figure 3). Effect assessments on non-target marine invertebrates exposed to CP have showed lethal responses in copepods (naupliar stage) at concentrations of 5.2 -37.3 ng L^{-1} after of 96 hours of exposure (Barata et al., 2002a;

Barata et al., 2002b; Medina et al., 2002), as well as effects on the feed rate and reproductive success in ranges of 7.4 and 62.5 ng L^{-1} (Barata et al., 2002b; Tucca, data no published), respectively. These data suggest an environmental risk for the invertebrate's community present in water column, mainly on early stages of copepods which have shown a high sensitive to pyrethroids.
Is important to note that results estimated with EVA passive samplers are provisory, since in situ calibration data are still in progress, where specific parameters are measured.
In conclusion, the use of passive samplers can be a powerful tool to detect trace concentrations of pesticides bioavailability in water and a reliable method for chemical risk assessment procedures.

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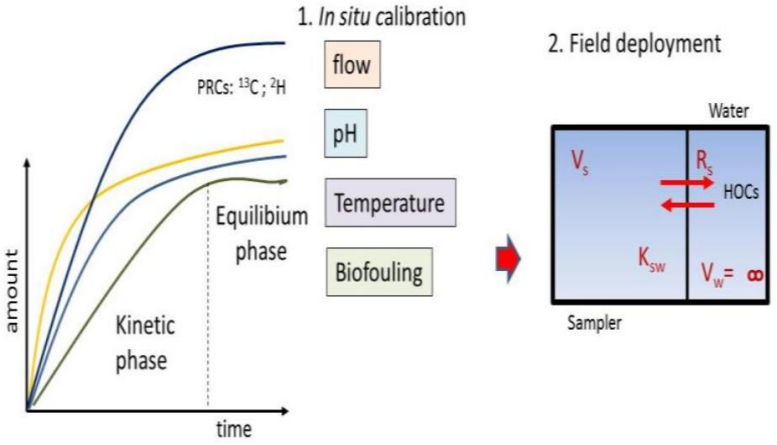

Figure 1. General scheme of uptake kinetic/equilibrium phase and exposure factors during *in situ* calibration (1). Specific parameters obtained from performance reference compounds (PCRs) to calculate C_{free} after that PSD were field deployment (2). Abbreviations: V_s=Passive sampler volume; R_s=Sampling rate; K_{sw}=Sampler-water partition coefficient; V_w= Water volume (V_w in the field is essentially infinite).

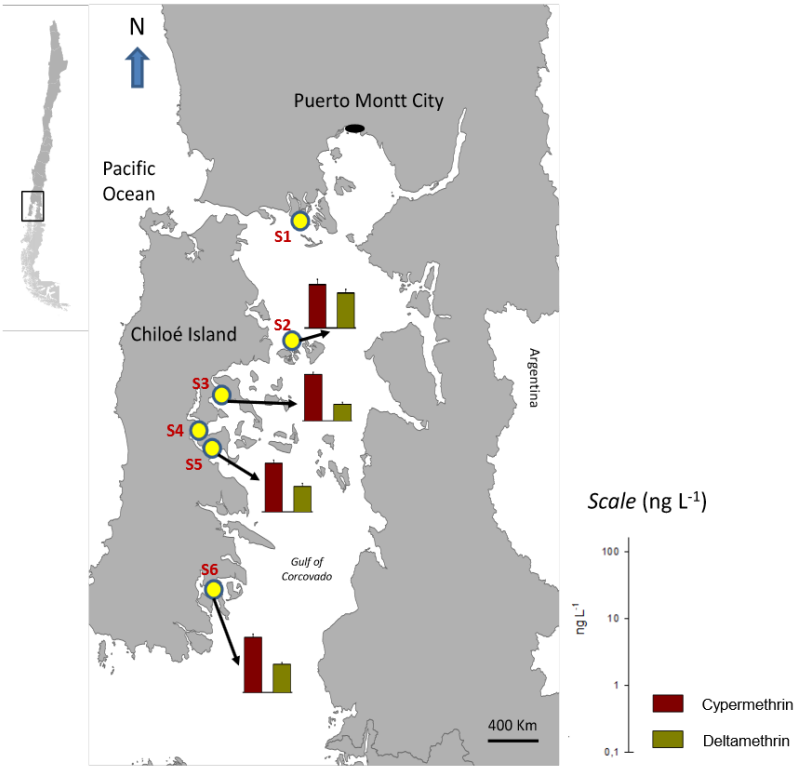

Figure 2. Levels of pyrethroid pesticides (ng L^{-1}) for different locations with intensive salmon farms in northern Chilean Patagonia.

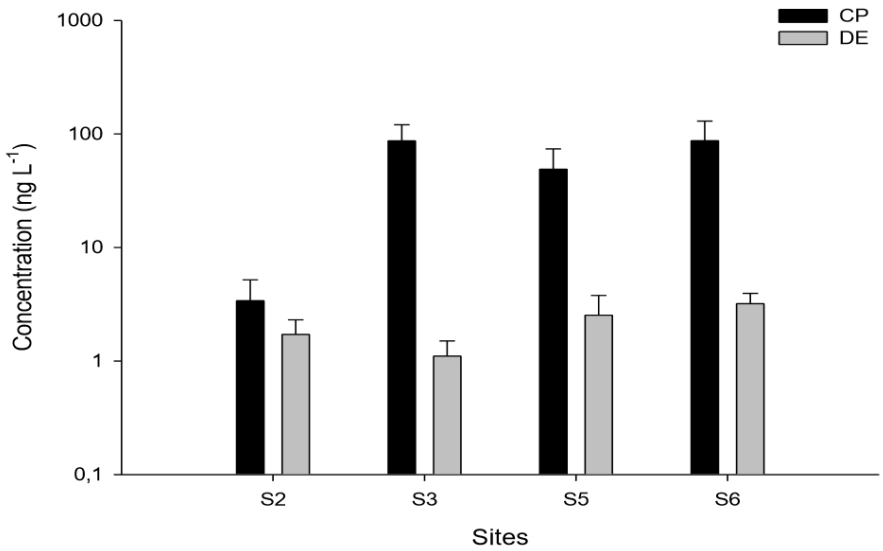

Figure 3. Pyrethroid concentrations (ng L^{-1}, ± standard error) detected in different sites with salmon farming in treatment. Acronyms: CP=Cypermethrin; DE= Deltamethrin.