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Fate and impact of antibiotics and pesticides used in marine aquaculture: An emergent threat to the coastal ocean (Destino e impacto de antibióticos y pesticidas utilizados en acuicultura marina: Potencial amenaza para el océano costero)

Tesis para optar al grado de Doctor en Oceanografía

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Fate and impact of antibiotics and pesticides used in marine aquaculture: An emergent threat to the coastal ocean

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Dirigée par Silvio PANTOJA et Camila FERNANDEZ

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ABSTRACT

Chile, as the second largest salmon farming country in the world, reports the highest use of antibiotics and pesticides, which can be harmful to both the environment and humans. These compounds tend to be sequestered by suspended particles, transported by currents and finally deposited in sediments, where they are later consumed by the benthic community. Changes in the bacterial community, emergence of resistance genes and impacts at the ecological level have been described for antibiotics and pesticides, although most focus on the local impact of salmon farming. This study sought to understand the dynamics and fate of antibiotics throughout the Puyuhaupi Fjord and to understand the partitioning behavior of the antibiotics florfenicol and flumequine through adsorption experiments that simulate the average temperature of the fjord. In addition, the occurrence of deltamethrin and cypermethrin in total suspended solids and filtering benthic organisms (bivalves and sponges) in the Puyuhuapi Fjord was evaluated. Finally, an experiment of respiration in the water column and marine sediments obtained in an area without aquaculture activity (Banyuls Bay, France) was developed to evaluate if the presence of antibiotics and pesticides can affect the degradation process of organic material, through changes in community respiration and remineralized components.

Our results show low concentrations of florfenicol (from trace to 23.1 ng L^{-1}) and flumequine (trace level) detected after 180 and 360 days (respectively) since their last medication at a distance between 2 and 23 km from the culture sites. The fugacity model used in our study area, together with the decay model, predicts that flumequine can remain in sediments for more than two months at sub-minimum inhibition concentrations (sub-MIC). This condition may promote bacterial selection for antibiotic resistance and eventually pose a risk to human health from the consumption of seafood products. The values of the partition constants K_d and K_{OC}, obtained by bacth experiments, suggest that the adsorption capacity of flumequine is twice that of florfenicol (Table 2, section 3.2), implying that flumequine has a greater tendency to be adsorbed and absorbed by sediments. From an environmental point of view, our results may imply that the fate of flumequine will be related to processes affecting particles, suspension transport and seafloor deposition, whereas florfenicol concentration be controlled by hydrodynamic processes such as dilution and transport by currents. In turn, a higher fraction of flumequine may be stored in the sediments in coastal areas housing salmon farming centers.

The pesticides deltamethrin and cypermethrin were incorporated through dips to control outbreaks of caligus (*Caligus rogercresseyi*) infection. Very low concentrations of deltamethrin were detected in total suspended solids (0.01 to 0.05 ng L^{-1}), which value would not have an effect on organisms (NOEC, LC_{50} and EC_{50}) or at the ecological level (NOEAEC), which may come from sediment resuspension or external input from adjacent areas with active salmon culture centers. Although cypermethrin was not used in Puyuhuapi Fjord, low concentrations were detected in bivalves and sponges (0.04 and 0.05 ng g^{-1}, respectively), values comparable to wild salmon caught for human consumption (0.04 ng g^{-1}). These results suggest an indirect exposure of the compound may be associated with external input from adjacent fjords or unreported treatments because cypermethrin can remain for more than two years in sediments with high organic material and low oxygen content. Preliminary results from the community breathing experiments suggest decreases in activity and/or changes in the biological component, especially in the bacterial community, since some differences in parameters such as dissolved organic carbon, ammonium, and nutrients are observed. However, possible changes in bacterial diversity have not been analyzed due to the pandemic conditions.

In future research, it is necessary to include in the study the fjords and canals adjacent to our study area, with and without aquaculture activity, which will allow the authorities to better evaluate the sanitary rests considering the interconnection of farming neighborhoods. It is also suggested to use both sponges and bivalves to evaluate the environmental conditions of an area, with or adjacent, to aquaculture activity. On the other hand, it is necessary to make modifications to the fugacity model used in our study, incorporating the presence of at least two layers in the water column.

RESUMEN

Chile, como segundo país con mayor producción en el cultivo de salmones a nivel mundial, reporta el mayor uso de antibióticos y pesticidas, lo que puede ser perjudicial tanto para el medioambiente como para el hombre. Estos compuestos tienden a ser secuestrados por las partículas suspendidas, transportados por las corrientes y, finalmente son depositadas en los sedimentos, donde eventualmente son consumidos por la comunidad bentónica. Cambios en la comunidad bacteriana, aparición de genes de resistencia e impactos a nivel ecológico han sido descritos para antibióticos y pesticidas, aunque la mayoría se enfocan en el impacto local de la actividad salmonera. Este estudio buscó entender la dinámica y el destino de los antibióticos en todo el fiordo Puyuhaupi y conocer el comportamiento particional de los antibióticos florfenicol y flumequina a través de experimentos de adsorción que simular la temperatura promedio de fiordo. Junto con esto se buscó evaluar la ocurrencia de deltametrina y cipermetrina en los sólidos totales suspendidos y los organismos bentónicos filtradores (bivalvos y esponjas) en el fiordo Puyuhuapi. Finalmente se desarrolló un experimento de respiración en columna de agua y sedimentos marinos obtenidos, una zona sin actividad acuícola (bahía Banyuls, Francia), para evaluar si la presencia de antibióticos y pesticidas pueden afectar el proceso de degradación del material orgánico, a través de cambios en respiración comunitaria y en las componentes remineralizadas.

Nuestros resultados muestran bajas concentraciones florfenicol (desde traza a 23.1 ng L^{-1}) y flumequina (nivel traza) detectados después de 180 y 360 días (respectivamente) desde su la última medicación a una distancia de entre 2 y 23 km de los centros de cultivo. El modelo de fugacidad utilizado en nuestra área de estudio, junto con el modelo de decaimiento, predicen que flumequina puede permanecer en los sedimentos más de dos meses a concentraciones de inhibición sub-Mínima (sub-MIC). Esta condición puedo promover la selección bacteriana por resistencia a los antibióticos y, eventualmente representar un riesgo para la salud humana por el consumo de productos marinos. Los valores de constantes de partición K_d y K_{OC}, obtenidos experimentalmente en nuestro estudio, sugieren que la capacidad de adsorción de flumequina es dos veces mayor que la de florfenicol (Tabla 2, sección 3.2), lo que implica que flumequina tiene una mayor tendencia a ser adsorbido por los sedimentos. Desde el punto de vista ambiental, nuestros resultados pueden implicar que el destino de la flumequina estará más asociado a procesos como el transporte de partículas y la deposición en el fondo marino, mientras que el florfenicol debería estar más

relacionado con procesos acuáticos como la dispersión y el transporte por las corrientes, lo que sugiere que, eventualmente, una mayor fracción de flumequina puede quedar almacenada en los sedimentos en la zona con centros de cultivo de salmones.

Los pesticidas deltametrina y cipermetrina se incorporaron a través de baños para controlar brotes de infección por caligus (*Caligus rogercresseyi*). Concentraciones muy bajas de deltametrina se detectaron en los sólidos totales suspendidos (0.01 a 0.05 ng L^{-1}), cuyo valor no tendría un efecto sobre los organismos (NOEC, LC_{50} y EC_{50}) o a nivel ecológico (NOEAEC), los que pueden provenir de la resuspensión de sedimentos o por aporte externo de áreas adyacentes con centros de cultivo activos. A pesar de no ser utilizada cipermetrina en fiordo Puyuhuapi, bajas concentraciones se detectaron en bivalvos y esponjas (0.04 y 0.05 ng g^{-1}, respectivamente) valores comparables a salmones silvestres capturados para consumo humano (0.04 ng g^{-1}). Estos resultados sugieren una exposición indirecta del compuesto puede estar asociado al ingreso externo desde fiordos adyacentes o bien tratamientos no reportados, debido a que cipermetrina puede permanecer más de dos años en sedimentos con alto material orgánico y bajo contenido de oxígeno. Resultados preliminares de los experimentos respiración comunitaria sugieren disminución en la actividad y/o cambios en la componente biológica, especialmente en la comunidad bacteriana, dado que se observan algunas diferencias en parámetros como carbono orgánico disuelto, amonio y los nutrientes. Sin embargo, los posibles cambios en la diversidad bacteriana no has sido analizados debido a las condiciones de pandemia.

En futuras investigaciones es necesario incluir en el estudio los fiordos y canales adyacentes a nuestra área de estudio, con a sin actividad acuícola, lo que permitirá a las autoridades evaluar de mejor manera los descansos sanitarios considerando la interconexión barrios de cultivo. También se sugiere utilizar tanto esponjas como bivalvos para evaluar el estado de una zona con actividad acuícola o adyacente a ella. Por otro lado, es necesario realizar modificaciones al modelo de fugacidad utilizado en nuestro estudio, incorporando la presencia de al menos dos capas en la columna de agua.

Résumé

Le Chili, deuxième pays d'élevage de saumons au monde, est celui qui utilise le plus d'antibiotiques et de pesticides, qui peuvent être nocifs pour l'environnement et l'homme. Ces composés ont tendance à être séquestrés par les particules en suspension, transportés par les courants et finalement déposés dans les sédiments, où ils sont finalement consommés par la communauté benthique. Des changements dans les communautés bactériennes, l'émergence de gènes de résistance et les impacts écologiques ont été décrits pour les antibiotiques et les pesticides, bien que la plupart se concentrent sur l'impact local de la salmoniculture. Le present travail vise à comprendre la dynamique et le devenir des antibiotiques dans le fjord de Puyuhaupi, à caractériser la partition des antibiotiques florfénicol et fluméquine par une approche expérimentale simulant les températures moyennes du fjord en hver et été. En outre, la présence des pesticides deltaméthrine et cyperméthrine dans les particules en suspension et les organismes benthiques filtrants (bivalves et éponges) dans le fjord de Puyuhuapi a été évaluée. Enfin, une expérience d'exposition des organismes vivants dans la colonne d'eau et dans les sédiments marins d'une zone sans activité aquacole (baie de Banyuls, France) a été réalisée pour évaluer si la présence d'antibiotiques et de pesticides affecte le processus de minéralisation de la matière organique, à travers des changements dans la respiration de la communauté et les composants reminéralisés.

Nos résultats montrent de faibles concentrations de florfénicol (de trace à 23,1 ng L^{-1}) et de fluméquine (niveau de trace) détectées après 180 et 360 jours (respectivement) depuis leur dernière médication à une distance comprise entre 2 et 23 km des sites d'aquaculture. Le modèle de fugacité utilisé dans notre zone d'étude, associé au modèle de décomposition, prévoit que la fluméquine peut rester dans les sédiments pendant plus de deux mois à des concentrations d'inhibition subminimales (sub-MIC). Cette situation peut favoriser la sélection bactérienne pour la résistance aux antibiotiques et, à terme, constituer un risque pour la santé humaine lié à la consommation de fruits de mer. Les valeurs des constantes de partage K_d et K_{OC}, obtenues par des expériences en lots dans notre étude, suggèrent que la capacité d'adsorption de la fluméquine est deux fois supérieure à celle du florfénicol (Tableau 2, section 3.2), ce qui implique que la fluméquine a une plus grande tendance à être adsorbée et absorbée par les sédiments. D'un point de vue environnemental, le devenir de la fluméquine sera plus associé aux processus affectant les particules, comme leur transport et leur déposition sur le fond marin, alors que la concentration de

florfénicol sera contrôlé par des processus hydrodynaques, comme la dilution et le transport par les courants. En conséquence, une fraction plus élevée de fluméquine pourrait être stockée dans les sédiments des zones côtières d'élevage de saumons.

Les pesticides deltaméthrine et cyperméthrine ont été utilisés dans des bains de saumon pour contrôler les foyers d'infection de Caligus (Caligus rogercresseyi). De très faibles concentrations de deltaméthrine ont été détectées dans les particules en suspension (0,01 à 0,05 ng L^{-1}), ce qui n'aurait aucun effet sur les organismes (NOEC, LC_{50} et EC_{50}) ou au niveau écologique (NOEAEC). Cette occurrence peut traduire la remise en suspension des sédiments ou un apport externe provenant de zones adjacentes, dans lesquelles des sites d'aquaculture sont actifs. Bien que la cyperméthrine n'ait pas été utilisée dans le fjord de Puyuhuapi, de faibles concentrations ont été détectées dans les bivalves et les éponges (0,04 et 0,05 ng g^{-1}, respectivement) avec des valeurs comparables à celles mesurées dans des saumons sauvage capturé pour la consommation humaine (0,04 ng g^{-1}). Ces résultats suggèrent une source depuis les fjords adjacents où des traitements n'ont pourtant pas été rapportés, car la cyperméthrine peut persister pendant plus de deux ans dans les sédiments à forte teneur en matière organique et à faible teneur en oxygène. Les résultats préliminaires des expériences de respiration de la communauté suggèrent une diminution de l'activité et/ou des changements dans les composants biologiques, en particulier dans la communauté bactérienne, puisque certaines différences dans les paramètres tels que le carbone organique dissous, la concentration en ammonium et en nutriments sont observées. Cependant, les changements possibles dans la diversité bactérienne n'ont pas été analysés en raison des conditions de la pandémie.

Dans les recherches futures, il sera nécessaire d'évaluer les apports de contaminants par les fjords et canaux adjacents à notre zone d'étude, avec et sans activité aquacole, ce qui permettra aux autorités de mieux évaluer les durées des périodes de ruptures sanitaires en considérant l'interconnexion entre les zones d'aquaculture. Il est également suggéré d'utiliser à la fois les éponges et les bivalves pour évaluer la qualité environnementale des zones aquacoles. D'autre part, il est nécessaire d'apporter des am aquacoleliorations au modèle de fugacité utilisé dans notre étude, en incorporant la présence d'au moins deux couches dans la colonne d'eau.

1.0 INTRODUCTION

1.1 Antibiotic and pesticides used in aquaculture

Food fish demand has been increasing since the ’80s, while natural fish capture seems to have reached a limit at ca. 90 million tons since the early 90’s (Ottinger *et al.*, 2016). Thus, the higher demand has been supplemented by aquaculture activities in the last decades (FAO, 2020; Figure 1).

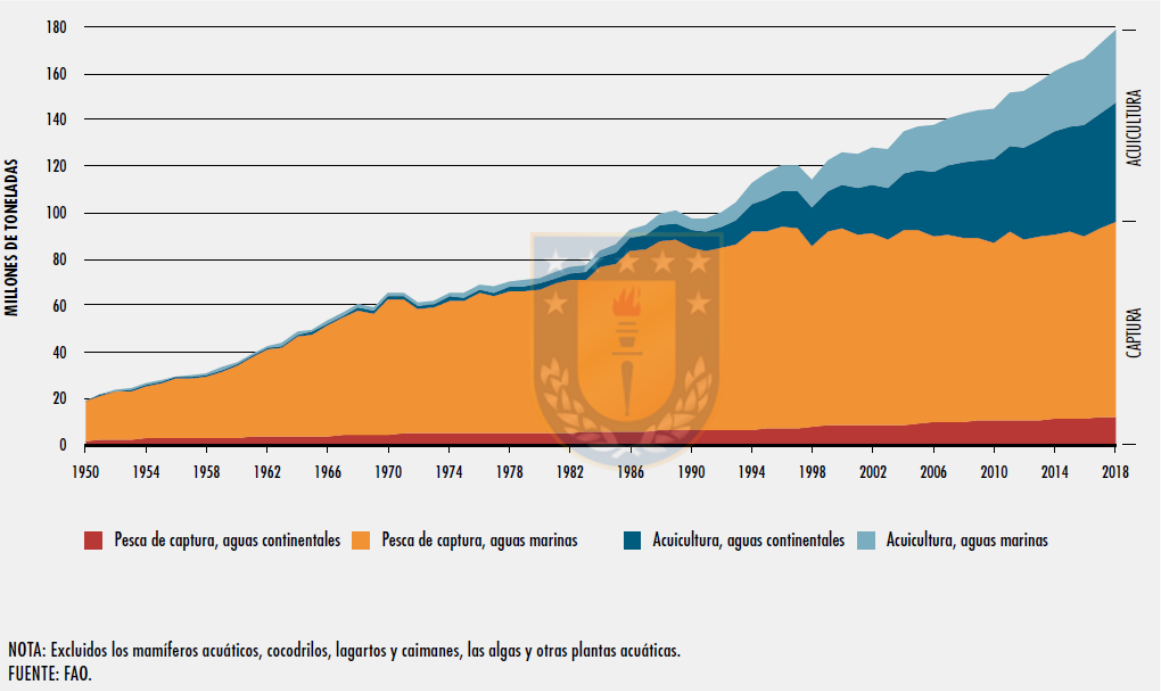


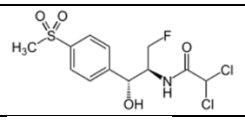
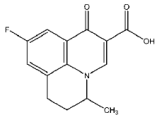
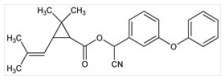
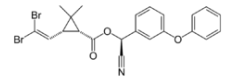
Figure 1. World capture fisheries and aquaculture production, extracted from FAO (2020).

Farming of marine salmon and trout produced 7.3 million tons in 2018, where Chile is the second salmon producer (38%) after Norway (39%) (FAO, 2018). Extensive and massive salmon production has long been known to generate local negative consequences in the marine environment, biodiversity, and the physicochemical properties of the sea bottom, resulting in an increase of the local carbon inventory, mostly through non consumed food pellets (Cromey *et al.*, 2002). Also, several studies have reported that a rise in nutrients has the potential to increase

primary production (Wang *et al.*, 2012; Iriarte *et al.*, 2013) and that a drop in O_2 have been observed in sediments (Buschmann *et al.*, 2006; Gaw *et al.*, 2014; Price *et al.*, 2015) in the vicinity of salmon cages (*e.g.*, Neori *et al.*, 2004; Nash *et al.*, 2005). Nevertheless, other studies suggest a minor or insignificant impact of these nutrients on algal blooms (Husa *et al.*, 2014; Skaala *et al.*, 2014). Currently, the industry has made efforts to improve technologies aiming to diminish nutrient inputs (Price *et al.*, 2015).

Other potential environmental impacts of salmon farming include a direct release of pesticides and pharmaceuticals, used to control outbreaks of parasites, bacterial infections, and viral diseases (Burridge *et al.*, 2010), that severely reduce production (FAO 2020). Antibiotics and pesticides used in Chile have different application forms and dosage, where antibiotics are mainly included in food pellets while pyrethroids are applied in baths, as shown in Table N°1.

Table 1. Antibiotics and pesticides more used in Chilean aquaculture industries

Compounds	**Chemical Structure**	**Group**	**Action Mechanism**	**Application and Dose[a]**
Florfenicol[b]		Phenicols	Protein synthesis inhibitors: action over subunit ribosome 50S	within food 10 mg kg^{-1} for 10 days
Flumequine[c]		Fluoroquilo-none	Inhibition in DNA replication and transcription	within food/ 25 mg kg^{-1} for 10 days
Cypermethrin[d]		Pyrethroids	Neurological impacts: blocks the electron transport chain acting over the sodium channel	health baths 0.005 mg L^{-1} x 30 a 60 min for 14 days
Deltamethrin[e]		Pyrethroids		health baths 0.003 mg L^{-1} x 30 min for 14 days

Reference: a: Bravo *et al.* (2005); b: Macorni *et al.* (1990); c: Barnard and Maxwell (2001); d: Singh and Agarwal (1991), e: Chalmers *et al.* (1987).

Once these compounds are released into the marine environment their environmental persistence and impact on non-target organisms is determined by their physicochemical properties (Table 2) that affect sorption, degradation processes, and sediment deposition (*e.g.*, Power and Chapman, 1992; Lutnicka *et al.*, 1999; Wen *et al.*, 2009; Sirtori *et al.*, 2012; Zhao *et al.*, 2013; Gaw *et al.*, 2014; Mitchell *et al.*, 2015).

Chile used in 2007 the highest amount of antibiotics compared to Norway, Canada and UK, with 385600 kg of antibiotics (active ingredient) for a salmon production of 380381 tons. In contrast, Norway with a higher salmon production (821997 tons) reported the use of 649 kg of

antibiotics (active ingredient) (Burridge *et al.*, 2010). The quantity of anti-lice declared in Chile was 132 kg of pesticides (active ingredient) while 600 kg of pesticides (active ingredient) were used in Norway (Burridge *et al.*, 2010).

Table 2. Physicochemical properties and biological effects of antibiotics and pesticides used in Chilean aquaculture industries.

Compounds		**Florfenicol**	**Flumequine**	**Cypermethrin**	**Deltamethrin**
(Chemical Formule)[a]		($C_{12}H_{14}Cl_2FNO_4S$)	($C_{13}H_{11}NO_5$)	($C_{22}H_{19}Cl_2NO_3$)	($C_{22}H_{19}Br_2NO_3$)
Molecular weight[a] (g mol^{-1})		358.21	261.25	416.3	505.19
Octanol/ water partition (Log Kow; L kg^{-1})		-0.12[b] to 0.19[c]	1.38 to 2.70[c]	6.6[e]	6.2[e]
Organic carbon partition (Log Koc; L kg^{-1})		-0.19 to -0.51[d]	0.99 to 2.99[d]	5.5[f]	5.8[d]
Water solubility (mg L^{-1})		1307[a]	Insoluble[a]	0.004[e]	>0.002[e]
No Observed Effect Concentration (NOEC; mg L^{-1})[a]	**Algae**	nd/	nd	1.3	nd
	Invertebrate	nd/	10	0.00004	0.0000041
	Fish	nd/	10	0.00003	>0.000032
Lethal Concentration (LC_{50}, mg L^{-1})[a]	**Algae**	nd/	Nd	nd	nd/
	Invertebrate	nd/	Nd	0.0128	nd
	Fish	> 780	Nd	0.0028	0.0015
Half maximal Effective Concentration (EC_{50}, mg L^{-1})[a]	**Algae**	>2.9	5.0	>0.1	9.1
	Invertebrate	>330	Nd	0.0003	0.00056
	Fish	nd	Nd	nd	0.00026
No-Observed Ecosystem Adverse-Effect Concentration (NOEAEC, mg L^{-1})[&, a]		nd	nd	0.00005	0.0032
Bioconcentratio Factor (BCF, L kg^{-1})[a]		nd	nd	1204	1400
Half-life (days)	**Water**	~ 74[g]	121[h]	22.1 (pH 8)[a]	17 to 48[a]
	Sediment	7.3[i]	150[j]	30[k] to > 730[l]	65 to 285[m]
	Biota	0.6[n]	1.25 to 0.6[ñ]	0.8 to 10[o]	nd

&: Mesocosmos study data; nd: No data. References: a: http://sitem.herts.ac.uk/aeru/vsdb/index.htm; b: Kołodziejska *et al.* (2013); c: Predicted ranges from USEPA (https://comptox.epa.gov/dashboard); d: $K_{OC} = 0.41\ K_{OW}$ (Karickhoff, 1981); e: Oros and Werner (2005); f: Maund *et al.* (2002); g: Kreider *et al.* (1996); h: Pouliquen *et al.* (2007); i: Hektoen *et al.* (1995); j: Halling-Sorenson *et al.* (1998); k: Mackay *et al.*, (2006); l: flocculated marine sediments (Hamaotene *et al.*, 2018); m: Benskin *et al.* (2016); n: Horsberg *et al.* (1994); ñ: Rogstad *et al.* (1993); o: USEPA (1989).

One of the serious concerns about the use of antibiotics in aquaculture includes the development of resistance in bacterial populations which, in turn, can limit the effectiveness of cultured species' immune systems (Cabello, 2006; Primavera, 2006) and eventually be transferred to humans (Burridge *et al.*, 2010). Conversely, deltamethrin and cypermethrin exposure in non-

target crustacean communities could produce changes in diversity (Van Geest *et al.*, 2014a) and affected the succession at an ecological level (Friberg-Jensen *et al.*, 2003; Van Geest *et al.*, 2014b), according to NOEC and LC_{50} values in different trophic levels (Table 2). These pesticides can bioaccumulate through trophic webs even after hours of exposition (Willis *et al.*, 2005; Alonso *et al.*, 2012; Burridge *et al.*, 2014; Ernst *et al.*, 2014).

Despite quantities of antibiotics and pesticides incorporated into marine systems, and due to partition, dispersion, and degradation processes summated to typical sampling effort and analytical difficulties, obtaining pesticide concentrations for each compartment (water, suspended, sediments, and non-target organism) is a real challenge. This can explain the few environmental values reported in the literature. In several cases, these values could be associated with fugacity-based models level III which was used to describe the dynamic and fate of different compounds in coastal marine environments after one day of medication. The model was tailored to a salmon processing environment with considerations for compound partition, treatment dosage, degradation, advective transport, rates of sedimentation and resuspension, and salmon density (Mackay and Paterson, 1991; Gouin and Harner, 2003; Hughes *et al.*, 2012; Zhang *et al.*, 2015; Kim *et al* 2017; Chen *et al.*, 2019; Wang *et al.*, 2020).

1.2 Fate and persistence of antibiotics

Antibiotics are administrated to salmon as a component of food pellets, and following each treatment, between 70% and 90% of non-metabolized compounds are released into the water column through urinal, branchial and fecal excretion (Pouliquen *et al.*, 2007, Grigorakis and Rigos, 2011; Miranda *et al.*, 2018). Between 5% and 20% of uneaten pellets were observed to sink to sediments (*e.g.*, Gowen *et al.*, 1994). Once released into the water column, and according to their physicochemical properties, these antibiotics can be partitioned between dissolved and particulate phases, suffer degradation processes, sedimentation processes, and also horizontal transportation (Sirtori *et al.*, 2012; Leal *et al.*, 2015; Liu *et al.*, 2015a, Mitchell *et al.*, 2015). During all dispersal processes (by horizontal transport), antibiotics can be affected by chemical, biological and abiotic hydrolytic degradations (*e.g.*, Wen *et al.*, 2009; Sirtori *et al.*, 2012; Zhao *et al.*, 2013; Mitchell *et al.*, 2015).

All these processes, summated to environmental conditions, determine the fate and persistence of antibiotics. Some studies have reported major preservation of antibiotics (longer

half-life) just under the cages with a poor quality of the sediments and evidence of anoxic conditions (black color and odor of H_2S), due to organic matter accumulation (Björklund *et al.* 1990; Samuelsen *et al.*, 1992). Flumequine, florfenicol, and others like oxytetracycline have a larger half-life under anoxic conditions (Björklund *et al.* 1990; Hektoen *et al.*, 1995; Coyne *et al.*, 2001; Burridge *et al.*, 2008).

Degradation of antibiotics and metabolite products have been poorly studied under natural environmental conditions, with a few studies in marine sediments under laboratory conditions (*e.g.*, Gaw *et al.*, 2014). One of them was florfenicol-amine (florfenicol metabolite) which has a persistence of months in the sediments in contrast to one week for florfenicol (*e.g.*, Hektoen *et al.*, 1995). Flumequine, in another hand, shows a major tendency to be associated with the particulate phase, sinking in sediments and, eventually, being more persistent (Björklund *et al.*, 1990; Coyne *et al.*, 2001; Burridge *et al.*, 2008). Intense degradation of florfenicol due to high hydrolysis rate has been reported to occur at pH above 8 (Mitchell *et al.*, 2015), while ionic metals compounds (*e.g.*, Ca^{2+}, Fe^{2+}) or organic colloids or humic and fulvic material act in the retention of antibiotics by sequestration (*e.g.*, Wang *et al.*, 2010; Leal *et al.*, 2015; Liu *et al.*, 2015; Mitchell *et al.*, 2015). Understanding the partitional behavior of antibiotics, like florfenicol and flumequine, under controlled temperature conditions similar to the Patagonia fjord can help to understand their fate in those environments.

Excessive and unrestricted use of antibiotics is a general problem in aquaculture (Ottinger *et al.*, 2016), especially in Chile, a country recognized for its high use of antibiotics (Burridge *et al.*, 2010). The annual use of antibiotics in the Patagonian regions of Los Lagos, Aysén, and Magallanes hold an average of ~373 tons, and has varied from 180 tons in 2009 to ~550 tons in 2014 and 2015, and gradually decreasing to ~323 tons in 2018 (Sernapesca, 2019). Some studies have been focusing on the local impact of salmon culture, however, few studies seek to understand the dynamic and fate of these compounds along of fjords, with high pressure of aquaculture activities.

1.3 Pesticides fate and occurrence in non-target organisms.

Pesticides are used, in Chile, as a treatment against infection in salmon cultures by *Caligus rogercresseyi*, which can generate severe skin damage and a major salmon susceptibility to suffering a bacterial and viral infection (Bravo, 2003; Johnson *et al.*, 2004; Lhorente *et al.*, 2014;

Dresdner *et al.*, 2019), which results in a decreasing to production and also in an increment in production costs (González and Carvajal, 2003; Rozas and Asencio, 2007; Revie *et al.*, 2009; González *et al.*, 2015). Some pesticides, like emamectin benzoate, have been used to target organisms like *C. rogercresseyi*, which has been proven to have developed some resistance. As a consequence, the industry has started to use new pesticides, like pyrethroids, using deltamethrin since 2007 and cypermethrin since 2009 (Bravo *et al.*, 2008, 2010). Others have also been used, such as diflumenzuron (chitin synthesis inhibitor) in 2010 and azamethiphos in 2013 (Helgesen *et al.*, 2014; Quiñones *et al.*, 2019).

Pyrethroids, as shown in Figure 2, are applied to salmons by bathing. The treated water is later released into the seawater, and their plume can be followed in the dissolved phase 1 km away from the locus of release and remain above detection limits for 48 hours (*e.g.* Willis *et al.*, 2005; Burridge *et al.*, 2014).

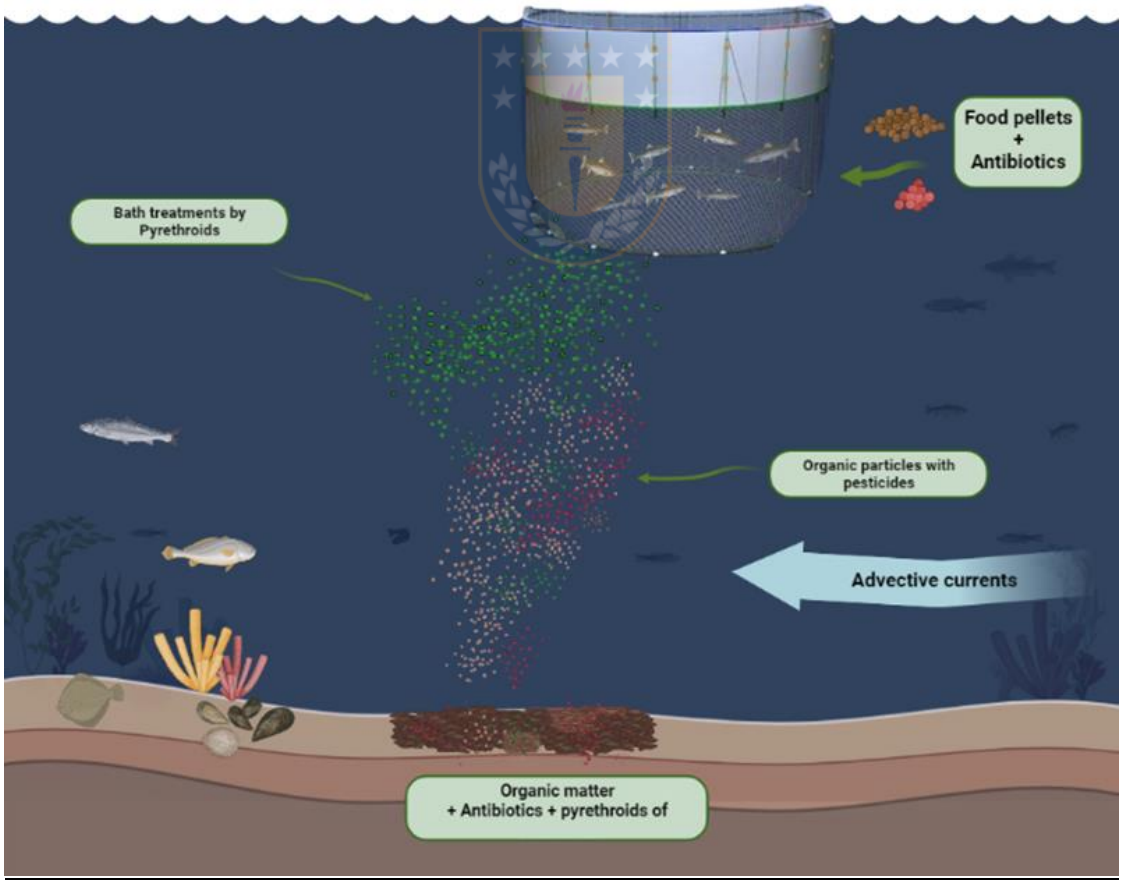


Figure 2. Conceptual model of fate and organism impact of pesticides used in aquaculture treatments (autoelaboration).

However, these compounds have a high Log Kow (about 6) summated to low solubility. Thus theyare highly susceptible to absorbin the organic matter of particles (Erst et al., 2014; Tucca et al., 2017; Méjanelle et al., 2020). After absorption, the pesticide carrier particles move the compounds by horizontal transport and by sinking to sediments. Sorption may determine thi fate and persistence of pyrthrrroids in the marine environment with a potential risk for non-target benthic organisms (Tucca et al., 2017; Urbina et al., 2019; Méjanelle et al., 2020).

The impact of pyrethroids released in the dissolved phase has been reported to have a potential effect over non-target adults and larvae crustacean (Mugni *et al.*, 2013; Gebauer *et al.* 2017; Parsons *et al.*, 2020), where it produces a reduction in the feeding and motility at least during 1h of salmon treatments, which can have some implications in an ecological level (Friberg-Jensen *et al.*, 2003; Van Geest *et al.*, 2014a). Other planktonic groups, such as phytoplankton showed growth stimulation and this process has implications on structure community through differences in sensitivity of the species (Wang *et al.*, 2010). It has been suggested that pesticides can produce changes in a photo and chemoautotrophs carbon fixation in the microbiota (Rain *et al.*, 2018). Recent studies have reported an efficient bacterial degradation of deltamethrin as a tool to remove residual content inside crabs (Ning *et al.*, 2020). Pyrethroids have the potential to bioaccumulate (Power and Chapman, 1992) due to their high affinity for organic matter and lipids (Log Kow 5 to 6). Filtering organisms are also exposed to pyrethroid contamination (*e.g.*, Mazzola and Sarà, 2001; Norambuena-Subibabre *et al.*, 2016). Contrary to the assumption that pyrethroid insecticides were converted to non-toxic metabolites by hydrolysis in mammals (Godin *et al.*, 2007), different pyrethroids were shown to be bioaccumulated by dolphins (Alonso *et al.*, 2012). Then assuming the high affinity by particles and subsequence sinking in the sediments, is possible to consider that these compounds can be incorporated by sessile filter organism, bioconcentrated or bioaccumulated, and eventually used as an environmental bioindicator in areas with active aquaculture conditions.

1.4 Impact of aquaculture pollutants on marine food webs and carbon cycle

A rising concern is whether pollutants and their metabolites released to aquatic environments are transferred to non-target edible shellfish and fish resources (*e.g.*, Lahti and Oikari, 2011; Cabello *et al.*, 2013), and thereby to humans (Burridge *et al.*, 2010). Dissolved pollutants enter non-target organisms of the pelagic food web through bioconcentration (Power and

Chapman, 1992) as shown for treatment bath plume, and this exposure is limited to a few days (Willis *et al.*, 2005). Due to their deposition to sediments, they are also incorporated by benthic organisms (Leung *et al.*, 2012; Kim *et al.*, 2014; Chen *et al.*, 2015). A continue exposure of antibiotics can induce changes in the sedimentary bacterial community (*e.g.*, Samuelsen *et al.*, 1992) and favor bacterial groups with resistant genes (*e.g.*, Chelossi *et al.*, 2003). Such perturbation has been reported close to aquaculture farms, especially by tetracycline (Miranda and Zeleman, 2002; Cabello *et al.*, 2016). Since part of the microbial community is affected by antibiotics, we would expect the activity of the affected population to be altered, and to be reflected in the overall degradation of organic matter by the microbial community (*e.g.*, Pantoja *et al.*, 2011, Arnosti, 2014), the first step of organic carbon decay.

The impact of dissolved pyrethroids is less clearly understood but the exposure of pelagic food web lasts at least hours (Willis *et al.*, 2005; Burridge *et al.*, 2014; Ernst *et al.*, 2014), and changes in non-target crustacean communities have been reported (Van Geest *et al.*, 2014). They can affect even crustacean benthic organisms, due to their high affinity for organic matter and lipids (Log K_{ow} 5 to 6). In benthic communities, meiofauna (<1000 to > 42 μm mesh) has a key role in carbon sequestration in sediments (Van Cappellen, 2003) and nutrient release due to remineralization (Webb and Montagna, 1993). The interaction with bacterial activity in sediments can stimulate organic matter degradation (Nascimiento *et al.*, 2012; Bonaglia *et al.*, 2014) and also compete to consume organic matter (*e.g.* Nascimiento, 2010). Copepods (arthropods) are second in abundance in sediment compared to nematodes (*e.g.*, Coull, 1999; Sajan *et al*, 2010; El-Serehy *et al.*, 2015) and preferred preys of invertebrates and fishes (Coull, 1999), and they could also be affected by pesticides that in turn affect mineralization or trophic structure.

Benthic response to antibiotics is complex because they inhibit some bacterial biochemical processes (see Table N°1) and also induce gene resistance (*e.g.*, Chellosi *et al.*, 2003; Marti *et al.*, 2014). Bacteria with antibiotic resistance occur in feces of treated salmons and are also detected in sediments underneath salmon farms (Miranda and Zemelman, 2002; Cabello, 2006; Primavera, 2006; Price *et al.*, 2015). Antibiotics more likely affect biomass and degradation of organic carbon and considering that aquaculture is a source of additional organic carbon to the ecosystem, a decrease in mineralization may lead to an even greater organic carbon accumulation.

Our understanding of the occurrence of toxic compounds released by aquaculture is limited to a few reports on pyrethroids in seawater, on florfenicol, and flumequine in sediments. Several

authors have pointed to the need for a more comprehensive inventory on the fate and partition of antibiotics and insecticides in the environment and on their degradation (*e.g.*, Ernst *et al.*, 2014; Gaw *et al.*, 2014). The impact of these pollutants on the carbon cycle, and especially on organic carbon mineralization is unknown.

1.5 The scientific problem and the strategy

Most of the studies about antibiotics have been focusing on the local impact of salmon culture. However, few studies seek to understand the dynamic and fate of these compounds along fjords with high pressure of aquaculture activities. Additionally, understanding the partitional behavior of antibiotics, like florfenicol and flumequine, under controlled temperature and salinity conditions similar to the Patagonia fjord can help to understand the fate of these compounds in those environments.

Assuming that pyrethroids have a high affinity with particles and, subsequently, those particles will sink into the sediments, these compounds have the potential to be incorporated by sessile filter organisms, bioconcentrated or bioaccumulated, and eventually used as an environmental bioindicator in areas with active aquaculture conditions.

The impact of antibiotics and pyrethroids on the carbon cycle, and especially, on organic carbon mineralization is unknown. The abundance and diversity of microbial populations could be affected by the antibiotic presence, producing a decrease in mineralization that may lead to an even greater organic carbon accumulation. A similar situation can be observed in marine sediments where copepods, the second highest group in abundance, could be affected by pesticides and, in turn, affect organic matter mineralization or trophic structure.

The question of the impact of pollutants released by aquaculture activities is not trivial. First, the marine environment dilutes the compounds to a point where they can be very difficult to detect. Second, at such low levels, the impacts of pollutants are subtle changes in ecological functions rather than usual toxicological effects (Rain-Franco *et al.*, 2018). Finaly, the industry has set-up management procedures to reduce the impact of salmon treatments, such as period of sanitary rests, usually of 3 months, after the collection of the fish. The overall impact of using pesticides and antibiotics may integrate toxicological effects close to the cages and during the treatment to other low levels impacts, farther away from the cages and at times stretching to after the sanitary rest

period. Scientific tools do not exist at present date to appraise those likely various effects. We therefore tried to tackle impacts using 3 complementary approaches (Figure 3):

- Environmental Measurements: assessing the occurrence of compounds (antibiotics) after the sanitary rest periods, all along the fjord, and not especially close to the cages. Also, pyrethooids occurrence in suspended particles and benthic filter-feeding organism were analyzed.
- Modeling: calculating exected concentration of antibiotics in seawater and sediments
- Experiments: determining potential impact of pesticides and antibiotics on remineralization, at concentrations representative of treatment periods, and of area in close vicinity of the cages.

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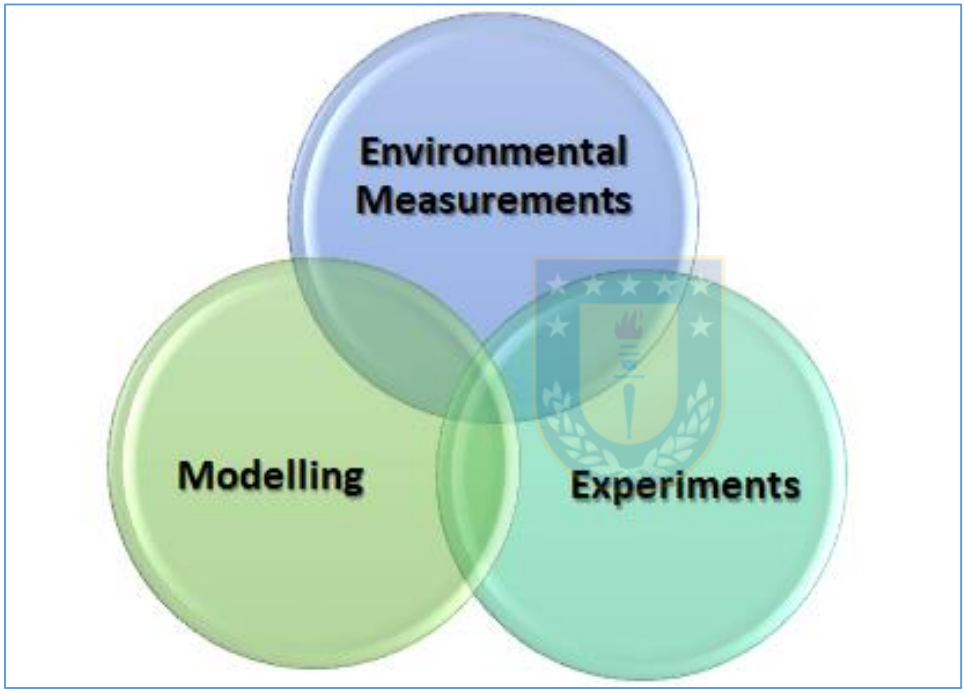

Figure 3. Strategy approach used in this study to response the scientific problems.

1.6 Hypotheses

Hypothesis 1:

Based on the expected partition coefficients of antibiotics and pyrethroids flumequine and pyrethroids are found mainly associated with organic material in particulate phases and benthic filter-feeders in Puyuhuapi Fjord, while florfenicol is mainly under the dissolved form.

Hypothesis 2:

Antibiotics and pesticides will affect the community respiration of microorganisms and meiofauna (crustaceans).

1.7 General goal

To understand the fate and dynamics of antibiotics and pesticides used by the aquaculture industry in the marine ecosystem and to evaluate their impact on key processes of the marine carbon cycle.

1.8 Specific goal

1) To determine the occurrence of florfenicol and flumequine in dissolved and particulate phases and in surface sediments along the Puyuhuapi fjord, to establish their fate supported by fugacity models.

2) To establish in laboratory experiments of sorption of florfenicol and flumequine under temperature and salinity similar to those of Puyuhuapi fjord.

3) To determinate pyrethroid contents in benthic sessile filter organisms and estimate possible bioconcentration.

4) To establish the effects of antibiotics and pesticides on community respiration in the water column and marine sediments, through an experimental approach with samples collected in Banyuls bay (France), an area without aquaculture activity.

2.0 MATERIAL AND METHODS

The present work was developed in two geographical areas; i) zone with active aquaculture activity, Puyuhuapi Fjord in Chilean Patagonia and, ii) zone without aquaculture activities in Banyuls Bay in France. The fate and dynamics of antibiotics and pesticides were studied in Puyuhuapy fjord and the effect of these compounds on the activity of bacterial communities and meiofauna was studied in Banyuls bay.

2.1 Study Area

2.1.1 Puyuhuapi fjord

Two field trips were conducted during August 2016 and March 2017 in the Puyuhuapi Fjord (44°57'S; 73°21'W), located in the Aysén Region of the Chilean Patagonia (Figure 4). The location has a total area of ca. 700 km^2, currently harboring 500 salmon cages (~ 9 % of fjord surface area). Since 2001, salmon aquaculture has been a prominent activity in this area, currently with 25 active culture centers and a salmon production of 26,670 tons during 2016 (Sernapesca, 2016a, b).

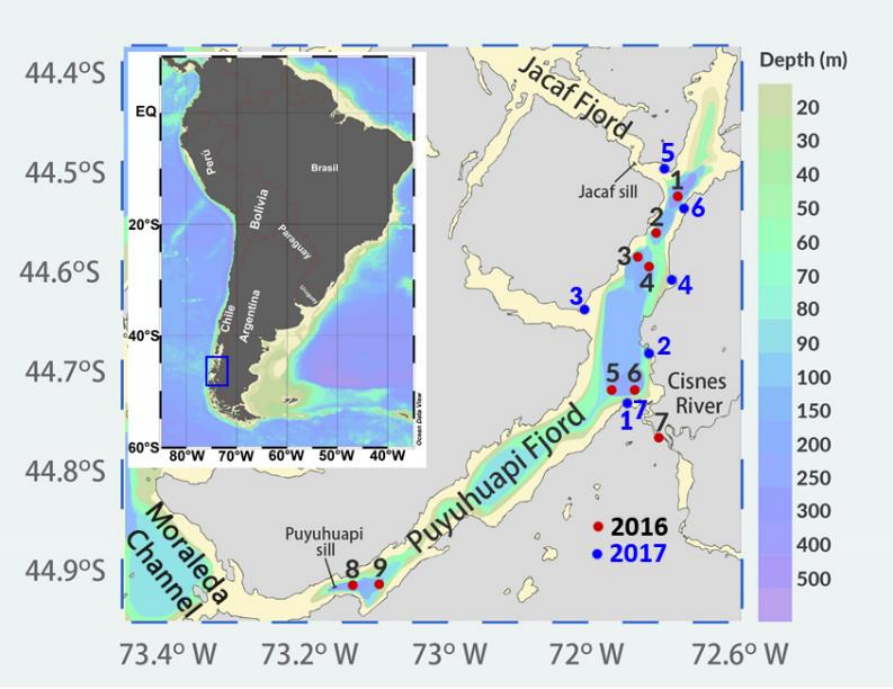

Figure 4. Study area and sampling locations. Black numbers and red circles were stations sampling during august 2016. Blue numbers and blue circles were sampling stations during march 2017.

Puyuhuapi fjord is connected to the north by the Jacaf fjord and to the south by the Moraleda Channel. Water circulation in the Puyuhuapi fjord follows an estuarine pattern characterized by a surface seaward flow of fresher waters from the continent and an intrusion of oceanic waters through the Jacaf and Moraleda Channels (Schneider *et al.*, 2014). Primary production in Puyuhuapi fjord averages 1.4 g C m^{-2} d^{-1} (Daneri *et al.*, 2012) and hypoxic conditions below 120m depth are promoted by the remineralization of organic matter and the presence of the Jacaf and Puyuhuapi sills (see Figure 3) which limit ventilation (Schneider *et al.*, 2014, Silva and Vargas 2014).

2.1.2 Banyuls bay in NW Mediterranean Sea

Field trips were conducted at the SOLA station (42°30′ N, 03°08′ E, 27 m depth) and the MESO station (42°29′ N, 03°09′ E, 35 m depth) located in Banyuls bay at NW Mediterranean Sea (Figure 5). These stations were part of long-term environmental monitoring by the Service d'Observation du Laboratoire Arago. The area corresponds to an oligotrophic zone with a ~1 µg chlorophyll *a* (chl *a*) L^{-1} (Obernosterer *et al.*, 2005) without aquaculture activities.

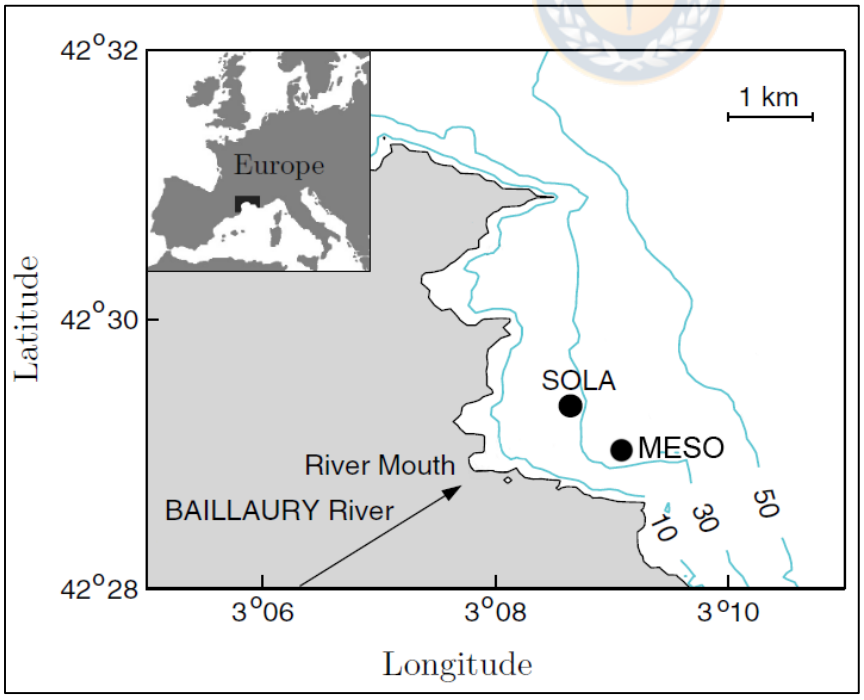

Figure 5. NW Mediterranean Sea study area. For experiments, the sediment samples were collected at the MESO station and water column samples were collected at the SOLA station. (modified from Guizien *et al.*, 2007).

2.2 Chapter I: Antibiotics florfenicol and flumequine in the water column and sediments of Puyuhuapi Fjord, Chilean Patagonia

We sampled Puyuhuapi Fjord during August 6-9, 2016, six months after the last programmed florfenicol treatment in the fjord between January and February 2016, and one year after the last treatment with flumequine (Sernapesca, 2016b). Water samples were collected at 8 sites along the fjord at four depths and from the surface of the Cisnes River, and surface sediments were taken at 4 sites (Figure 4). Seawater samples were collected with 10L-Niskin bottles and two-liter subsamples were then filtered onto previously combusted GF/F filters (0.7 μm pore size). Filters were stored at -20°C in the dark until analysis. The filtrate was acidified to pH 3 with 40% H_2SO_4, amended with 0.5 g L^{-1} Na_2EDTA to chelate major cations, and then stored at 4ºC. Surface sediments were collected using a Rumohr corer, and the top one-centimeter sections were removed with a core extruder. Sediment samples were then stored at -20°C in the dark until analysis. Physical characterization of the sampling sites was conducted by measuring temperature, salinity, and dissolved oxygen in the water column using a Seabird SBE Model 25 CTD.

2.2.1 Analysis of antibiotics

Dissolved organic matter was pre-concentrated onto 3 mL (60 mg) solid-phase extraction columns (SSDVB063, Styre Screen) in a VaccElut Cartridge Manifold (Agilent). C18 cartridges were previously conditioned with 5 mL methanol followed by 5 mL ultra-pure water. Adsorbed material was eluted with 10 mL methanol at a flow rate of 2 drops per second into silanized vials and directly evaporated under a nitrogen stream at 40°C. The residue was dissolved in 1000 μL methanol and filtered through 0.22 μm PVDF syringe filters, gently saturated with N_2, and then kept at -20°C until analysis (Zhou *et al.*, 2012). The particulate organic matter on filters and within sediment (ca. 1 g dry weight) was extracted using ultrasound extraction (3 times) with 5 mL citric acid buffer (pH 3) and 5 mL acetonitrile for 15 min, and then centrifuged for 10 min at 1400 rpm. Supernatants were diluted to 100 mL using milli-Q water, amended with 0.5 g L^{-1} Na_2EDTA, and loaded into SPE columns (SSDVB063, Styre Screen). Elution and storage of eluates were as described above for the dissolved phase.

Analyses of florfenicol and flumequine were conducted using an UHPLC Shimadzu (Kyoto, Japan) Nexera X2 LC-30 AD system coupled to a single quadrupole mass spectrometer

LCMS-2020 with an electrospray ionization (ESI) interface. Separation was carried out on a Phenomenex (Torrance, CA, USA) Kinetex EVO C18 core-shell column (150 mm x 2.1 mm, 2.6 µm) connected to a Kinetex C_{18} guard column, both operating at 40° C. Mobile phase A was 0.1% formic acid in Milli-Q water, and phase B was formic acid 0.1% in acetonitrile. The gradient used was 10% B for 1 min, increased to 70% B over 3 min, and then maintained for 10 min at a flow rate of 0.2 mL min^{-1}. The ESI interface was used simultaneously in positive and negative mode to measure flumequine (positive) and florfenicol (negative). Mass spectrometry analysis was implemented in selected ion monitoring (SIM) acquisition mode to monitor molecular ions at m/z 262 $[M+H]^+$ for flumequine and 356 $[M-H]^-$ for florfenicol. Samples in which florfenicol and flumequine were detected were further analyzed by UHPLC-MS-MS for confirmation, under the same chromatographic conditions as for UHPLC-MS. Chromatography was performed in a Shimadzu Nexera X2 UHPLC LC-30 AD system coupled to a LCMS8030 mass spectrometer with ESI. Detection was carried out by tandem MS in multiple reaction monitoring (MRM) mode using the following parent and product ions m/z values for flumequine (m/z 262→202) and florfenicol (m/z 356→185). MS operating conditions were set as follows: ESI voltage 4.5 kV, collision energy 30.0 V, nebulizer gas (N_2) flow: 3.0 L min^{-1}, drying gas (N2) flow: 15 L min^{-1}, desolvation line (DL) temperature: 250 °C and heat block temperature: 400 °C. Data were acquired, recorded and analyzed using Shimadzu LabSolution 5.8 software. Quantification was carried out using a calibration curve with serial dilutions of florfenicol (CAS N° 73231-34-2) and flumequine (CAS N° 42835-25-6). Reproducibility was 3 to 5 % (coefficient of variation), routinely determined from three to five replicate analyses. Recovery yield of antibiotics from seawater was estimated by adding 1 mL of a 100 µg L^{-1} antibiotic standard solutions to 1L Milli-Q water (in triplicate) and maintained for 24h in the dark under continuous shaking. Antibiotics were extracted and analyzed as described in the above methodology, resulting in recoveries of 79% for florfenicol and 66% for flumequine. Detection and quantification limits were calculated using signal-to-noise ratios (S/N) of 3 and 10, respectively. Detection limits for florfenicol were 2 ng L^{-1} in seawater and 1 ng gdw^{-1} in sediments and for flumequine were 12 ng L^{-1} and 6 ng gdw^{-1}. Limits of quantification for florfenicol were 6 ng L^{-1} in seawater and 3 ng gdw^{-1} in sediments and for flumequine were 36 ng L^{-1} and 18 ng gdw^{-1}.

Regarding QA/QC, all solvents used for chromatography were LC grade, Milli-Q water with a resistivity of 18.2 MΩ cm (25ºC), TOC < 5 ppb and bacterial count <0.01 CFU mL^{-1}. All

reagents and chemicals were at least analytical grade (ACS) and all instruments are annually calibrated as recommended by the by manufacturer. QC was performed using blank (methanol) and internal reference samples (florfenicol and flumequine standards diluted at calibration middle-level), which were analyzed in triplicate at initial, middle and end of each sample analysis batch. Thus, the equipment performance was constantly evaluated including carry-over effect, resulting in relative standard errors ≤5% for concentration and ≤1% for retention time.

2.2.2 Multimedia fugacity model

A fugacity-based model level III (Mackay and Paterson, 1991; Gouin and Harner, 2003; Hughes *et al.*, 2012; Zhang *et al.*, 2015) was designed to predict the dynamic and fate of antibiotics in farmed fish, and in the water column and sediment after one day of medication. The model was tailored to a salmon processing environment with considerations for antibiotic partition, dosage, degradation, advective transport, rates of sedimentation and resuspension, and salmon density. Calculations were made for simultaneous treatments with medicated feed pellets in 25 salmon farms, each consisting of 20 cages, as found in Puyuhuapi Fjord (Sernapesca, 2016b). Dosages in fish feed were 10 mg kg^{-1} florfenicol and 30 mg kg^{-1} flumequine, and the biomass of almonids was between 11 and 17 kg m^{-3} per cage with 15% mortality rate over a two-year production period (Subpesca, 2016). The model considers both diffusive and advective transports of antibiotics from seawater in sediments, and removal by microbial degradation, modeled as first order reaction rate. Physical and chemical properties of antibiotics and environmental parameters used in the model for Puyuhuapi Fjord are shown in Tables S2, S3 (Supplementary material). See more detail in the result section chapter I.

2.2.3 Monte Carlo Simulation

A probabilistic simulation was carried out to assess uncertainties and sensitivity of the model based on probability distributions of the input parameters (Table S3) and their contribution to variability in modeling outcomes Lognormal and triangular distributions were assumed for input parameters. These analyses determined 95% confidence intervals (CI95%) from the probabilistic distribution of model outcomes (Figure 3 main paper). Simulations were run 100,000 trials using Crystal Ball 11.1.1 software (Gentry *et al.*, 2008).

2.2.4 Simulation test for permanence time of antibiotics

A second modeling experiment involved ten days of daily additions of florfenicol and fifteen days of flumequine, a common treatment protocol for the industry in southern Chile (Contreras and Miranda, 2011). This simulation was used to evaluate whether antibiotics remain at inhibiting or sub-inhibiting concentrations in seawater and surface sediment after the end of the treatment, and if so, for how long they remained above either of these thresholds. During consecutive daily treatments, microbial decay of predicted antibiotic contents in both water and sediment was calculated assuming to follow first-order reaction kinetics using half-life values from literature (Table S2). Environmental concentrations estimated for seawater and sediment through modeling were used as initial values for temporal decay simulation of antibiotics. During each treatment in cages the degradation rate constant (k, d^{-1}) was computed using equation 1:

$$ln\frac{C_i}{C_0} = -k\ t \quad (1)$$

where C_0 is the estimated concentration from the multimedia model of compartment-i, and k values are calculated as $0.693/t_{1/2}$.

2.3 Chapter II: Batch experiment study of water-sediment partition of flumequine and florfenicol, two antibiotics used in salmon aquaculture in Chile

Seawater was collected from the surface, and marine sediment was collected at ca 90m depth on the 8th October, 2018 at the Oceanographic Time Series Station 18 (36° 29.94′ S, 73° 07.8′ W) of the COPAS Center for Oceanographic Research in the eastern South Pacific (FONDAP ANID Chile). Experimental procedures were conducted in the Laboratory of Marine Organic Geochemistry at the University of Concepción. Experiments were conducted under sterile and dark conditions, under constant orbital agitation (200 rpm), at 8°C and 15°C, in both pure water (milliQ water, salinity = 0‰) and marine water (salinity =35.34‰)

2.3.1 Sediment-water batch experiments

Glassware was cleaned by calcination to 450°C for 4 h and exposed to UV radiation for 30 minutes before use. Natural seawater was filtered through a PVDF membrane filter of 0.22 µm pore size and then autoclaved. Natural marine sediments were autoclaved and exposed to UV radiation for 30 minutes before use. Ultrapure water was obtained from a Milli-Q device (18Ω). Incubations were carried out on a Thermo Scientific Chamber MaxQ 6000. A mix of flumequine (Sigma-Aldrich CAS 42835-25-6) and florfenicol (Sigma-Aldrich CAS 73231-34-2) was prepared as a primary standard stock solution of 40 mg L^{-1} of each antibiotic.

Experiments started on the 23rd of October, 2018. Forty mL of seawater or ultrapure water were added to glass flasks of 80 mL. The same water volume was amended with 3 g of wet marine sediments to prepare the water + sediment treatments. Each treatment was prepared in triplicate (Figure 6).

Control treatments consisted of flasks containing pure water or seawater, with or without sediment, but without antibiotic addition. For other treatments, antibiotics were added to the tubes to a final concentration of 1.4 (8°C incubation) or 1.2 mg L^{-1} (15°C incubation) of each florfenicol and flumequine. Flasks were gently mixed, maintained for 30 min under dark conditions, and 1 mL of water was sampled for the initial time. Further sampling times were at 1, 2, 3, 4, 24, and 48h. The 1 mL water sample was diluted 10X using an acetonitrile: water mix (50:50, v/v), then a subsample of 1 mL was directly injected in the UHPLC-MS.

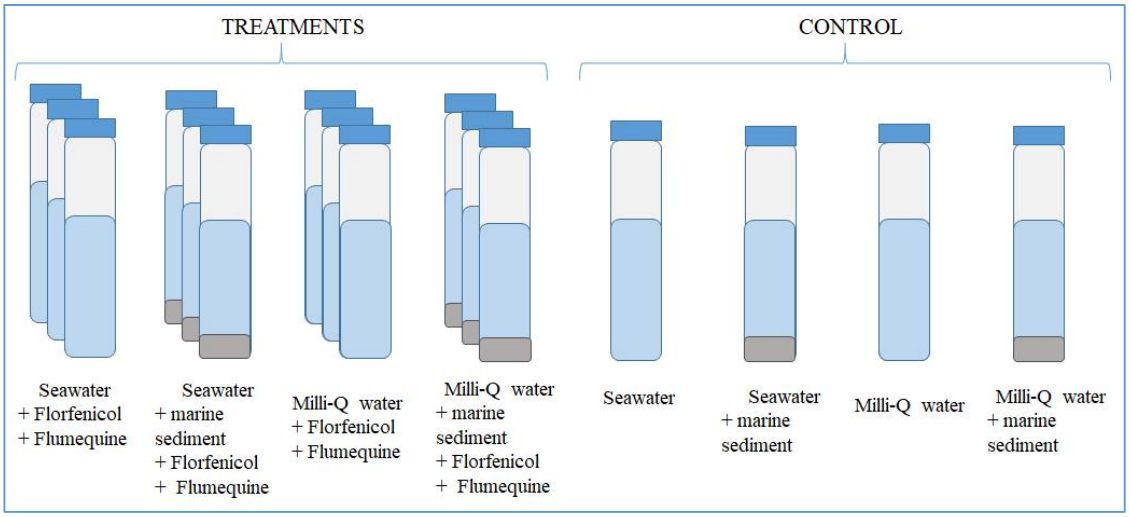

Figure 6. Experimental design used in batch experiments carried out in the Geochemistry Organic Marine Laboratory at the University of Concepcion. After 30 min of incorporating the antibiotics, 1 mL of water was sampled (initial time). Further samples were collected every hour until 4h, at 24h and 48h. This experiment was done at 8°C and repeated at 15°C.

2.3.2 Analysis of antibiotics

Antibiotics were analyzed using a Shimadzu (Kyoto, Japan) Nexera X2 LC-30 AD UHPLC system coupled to a single quadrupole mass spectrometer LCMS-2020 with an electrospray ionization (ESI) interface. The mobile phase A was Milli-Q + Formic acid 0.1%, and B was Acetonitrile + Formic Acid 0.1%. The analysis started using 10% B for 1 min, increasing to 70% around 3 min, maintained for 10 min, and finally decreased to 10% B for 5 min. Florfenicol and flumequine were separated on a Phenomenex (Torrance, CA, USA) Kinetex EVO C18 core-shell column (150 mm x 2.1 mm, 2.6 µm) connected to a Kinetex C18 guard column, operating at 40° C. Ionization ESI in positive and negative mode was used simultaneously to measure florfenicol (negative) and flumequine (positive). Mass spectrometry analysis was implemented in selected ion monitoring acquisition mode to monitor molecular ions at m/z 262 $[M+H]^+$ for flumequine and 356 $[M-H]^-$ for florfenicol. Reproducibility was 3 to 5 % (coefficient of variation), routinely determined from three to five replicate analyses (Jara *et al*., 2021).

2.3.3 Statistical analyses

Tests of both homogeneity of variances (Levene test) and normality of variables (Shapiro–Wilk test) were not fulfilled. We therefore tested for significant differences between categorical factors (matrix, antibiotic type, and temperature) using the nonparametric Kruskal–Wallis test (95% significance), and for differences between experimental treatments (Seawater, pure water, seawater + sediment, and pure water + sediment) using the paired sample Wilcoxon test (95% significance).

2.3.4 Calculation of K_d and K_{OC}

When the difference between treatment with and without sediment was proven to be significant using the Wilcoxon test, concentrations of sorbed antibiotics were calculated as the difference between the concentration of a dissolved antibiotic in a given treatment without sediment and that with sediment. This was calculated for each replicate at each of the equilibrated times (4, 24, and 48 h). For instance, for the treatment seawater at 4H and the first replicate, it was calculated as:

$$[FLU_{\text{in sed SW W}}]_{4H,R1} = \frac{[FLU_{\text{SW W}}]_{4H,R1} - [FLU_{\text{SW W+SED}}]_{4H,R1}}{\text{SedW}}$$

where,

$[FLU_{\text{SW W}}]_{4H,\,R1}$ is the dissolved antibiotic concentration (FLU stands for flumequine) in the first replicate of the treatment (SW W stands for seawater winter temperature) without sediment sampled at 4H, expressed in μg L^{-1};

$[FLU_{\text{SW W+SED}}]_{4H,\,R1}$ is the dissolved antibiotic concentration in the first replicate of the same treatment with sediment, sampled at 4H, expressed in μg L^{-1};

SedW is the dry weight of sediment used in the treatment, expressed in kg L^{-1};

$[FLU_{\text{in sed SW W}}]_{4H,\,R1}$ is the calculated content of flumequine sorbed to the sediment, expressed in μg kg^{-1}, for the treatment SW W, at 4H, for the first replicate.

The dissolved-particle partition constant K_d was calculated as the ratio of sorbed antibiotic concentration to the dissolved antibiotic concentration, for a given treatment:

$$K_d = \frac{Ci_{T,sed}}{Ci_{T,sw}}$$

Where:

$Ci_{T,sed}$ Is the concentration of the compound i in the sediment, averaged for all equilibrium times (T=, 4,8, 24 and 48h) in the experiments carried out at temperature T.

$Ci_{T,sw}$ Is the concentration of the compound i in the seawater (experiment condition without sediment), averaged for all equilibrium times (T=, 4, 8, 24 and 48h) in the experiments carried out at temperature T.

δK_d is the error on K_d, and is calculated using propagation error formula using derivatives:

$$\delta K_d = K_d \times \sqrt{(\frac{\delta Ci_{T,sed}}{Ci_{T,sed}})^2 + (\frac{\delta Ci_{T,sw}}{Ci_{T,sw}})^2}$$

Where:

$\delta Ci_{T,sed}$ is the error on $Ci_{T,sed}$ and its calculation is explained below.the error

$\delta Ci_{T,sw}$ is the standard deviation of the measurements of $Ci_{T,sw}$.

Calculation of $\delta Ci_{T,sed}$

$$Ci_{T,sed} = Ci_{T,sw+sed} - Ci_{T,sw}$$

The expression of $\delta Ci_{T,sed}$ is, using derivatives:

$$\delta Ci_{T,sed} = \sqrt{(\delta Ci_{T,sw+sed})^2 + (\delta Ci_{T,sw})^2}$$

The organic carbon partition constant K_{OC} represents the ratio of sorbed antibiotic concentration in the organic phase, divided by the dissolved antibiotic concentration, for a given treatment and a given replicate:

$$K_{OC} = \frac{K_d}{OC}$$

where, OC is the concentration of organic carbon in the dry sediment, expressed in kg kg^{-1} and K_{OC} is expressed in L (of seawater)/ kg (of organic carbon). At station 18, OC= 0.03 kg kg^{-1}= 3%.

2.4 Chapter III-B: Occurrence of pesticides in marine benthic filter-feeders in the Puyuhuapi fjord (44°57'S; 73°21'W), Chilean Patagonia

2.4.1 Field Sampling

Seawater and benthic filter-feeding organisms were collected in seven sampling stations in the Puyuhuapi fjord between March 23th and 30th, 2017 (Figure 7, Table 3), onboard the vessel L/M Don Osvaldo (with a length of 15 m and the beam of 3.5 m; registration CIS-1719 CA 2028). Seawater samples were collected between 6 and 15 m depth with Niskin bottles (10 L), prefiltered with a 100 μm sieve, and filtered using a precombusted filter GF/F 0.7 μm pore size. The filter was kept at -20°C for pesticides and total organic carbon measurement. Sponge species (three species for each station) and two bivalve species (*Mytilus chilensis* and *Chlamys patagonica*) were collected by scuba diving, freeze-dried and kept in dark conditions. On each station, three specimens of sponge were collected, and six specimens per bivalve species. Pesticides, lipids, and elemental analysis (CHN) were conducted in the Benthic Ecogeochemistry Laboratory (LECOB) at the Observatoire Océanologique de Banyuls-sur-mer, Sorbonne Université (France).

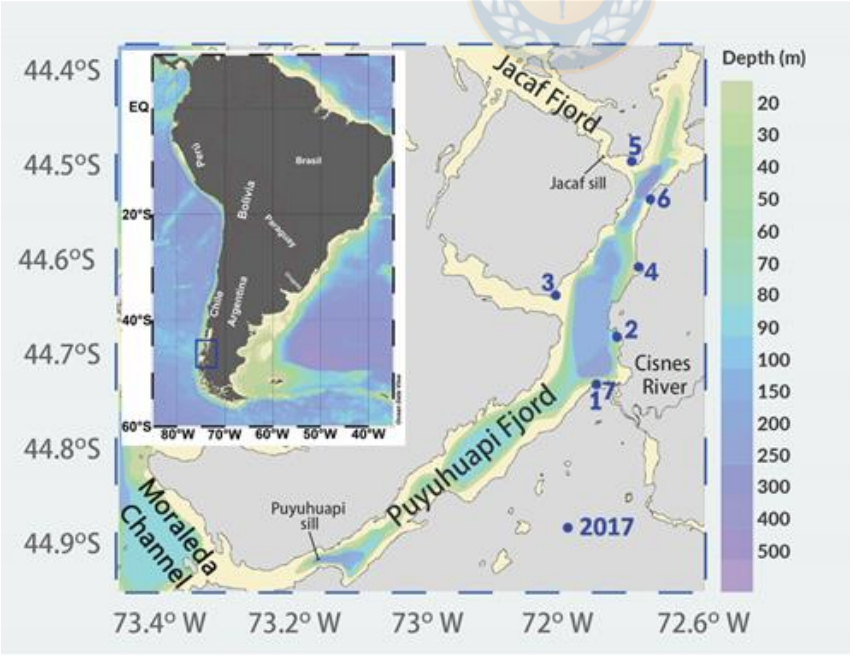

Figure 7. Study area and stations where were collected seawater and organisms' samples during March 2017.

Table 3. Location of sampling sites in the Puyuhuapi Fjord during March 2017. Three specimens for each sponge species and six specimens for each bivalve species were collected by scuba-diving.

Sampling site	Sampling Day	Latitude (S)	Longitude (W)	Total suspended particles depth (m)	Organisms
1	23	44° 44.699'	72° 44.356'	6	Sponges[a,b,c] and bivalves[h]
2	24	44° 41.481'	72° 42.237'	6	Sponges[a,b,f] and bivalves[h]
3	25	44° 38.570'	72° 47.829'	15	Sponges[a,b,d] and bivalves[h,i]
4	28	44° 36.412'	72° 40.629'	15	Sponges[a,b,d] and bivalves[h,i]
5	29	44° 33.024'	72° 39.386'	15	Sponges[b,d]
6	29	44° 44.838'	72° 44.758'	6	Sponges[a,b,e]
7	30	44°44.702'	72°44.420'	15	Sponges[a,b,g]

Sponge's: a) *Cliona chilensis*; b) *Axintella crinita*; c) *Amphilectus rugosus*; d) *Tedania spinata*; e) *Biemna sp*; f) *Unidentified D*; g) *Unidentified S.* Bivalves: h) *Mytilus chilensis* ; i) *Chlamys patagonica*.

2.4.2 Total lipids analysis.

The sulphophosphovanillin method (colorimetric method) considers the relation of total lipids with cholesterol and gravimetric standards as described by Baner and Blackstock (1973). Briefly, this method is based on (i) lipid extraction: 1.5 mL of chloroform-methanol mixture (2:1) were added to 25 mg of freeze-dried samples, maintained in orbital agitation during 20 min, centrifugated for 5 min at 1000 rpm, and 1 mL of supernatant was dried at 90°C, (ii) hydrolysis: 500 µL sulfuric acid (97%) was added in order to hydrolyze ester lipids from dry extracts, closing the screw cap, and heating for 10 min at 90°C. Samples were immediately cooled, (iii) complex formation: 100 µL of the hydrolyzed solution were recovered, and 2.5 mL of phosphor-vanilla solution were added to produce stable color by mixing, and measured in the spectrophotometer at 520 nm after 30 min in disposable cuvettes of 10-mm. A cholesterol stock solution (0.9 mg mL^{-1}) was used to prepare the calibration curve with a range of concentration of 20 to 1000 µg chol. mg dw^{-1}. This calibration curve had been treated with the same procedure previously described after drying the sample at 90°C in step (ii). Data were normalized by organic matter content in the samples.

2.4.3 Pesticide analysis.

The extraction and purification of pesticides from suspended solids and benthic organisms were performed in the LECOB laboratory during autumn 2018. Solid lyophilized suspended samples were extracted by ultrasound for 20 min with 5 mL dichloromethane (DCM), centrifuged for 5 min at 5500 rpm. A similar process was used for sponges and soft body of bivalves samples (0.5 g, lyophilized), but a DCM-hexane mixture (10:1) was used for the extraction. After extraction, 50 µL of internal standard (0.001 µg mL^{-1}) were added to extracts, reducing the volume to ~1 mL using a rotary evaporator, and a few quantities of anhydrous sodium sulfate were added and keep overnight. Finally, the extract was conserved in iso-octane.

Extracts were cleaned using a column packet from bottom to top with 5 g of water-deactivated silica gel, 3 g of water-deactivated alumina, and 0.5 g of anhydrous sodium sulfate. The column was cleaned with 5 mL of hexane. The extract was eluted in four phases, (i) F1, 25 mL of hexane, (ii) F2, 32 mL of hexane: DCM mixture (3:1), (iii) F3, 25 mL of DCM and, (iv) F4, 15 mL of methanol. The total volume was reduced with a rotary evaporator, conserved in an iso-octane and kept at -20°C until analysis.

Table 4. Quantification transition and Detection and Quantification of limit (LOD and LOQ, respectively) of pyrethroids in organisms (ng g lipid dw^{-1}) and particles (ng L^{-1}).

Compounds	**Quantification transition**	**LOD**	**LOQ**	**LOD**	**LOQ**
Unit	(*m/z*)	(ng g lipid dw^{-1})	(ng g lipid dw^{-1})	(ng L^{-1})	(ng L^{-1})
Allethrin	301 → 168	0.00856	0.01199	0.00008	0.00012
Bifenthrin	205 → 121	0.01741	0.03483	0.00017	0.00033
Cyhalomethrin	205 → 141	0.01741	0.34829	0.00017	0.00334
Cyfluthrin	207 → 35	0.03173	0.31729	0.00030	0.00305
Cypermetrhin	207 → 35	0.06181	0.47897	0.00059	0.00460
Fenvalerate	211 → 167	0.06181	3.09066	0.00059	0.02967
Deltamethrin	217 → 81	0.06181	0.09272	0.00059	0.00089

Fractions containing pesticides were analyzed by gas chromatography coupled to a triple quadrupole mass spectrometer (GC/ MS-MS) operated in negative ionization mode with ammonium as the ionizing agent (NCI) (Dallegrave *et al.*, 2016). Briefly, the Multiple Reaction Monitoring mode records consecutive fragmentations of parent ions to daughter ions, ultra-specific

pairs of compounds. Quantification is performed against deuterated internal standards (d6-Cypermethrin, m/z: 213 → 35) and correcting responses for each pyrethroid (Table 4).

Pesticide calibration curves were established by analyzing certified solutions. Analytical quality control is based on reproducibility, recovery and blank levels (Feo *et al.*, 2010, Dallegrave *et al.*, 2016, Aznar-Alemany *et al.*, 2017).

2.5 Chapter IV: The impact on the carbon cycle of antibiotics and pyrethroids used in aquaculture activities.

A community respiration experiment was performed to establish the impact of antibiotics (oxytetracycline, florfenicol, and flumequine) and pyrethroids (cypermethrin and deltamethrin) in carbon cycle processes. Seawater and marine sediment were performed in the Benthic Ecogeochemistry Laboratory (LECOB) at the Oceanographic Observatory of Banyuls, Sorbonne Université (France). Analysis for nutrients, ammonium, and dissolved organic carbon (DOC) were conducted by Microbial Oceanography Laboratory (LOMIC) and flux cytometry was conducted in BioPIC laboratory, both laboratories at the Oceanographic Observatory of Banyuls, Sorbonne Université (France). Diversity and abundance of meiofauna were measured and bacterial abundance and diversity will be determined by DNA analysis.

2.5.1 Field Sampling

Fifteen sediment cores were obtained in the MESO station (42°29′ N, 03°09′ E, 35 m depth) by scuba-diving on June 19th, 2018. Whereas, on July 2nd, 20 L of seawater samples were collected at 3 m depth with a Niskin bottle (10 L) in the SOLA station (42°30′ N, 03°08′ E, 27 m depth). Seawater samples were prefiltered with a 200 μm sieve.

2.5.2 Experiment procedures

The microcosm experiments (seawater and marine sediments) were performed under dark conditions (to prevent algae growth) and at a constant temperature of 14°C, through immersion in a water recirculation tank. Each microcosm was acclimatized for 24h. Solvent and drugs were added as shown in Figure 8.

Daily dissolved oxygen was measured using an equipment Unisense Microsensor Multimeter Picoammeter PA2000 and microelectrode Unisense OX-100-12593. Samples were obtained for the initial condition (baseline) and for the final time of the experiments to measure nutrients, ammonium, dissolved organic carbon, and biological parameters (meiofauna and bacterial abundance and diversity).

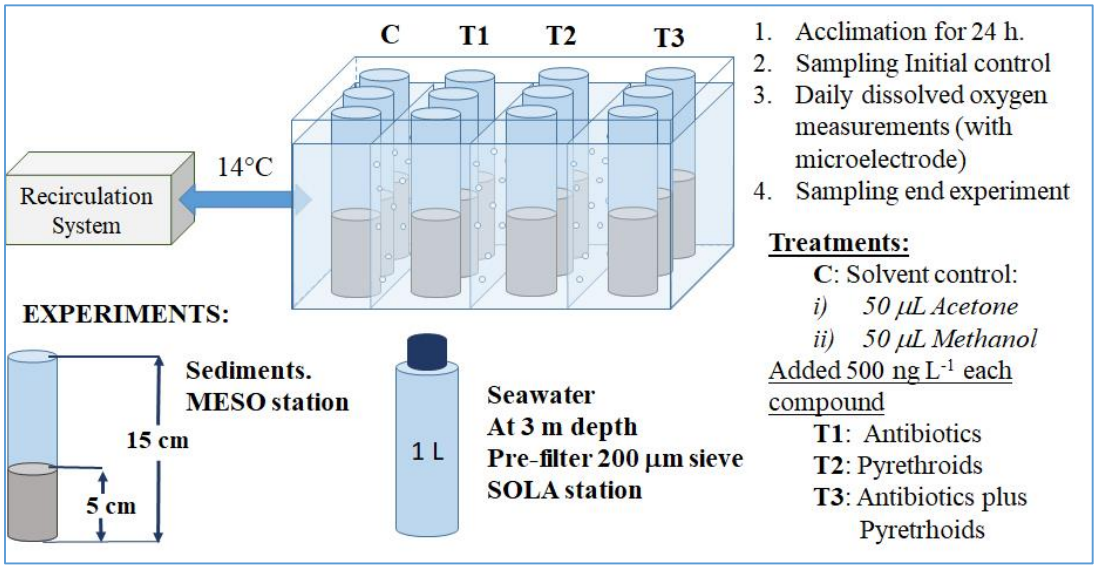

Figure 8. Design of experiments conducted in sediments and seawater microcosms. Oxytetracycline, florfenicol and flumequine (antibiotics), and cypermethrin and deltamethrin (pyrethroids) were added at a final concentration of 500 ng L^{-1} for each compound. Samples were obtained for initial conditions (baseline) and for the final time of the experiments.

2.5.3 Meiofauna community analysis

Subsamples of each core (3 cm) were cut off and preserved in 70% alcohol. These samples were washed on a 1 mm and 40 µm sieve with fresh water. The separation of meiofauna and fine sediments, after washing of samples, was extracted with Ludox (specific gravity:1.15). Briefly, sediments plus meiofauna and 20 mL of water were transferred to a 500 mL centrifuge tube, added 100 mL of Ludox and 3 gr of Kaolin, mixed, and centrifuged for 5 min at 6000 rpm. The supernatant containing the organisms was washed on a 40 µm sieve with fresh water. The organisms retained on the sieve were transferred to a plastic tube and preserved with 70% alcohol. Ten drops of Rose Bengel were added to each sample for easier recognition of the organisms. The meiofauna obtained was identified and counted under a stereomicroscope at a minimum 10-25 x magnification and expressed as specimens per unit volume.

2.5.4 Bacterial community analysis

Total bacterial abundance and high and low acid nucleoids content were analyzed by flux cytometry at BioPIC laboratory at the Oceanographic Observatory of Banyuls, Sorbonne Université (France). Additionally, the abundance and diversity of phytoplankton were analyzed. Bacterial community diversity will be measured at Microbial Oceanography Laboratory in Oceanographic at the Observatory of Banyuls, Sorbonne Université (France), as soon as possible under these pandemic conditions.

2.5.5. Statistical analyses

Tests of both homogeneity of variances (Levene test) and normality of variables (Shapiro–Wilk test) were fulfilled, then One-way Anova test for nutrients and two-ways Anova test for Oxygen concentration values was applied for both water column and sediment experiments. We therefore tested for significant differences between categories Control Solvents, Antibiotic, Pesticides and Antibiotic + Pesticides treatments using the parametric Turkey test (95% significance). Anosim test with a 95% significance was applied for biological components for both water column and sediment experiments.

3.0 RESULTS

3.1 Chapter I: Antibiotics florfenicol and flumequine in the water column and sediments of Puyuhuapi Fjord, Chilean Patagonia

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Abstract

Chile is a major global producer of farmed salmon in the fjords of Patagonia, and also a major consumer of antibiotics. We tested whether the antibiotics florfenicol and flumequine persisted in the large Puyuhuapi Fjord after the six months that followed mandatory concerted treatment by all salmon farms present in the fjord; we then estimated residence times of antibiotics in the system. Antibiotics were detected in 26% of analyzed samples, only within the particulate phase, with concentrations of florfenicol up to 23.1 ng L^{-1} where quantified. Flumequine was present in one sample at trace concentration; neither were detected in the dissolved phase nor in surface sediments. A fugacity-based model predicted that flumequine remains in surface sediments at sub-Minimal Inhibiting Concentrations (sub-MIC) shown to promote selection for antibiotic resistance in bacteria. Our observations pose new questions such as whether surface sediments might act a reservoir of antibiotic resistomes of bacteria, and whether bacteria bearing antibiotic resistance genes could eventually become a risk for human health through consumption of marine products.

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Antibiotics florfenicol and flumequine in the water column and sediments of Puyuhuapi Fjord, Chilean Patagonia

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HIGHLIGHTS

- Antibiotics were detected in 26% of analyzed samples in Puyuhuapi Fjord.
- Maximum quantified concentration was 23 ng L^{-1}.
- A fugacity-based model predicted that flumequine remains in sediments at Sub-MIC.

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ABSTRACT

Chile is a major global producer of farmed salmon in the fjords of Patagonia, and therefore a major consumer of antibiotics. We tested whether the antibiotics florfenicol and flumequine persisted in the large Puyuhuapi Fjord after the six months following mandatory concerted treatment by all salmon farms present in the fjord. Antibiotics were detected in 26% of analyzed samples, but only within the particulate phase, with concentrations of florfenicol of up to 23.1 ng L^{-1}, where detected. Flumequine was present in one sample at trace concentration, and neither antibiotic was detected in the dissolved phase nor in surface sediments. A fugacity-based model predicted that flumequine should theoretically remain in surface sediments at the sub-Minimal Inhibiting Concentrations (sub-MIC) previously shown to promote selection for antibiotic resistance in bacteria. Our observations suggest that surface sediments might act as a reservoir for antibiotic resistomes of bacteria, and that bacteria bearing antibiotic resistance genes could eventually become a risk for human health through the consumption of marine products.

1. Introduction

Aquaculture activities are being conducted on an increasingly larger scale because of global food security concerns (FAO, 2020). Consequently, increased production levels are becoming more reliant on the use of antibiotics that can reach the surrounding environment (e.g., Du et al., 2017). These antibiotics potentially select and modify the environmental distribution of antibiotic-resistant bacteria (Costanzo et al., 2005; Smith, 2008; Tamminen et al., 2011; Shah et al., 2014) which ultimately pose a risk for human health through potential gene transfer to human pathogenic bacteria (Miranda and Zemelman, 2001; Yang et al., 2013; Tomova et al., 2015; Miranda et al., 2018).

Elevated abundances of antibiotic resistant bacteria have been detected both in the water column (Nygaard et al., 1992; Samuelsen

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et al., 1992; Schmidt et al., 2000; Petersen et al., 2002; Cabello et al., 2016), and in marine sediments beneath culture cages (Björklund et al., 1991; Herwing et al., 1997; Schmidt et al., 2000). These bacteria can persist in the environment even after the decomposition of antibiotics (Tamminen et al., 2011).

The fate of antibiotics in the coastal ocean depends on physicochemical partitioning between dissolved and particulate phases, biological degradation, sedimentation, and horizontal transport (Sirtori et al., 2012; Leal et al., 2015; Liu et al., 2015, Mitchell et al., 2015). Antibiotics are administrated to farmed salmon as a component of food pellets, and following each treatment, a fraction of the consumed antibiotics is released into the water column though urinal, branchial and faecal excretion (Pouliquen et al., 2007, Grigorakis and Rigos, 2011; Miranda et al., 2018). Sinking of unconsumed or partially consumed pellets undergo degradation, particle scavenging and dispersion, also leading to release of antibiotics (Leal et al., 2015; Mitchell et al., 2015). Almost 5% of the total administered food pellets remain unconsumed and are subsequently deposited to sediments beneath the cages (Cabello et al., 2013; Miranda et al., 2018) where they can be ingested – together with antibiotics – by benthic and demersal organisms (Leung et al., 2012; Chen et al., 2015). The antibiotics florfenicol and flumequine are affected by sorption into particles, photodegradation, and microbial activities (Maki et al., 2006) that presumably include fungal degradation (Nnenna et al., 2011). Laboratory incubations of surface marine sediments have determined the half-life of florfenicol in marine sediments to be about one week (Hektoen et al., 1995), with that of flumequine being about 150 days (Halling-Sørensen et al., 1998).

The antibiotics florfenicol and flumequine are used for the treatment of Piscirickettsiosis (SRS- Salmon *Rickettsial septicaemia*) (Rozas and Enríquez, 2014). Florfenicol is also used for the treatment of Bacterial Kidney Disease, BKD (Sernapesca, 2017a). Chile is the second largest salmon producer globally after Norway (FAO, 2018), and is therefore a major consumer of antibiotics when normalized to harvest biomass (Miranda et al., 2018). Annual consumption of antibiotics in Chilean salmon farms varied between 557 tonnes during 2015, and 323 tonnes during 2018, with an antibiotic consumption index (i.e., antibiotic used/salmonid harvest) of 0.05% in 2017 (Sernapesca, 2019). High incidences of Piscirickettsiosis has resulted in florfenicol as presently being one of the most widely used antibiotics (Rozas and Enríquez, 2014; Sernapesca, 2017b).

In the present study, we investigated the fate of the antibiotics florfenicol and flumequine in Puyuhuapi Fjord. The study was designed to assess whether these antibiotic loads could be considered an environmental threat by promoting selection of antibiotic-resistant bacteria when antibiotics were at least at sub-Minimal Inhibiting Concentrations (sub-MIC, e.g. Cairns et al., 2018). Whilst MIC of antibiotics can inhibit visible growth of some bacteria (e.g., Wiegand et al., 2008), sub-MIC selection for antibiotic resistance mutations results from effects on genetic and phenotypic variability, cell-to-cell signaling, biofilm formation, quorum sensing and gene expression (Cairns et al., 2018).

Measurements of concentrations of antibiotics, and their potential threat, were assessed through chemical determinations of florfenicol and flumequine in the fjord, and through the use of a multimedia fugacity-based model on non-equilibrium and steady-state conditions (Mackay and Paterson, 1991; Mackay, 2001; Gouin and Harner, 2003; Hughes et al., 2012; Zhang et al., 2015). This modelling effort was developed to evaluate the specific compartments of the coastal ocean where antibiotics might be more likely to be detected, and to estimate their potential persistence in the water column and in sediments.

2. Materials and methods

2.1. Study area

Puyuhuapi Fjord (44°57.57′S; 73°21′W) is located in the Aysén Region of Chilean Patagonia and has a total area of ca. 700 km^2, currently harboring 500 salmon cages (~9% of fjord surface area). Since 2001, salmon aquaculture has been a prominent activity in this area, currently with 25 active culture centers and a salmon production of 26,670 tonnes during 2016 (Sernapesca, 2016a, b). The fjord is connected to the north through the Jacaf Channel and to the south through the Moraleda Channel (Fig. 1). Water circulation in Puyuhuapi Fjord follows an estuarine pattern characterized by surface seaward flow of fresher waters from the continent and intrusion of oceanic waters through the Jacaf and Moraleda Channels (Schneider et al., 2014). Primary production in Puyuhuapi Fjord averages 1.4 g C m^{-2} d^{-1} (Daneri et al., 2012). Hypoxic conditions below 120 m depth are promoted by remineralization of organic matter and the presence of the Jacaf and Puyuhuapi sills (Fig. 1) which limit ventilation (Schneider et al., 2014; Silva and Vargas 2014).

Official reports from the Undersecretariat of Fisheries and Aquaculture indicate that ca. 20 tonnes florfenicol were used during 2015, and ca. 4 tonnes during 2016 in Puyuhuapi Fjord (Sernapesca, 2016c), while almost 17 tonnes florfenicol and 0.45 tonnes flumequine were used in Jacaf Fjord (Sernapesca, 2016c, 2017b, 2018) as treatment for an outbreak of Piscirickettsiosis reported in 2016 (Sernapesca, 2017a).

2.2. Sampling

Sampling was conducted in Puyuhuapi Fjord between August 6–9, 2016, six months after the last programmed florfenicol treatment within the fjord that occurred between January and February 2016, and one year after the last treatment with flumequine (Sernapesca, 2016b). Water samples were collected at 4 depths from 8 sites along the fjord and from surface water of the Cisnes River. Samples from surface sediments were taken at 4 sites within the fjord (Fig. 1, Table 1). Seawater samples were collected using 10L-Niskin bottles, and 2-L subsamples subsequently filtered onto previously combusted GF/F filters (0.7 μm pore size). Filters were stored at −20 °C in the dark until analysis. Filtrate was

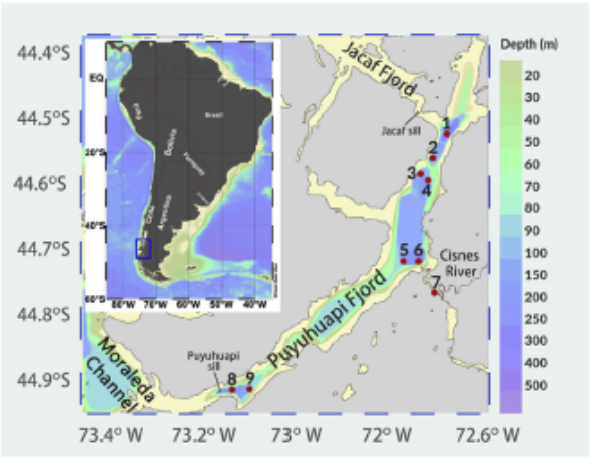

Fig. 1. Location of sampling sites in Puyuhuapi Fjord. See also Table for details of sites 1–9. The map was generated using the Ocean Data View software (Schlitzer, 2018).

Table 1
Water and surface sediment (0–1 cm) samples analyzed for florfenicol and flumequine in Puyuhuapi Fjord. Although both particulate and dissolved phases were analyzed (see methods), antibiotics were detected only in suspended particles and shown in ng L^{-1} ± standard deviation of duplicate injections. ND: Not detected. Trace indicates concentration lower than quantification limit (details in method section).

Sampling site and geographical location	Water depth (m)	Florfenicol	Flumequine
1	2	ND	Trace
44° 31.607′ S	10	ND	ND
72° 42.312′ W	50	ND	ND
	140	ND	ND
2	2	10.4 ± 0.5	ND
44° 33.854′ S	100	23.1 ± 2.5	ND
72° 42.127′ W	140 (surface sediment)	ND	ND
3	2	ND	ND
44° 35.309′ S	10	ND	ND
72° 43.587′ W	70	ND	ND
	140	ND	ND
	270 (surface sediment)	ND	ND
4	2	ND	ND
44° 35.906′ S	10	ND	ND
72° 42.721′ W	70	ND	ND
	140	ND	ND
	190 (surface sediment)	ND	ND
5	2	ND	ND
44° 43.341′ S	10	ND	ND
72° 45.866′ W	50	ND	ND
	100	ND	ND
	130 (surface sediment)	ND	ND
6	2	ND	ND
44° 43.330′ S	10	ND	ND
72° 43.896′ W	50	ND	ND
	160	ND	ND
7	0 (Cisnes River)	ND	ND
44° 46.483′ S			
72° 42.000′ W			
8	2	7.5 ± 0.1	ND
44° 55.132′ S	10	7.4 ± 0.9	ND
73° 07.553′ W	100	9.1 ± 0.2	ND
9	2	ND	ND
44° 55.067′ S	10	Trace	ND
73° 05.318′ W	50	Trace	ND
	150	ND	ND

acidified to pH 3 with 40% H_2SO_4, amended with 0.5 g L^{-1} Na_2EDTA to chelate major cations, and then stored at 4 °C. Surface sediments were collected using a Rumohr corer, and the top 1 cm sections removed with a core extruder. Sediment samples were then stored at −20 °C in the dark until analysis. Physical characterization of the water column at each sampling site was conducted by measuring temperature, salinity and dissolved oxygen using a Seabird SBE Model 25 CTD (Table S1).

2.3. Analysis of antibiotics

Dissolved organic matter was pre-concentrated onto 3 mL (60 mg) solid phase extraction columns (SSDVB063, Styre Screen) in a VaccElut Cartridge Manifold (Agilent). C_{18} cartridges were previously conditioned with 5 mL methanol followed by 5 mL ultrapure water. Adsorbed material was eluted with 10 mL methanol at a flow rate of 2 drops per second into silanized vials, and directly evaporated under a nitrogen stream at 40 °C. The residue was dissolved in 1000 μL methanol and filtered through 0.22 μm PVDF syringe filters, gently saturated with N_2, and then kept at −20 °C until analysis (Zhou et al., 2012).

The particulate organic matter on filters and within sediment (ca. 1 g dry weight) was extracted using ultrasound extraction (3 times) with 5 mL citric acid buffer (pH 3) and 5 mL acetonitrile for 15 min, and then centrifuged for 10 min at 1400 rpm. Supernatants were diluted to 100 mL using Milli-Q water, amended with 0.5 g L^{-1} Na_2EDTA, and loaded into SPE columns (SSDVB063, Styre Screen). Elution and storage of eluates were as described above for the dissolved phase.

Analyses of florfenicol and flumequine were conducted using an UHPLC Shimadzu (Kyoto, Japan) Nexera X2 LC-30 AD system coupled to a single quadrupole mass spectrometer LCMS-2020 with an electrospray ionization (ESI) interface. Separation was carried out on a Phenomenex (Torrance, CA, USA) Kinetex EVO C_{18} core-shell column (150 mm × 2.1 mm, 2.6 μm) connected to a Kinetex C_{18} guard column, with both operating at 40 °C. Mobile phase A was 0.1% formic acid in Milli-Q water, and phase B was formic acid 0.1% in acetonitrile. The gradient used was 10% B for 1 min, increased to 70% B over 3 min, and then maintained for 10 min at a flow rate of 0.2 mL min^{-1}. The ESI interface was used simultaneously in positive and negative mode to measure flumequine (positive) and florfenicol (negative). Mass spectrometry analysis was implemented in selected ion monitoring (SIM) acquisition mode to monitor molecular ions at m/z 262 $[M+H]^+$ for flumequine and 356 $[M-H]^-$ for florfenicol.

Samples in which florfenicol and flumequine were detected were further analyzed by UHPLC-MS-MS for confirmation, under the same chromatographic conditions as adopted for UHPLC-MS. Chromatography was performed in a Shimadzu Nexera X2 UHPLC LC-30 AD system coupled to a LCMS8030 mass spectrometer with ESI. Detection was carried out by tandem MS in multiple reaction monitoring (MRM) mode using the following parent and product ions m/z values for flumequine (m/z 262 → 202) and florfenicol (m/z 356 → 185). MS operating conditions were set as follows: ESI voltage 4.5 kV, collision energy 30.0 V, nebulizer gas (N_2) flow: 3.0 L min^{-1}, drying gas (N_2) flow: 15 L min^{-1}, desolvation line (DL)

temperature: 250 °C and heat block temperature: 400 °C. Data were acquired, recorded, and analyzed using Shimadzu LabSolution 5.8 software. Quantification was carried out using a calibration curve with serial dilutions of florfenicol (CAS N° 73231-34-2) and flumequine (CAS N° 42835-25-6). Reproducibility was 3–5% (coefficient of variation), routinely determined from three to five replicate analyses. Recovery yield of antibiotics from seawater was estimated by adding 1 mL of a 100 μg L^{-1} antibiotic standard solutions to 1L Milli-Q water (in triplicate) and maintained for 24 h in the dark under continuous shaking. Antibiotics were extracted and analyzed as described in the above methodology, resulting in recoveries of 79% for florfenicol and 66% for flumequine. Detection and quantification limits were calculated using signal-to-noise ratios (S/N) of 3 and 10, respectively. Detection limits for florfenicol were 2 ng L^{-1} in seawater and 1 ng gdw^{-1} in sediments, and for flumequine were 12 ng L^{-1} and 6 ng gdw^{-1} respectively. Limits of quantification for florfenicol were 6 ng L^{-1} in seawater and 3 ng gdw^{-1} in sediments and for flumequine were 36 ng L^{-1} and 18 ng gdw^{-1}.

Regarding QA/QC, all solvents used for chromatography were LC grade, Milli-Q water with a resistivity of 18.2 MΩ cm (25 °C), TOC < 5 ppb and bacterial count <0.01 CFU mL^{-1}. All reagents and chemicals were at least analytical grade (ACS) and all instruments are annually calibrated as recommended by the manufacturer. QC was performed using blank (methanol) and internal reference samples (florfenicol and flumequine standards diluted at calibration middle-level), which were analyzed in triplicate at initial, middle and end of each sample analysis batch. Thus, the equipment performance was constantly evaluated, including carry-over effect, resulting in relative standard errors ≤5% for concentration and ≤1% for retention time.

2.4. Multimedia fugacity model

A fugacity-based model level III (Mackay and Paterson, 1991; Gouin and Harner, 2003; Hughes et al., 2012; Zhang et al., 2015) was developed to predict equilibrium distribution of antibiotics in farmed fish, and in the water column and sediment after one day of medication. The model was tailored to a salmon processing environment, with considerations given to antibiotic partition, dosage, degradation, advective transport, rates of sedimentation and resuspension, and salmon density. Calculations were made for simultaneous treatments with medicated feed pellets in 25 salmon farms, each consisting of 20 cages, as found in Puyuhuapi Fjord (Sernapesca, 2016b). Dosages in fish feed were 10 mg kg^{-1} florfenicol and 30 mg kg^{-1} flumequine, and the biomass of salmonids was between 11 and 17 kg m^{-3} per cage, with 15% mortality rate assumed over a two-year production period (Subpesca, 2016). The model considers both diffusive and advective transport of antibiotics from seawater into sediments, and removal by microbial degradation, modelled as a first order reaction rate. Physical and chemical properties of antibiotics and environmental parameters used in the model for Puyuhuapi Fjord are shown in Tables S2 and S3 (Supplementary material).

Modelled antibiotic concentrations in seawater, sediment, fish and suspended particles (C_i, mol m^{-3}) were calculated on the basis of their fugacity values in each compartment (f_i, Pa). *fi* is interpreted as the escaping tendency of chemicals from one phase to another, and combines with the fugacity capacity (Z_i, mol Pa^{-1} m^{-3}) to give the concentration of each specific compartment-*i* (Mackay, 2001):

$$C_i = f_i \times Z_i \quad (1)$$

Z_i values were calculated for the compartments of water (dissolved), suspended particles, sediment and fish, as shown in the set-up depicted for florfenicol and flumequine (Fig. 2), on the basis of physical-chemical and environmental parameters (Table S4):

2.4.1. Transport and degradation

Transport of antibiotics (F, mol d^{-1}) – by sorption from seawater into sediment and by diffusive uptake from water by salmon – was estimated using $F = D\ (f_1 - f_2)$, where D is a transport rate parameter (mol Pa^{-1} d^{-1}), f_1 the fugacity of antibiotics in water, and f_2 their fugacity in either fish or sediments. Advective transfer processes are water current (G_W, m^3 d^{-1}), and rates of deposition (G_D, m^3 d^{-1}) and resuspension of particles (G_R, m^3 d^{-1}) across the water-sediment interface:

$$F_{transport} = G_i\ Z_i\ f_i = D_{transport}\ f_i \quad (2)$$

where G_i is flow rate (m^3 d^{-1}), Z_i is fugacity capacity (mol Pa^{-1} m^{-3}) to define the flux F from the D value $D_{transport}$ (mol Pa^{-1} d^{-1}) representing currents in water and particulate flux at the water-sediment interface (i.e., deposition and resuspension). Degradation flux ($F_{degradation}$) for each compartment-*i* is represented as:

$$F_{degradation} = k_i\ VC = k_i\ V_i\ Z_i\ f_i = D_{degradation}\ f_i \quad (3)$$

k_i is the reaction rate constant (d^{-1}) for degradation of both antibiotics, and V_i is the volume of each compartment-*i* (m^3). D values were multiplied by fugacity (f_i) for each compartment-*i* to obtain the rate in units of mol d^{-1} (further transformed to kg d^{-1}). In fish, the food uptake D value (D_{food}) was estimated from $G_W Z_{food}$, where Z_{food} was calculated from the lipid content of pellets (20–30%, f_{food}), Z_W, and the octanol-water partition coefficient (K_{OW}). Uptake efficiency (E_{food}) of 95% was assumed in the model.

2.4.2. Fugacity and predicted concentrations

The mass balance models adopted to estimate f in water (f_W), sediment (f_S) and fish (f_F) were defined from previously calculated D values (mol Pa^{-1} d^{-1}) as shown in equations (4)–(6),

$$f_W = \frac{E_{W-5\%} + G_W C_{in} + f_S(D_{resupension}) + f_F(D_{gillfish-water} + D_{excretion})}{(D_{deposition} + D_{advection} + D_{degradation})} \quad (4)$$

$$f_S = \frac{E_{S-5\%} + f_W(D_{deposition})}{(D_{resuspension} + D_{degradation} + D_{burial})} \quad (5)$$

$$f_F = \frac{f_W(D_{food-95\%} + D_{gill\ water-fish})}{(D_{gill\ fish-water} + D_{metabolization} + D_{growth} + D_{excretion})} \quad (6)$$

where E represents the emission rates (mol d^{-1}), assuming uptake efficiency of 95% of the chemical by fish ($D_{food\text{-}95\%}$), and a loss of 5% through medicated feed pellets unconsumed by salmonids, which then became a direct input to water or sediment compartments ($E_{W\text{-}5\%}$ and $E_{S\text{-}5\%}$, respectively). G_W is the advection inflow rate (m^3 d^{-1}), C_{in} is the advection inflow concentration (mol m^{-3}), and D_{gill} the diffusive transport of antibiotics between water and fish. For f_F it was assumed that fugacity of the antibiotic in food (f_{food}) was equal to f_W.

2.4.3. Monte Carlo simulation

A simulation was carried out to assess uncertainties and sensitivity of the model based on probability distributions of the input parameters (Table S3) and their contribution to variability in modelling outcomes. Lognormal and triangular distributions were assumed for input parameters. These analyses determined the 95%

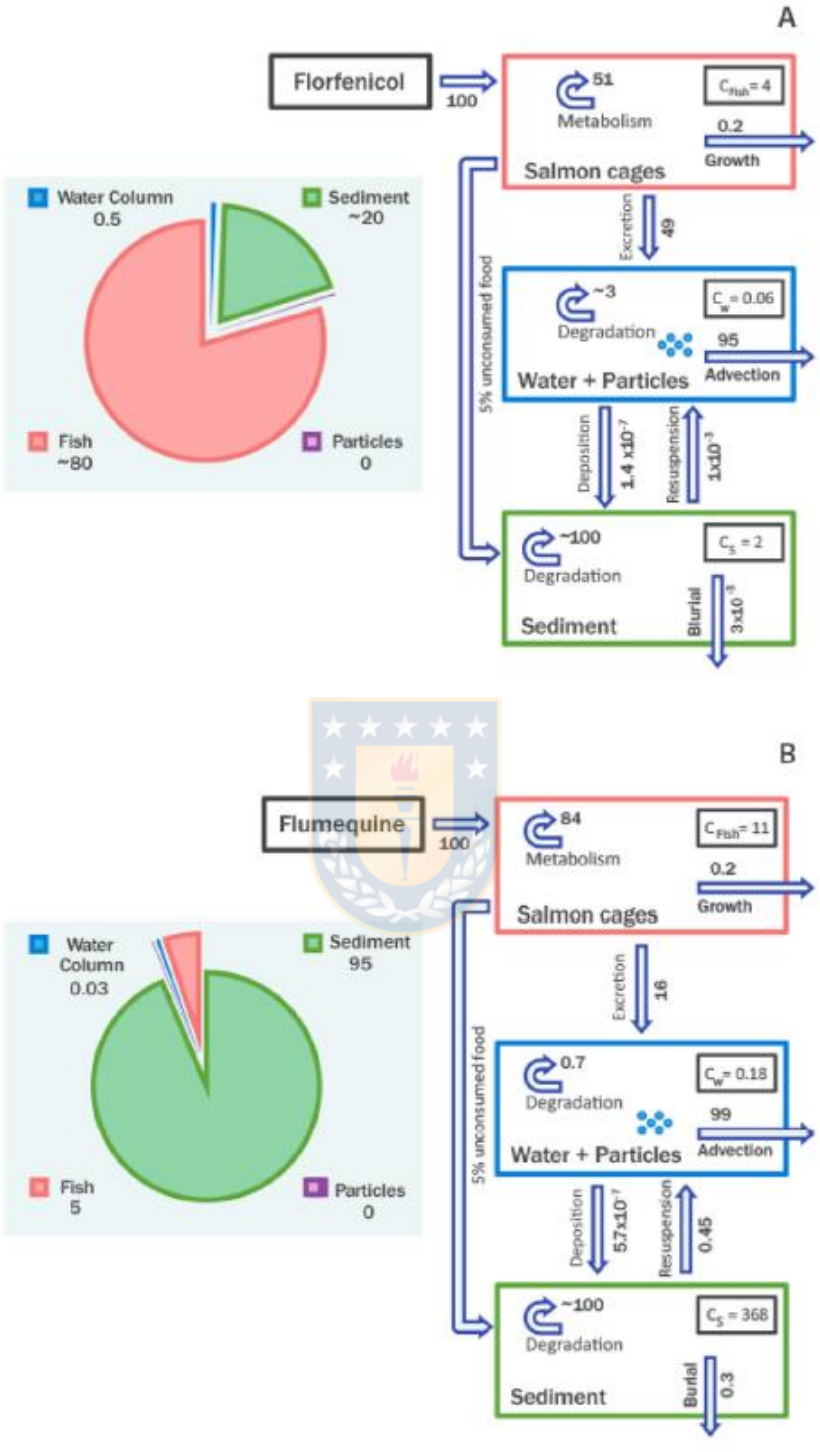

Fig. 2. The output of modelling of fluxes and contents of florfenicol (A) and flumequine (B) after one day of medication of 951.3 kg florfenicol and 2854 kg flumequine in 25 culture centers. Fluxes of antibiotics are given as percentage of added antibiotics (blue arrows). Antibiotic contents in fish (C_{Fish}) and surface sediment (C_S) are presented in ng gdw^{-1}, with concentrations in seawater (C_w) presented in ng L^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

confidence intervals ($CI_{95\%}$) from the probabilistic distribution of model outcomes (Fig. 3 main paper). Simulations were run 100,000 trials using Crystal Ball 11.1.1 software (Gentry et al., 2008).

2.4.4. Simulation test for permanence time of antibiotics

A second experiment modelled ten days of daily additions of florfenicol and fifteen days of flumequine, a common treatment protocol for the industry in southern Chile (Contreras and Miranda, 2011). This simulation was used to evaluate whether antibiotics remain at inhibiting or sub-inhibiting concentrations in seawater and surface sediment beyond the end of the treatment, and if so, for how long they persisted above either of these thresholds.

During consecutive daily treatments, microbial decay of predicted antibiotic contents in both water and sediment was calculated assuming that such decay followed first-order reaction kinetics using published values for half-life (Table S2). Concentrations estimated for seawater and sediment through modelling were used as initial values for temporal decay simulation of antibiotics. During each treatment in cages the degradation rate constant (k, d^{-1}) was computed using equation (7):

$$\ln \frac{C_i}{C_0} = -k\,t \tag{7}$$

where C_0 is the estimated concentration from the multimedia model of compartment-i, and k calculated as $0.693/t_{1/2}$.

3. Results

3.1. Presence of antibiotics in Puyuhuapi Fjord

For analysis of florfenicol and flumequine, we processed 32 samples of seawater, 1 of river water, and 4 of surface sediment. Antibiotics were detected only in suspended seawater particles, and were not detected in the dissolved phase nor in surface sediments. Antibiotics were found in 26% of analyzed samples, in four out of nine sampling sites, and at levels ranging from trace concentrations to 23.1 ng L^{-1} for florfenicol (sites 2, 8, and 9), and at only trace concentrations for flumequine (site 1). Where present, florfenicol was found at all depths (0–100 m) in the water column, with flumequine detected in only one sample at 2-m depth (Table 1).

3.2. Modelled fluxes of florfenicol and flumequine

Modelling output predicted average concentrations of 0.06 ± 0.03 ng L^{-1} of florfenicol and 0.18 ± 0.08 ng L^{-1} of flumequine in the water column after 1 day of medication in 25 salmon farms. Advection was a significant controlling factor determining the short-term fate of antibiotics, by transporting >90% of florfenicol and flumequine from cages into the surrounding water column (Fig. S1). In fish, flumequine was metabolized twice as fast as florfenicol. Unconsumed food pellets from salmon cages were the major predicted source of antibiotics to surface sediments,

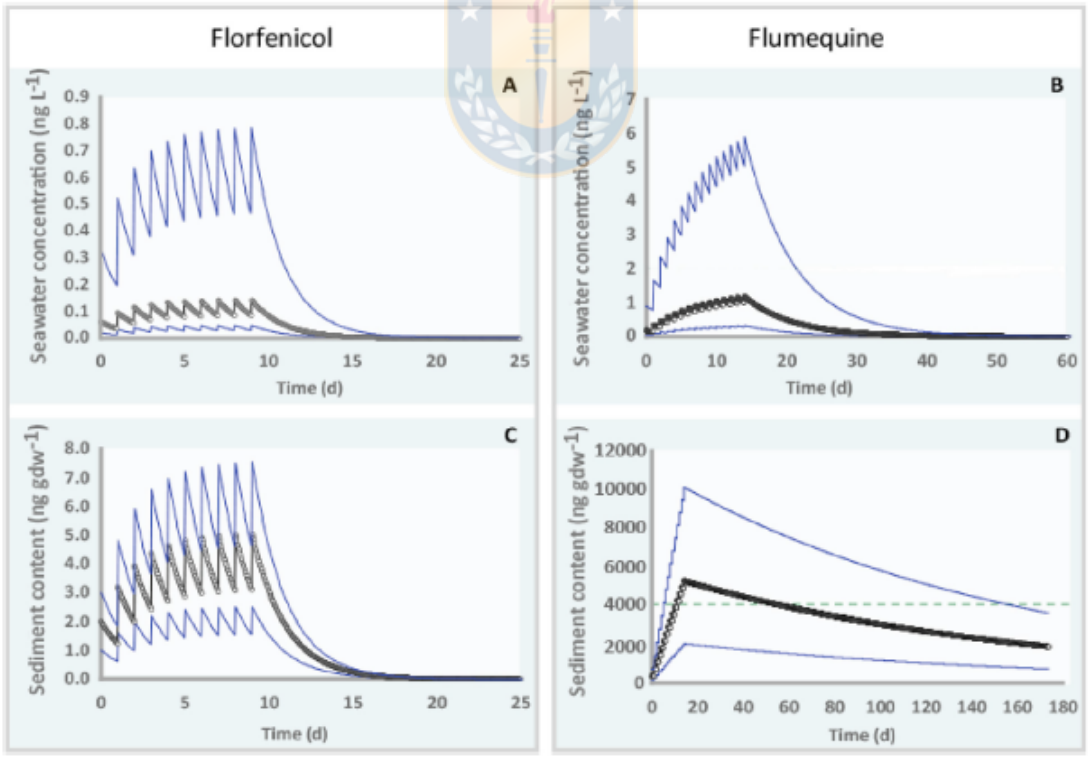

Fig. 3. Simulation of consecutive treatments with florfenicol and flumequine in salmon cages with predicted water column concentrations (A, B) and sediment content (C, D) after addition of antibiotics. Black lines show average values predicted by the scenario of average salmon density of 14 kg m^{-3} within the minimum and maximum permitted salmon density in cages (blue lines, 11 and 17 kg fish m^{-3}). The horizontal green dotted line shows sub-MIC threshold in surface sediment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

resulting in predicted contents of 2 ± 0.01 ng florfenicol gdw^{-1} and 368 ± 82 ng flumequine gdw^{-1} (Fig. 2).

3.3. Timescale of persistence of antibiotics in the fjord

Modelled decay following 10 consecutive days of antibiotic treatment in Puyuhuapi Fjord showed that florfenicol would be predicted to remain below detection limits in the water column (<2 ng L^{-1}), and above detection limit in surface sediments (1 ng gdw^{-1}) for about 12 days. For an average salmon density of 14 kg m^{-3}, maximum values were ~0.1 ng L^{-1} in seawater and ~4 ng gdw^{-1} in surface sediment during the 10-d treatment period (Fig. 3 A, C). Flumequine, administered for 15 consecutive days would be predicted to remain below detection limit (12 ng L^{-1}) in the water column (Fig. 3B). Under scenarios of either average (14 kg m^{-3}) or highest permitted salmon density (17 kg m^{-3}), predicted flumequine concentration peaked at ~ 10,000 ng gdw^{-1} in sediments and remained above the sub-MIC threshold of ~4000 ng gdw^{-1} for up to 150 days (Fig. 3D).

4. Discussion

4.1. Contents of florfenicol and flumequine during sanitary rest

Our observations of detectable florfenicol and flumequine in a relatively small proportion of samples is in general agreement with previous published studies in a range of marine environments under human influence around the world (Table 2). However, much higher concentrations of florfenicol ranging from 30 μg L^{-1} to 11 mg L^{-1} have been reported in water samples collected 500 m from fish farming sites in Dailan Bay, northern China, during active treatment (Zong et al., 2010). Data are more limited for dissolved flumequine in seawater, but in Cadiz Bay (Spain), flumequine was detected in 14% of samples, with the highest concentration being 3.6 ng L^{-1} (Biel-Maeso et al., 2018). Published data are too limited to compare to the trace amounts of flumequine detected by our study in only one water phase sample.

Florfenicol and flumequine were not detected in surface sediments at sites located between 2 and 23 km from the salmon farm perimeters. These data are consistent with previous observations in the Calbuco Archipelago in northern Patagonia (41°48′S) where no florfenicol and only trace amounts of flumequine were detected in surface sediments in areas located between 20 m and 8 km from a salmon farm (Buschmann et al., 2012). Our study is also consistent with data from sites located 500 m from freshwater trout farms in Italian rivers, where flumequine contents in sediments ranged from undetectable to 0.8 ng gdw^{-1} (Lalumera et al., 2004). In the 5 water samples where antibiotics were quantified, concentrations (7.4–23.1 ng L^{-1}, Table 1) were below minimal inhibiting concentrations (sub-MIC) reported for bacteria (e.g. 2 mg kanamycin L^{-1}, Cairns et al., 2018) ca. 180 days after administration of florfenicol, and ca. 365 days after the last treatment of flumequine. Concentrations during the period between the addition of antibiotics and our sampling (six to twelve months later) was considered using modelled decay of florfenicol and flumequine in the fjord (discussed in section 4.3).

4.2. Modelling fluxes of florfenicol and flumequine

Fugacity-based models have been successfully used to describe distributions of non-ionic hydrophobic pollutants (Gouin and Harner, 2003; Huang et al., 2019; Hughes et al., 2012). Given a tendency to strongly adsorb onto particulate organic carbon, the fate of flumequine appears to be adequately described by such a model, whereas the interaction of florfenicol with particles could be underestimated by not considering ionic and hydrogen bonding (Toll, 2001). Nonetheless, the fugacity-based model III has appropriately predicted florfenicol concentrations in Chinese rivers (Zhang et al., 2015).

In the present modelling study, water column and sediment compartments showed similar general distribution patterns for both antibiotics, with advective fluxes accounting for >90% of losses from cages to the water column, and an almost total degradation of deposited antibiotics in surface sediments under the cages (Fig. 2).

Table 2
Concentrations of florfenicol and flumequine in water and sediments measured in the present study compared with several other environments. NA: Not analyzed, ND: Not detected.

Country	Location	Flumequine	Florfenicol	Remarks	References
Water (ng L^{-1})					
China	Yangtze Estuary	NA	0.4–90	Industrialized zone, highly populated. Sampling along the coast	Yan et al. (2013), Zhao et al. (2015)
	Jiulong River Estuary	NA	6–15	Surrounded by pig farms along rivers. Samples collected in the intertidal zone, during low tide	Zheng et al. (2011)
	Beibuwan Gulf	NA	ND- 35	Surrounded by shrimp and fish farms	Li et al. (2016)
	Yellow Sea	NA	ND - 42	Samples were collected 5 km from aquaculture zone	Du et al. (2017)
	Dailan Bay	NA	ND - 11000000	Sampling conducted in the vicinity of aquaculture activities.	Zong et al. (2010), Na et al. (2013), Xie et al. (2018)
Spain	Gulf and Bay of Cadiz	ND - 4	NA	Area influenced by human activities	Biel-Maeso et al. (2018),Martínez-Bueno et al. (2009)
Chile	Puyuhuapi Fjord	ND	ND	Dissolved phase six months after medications.	This study
		ND - Trace	ND - 23	Particulate phase-six month after medications	
Sediments (ng gdw^{-1})					
China	Yang River estuary	ND - 0.3	NA	Samples taken near aquaculture activity	Liu et al. (2018)
	Beibuwan Gulf	NA	ND - 35	Surrounded by shrimp and fish farms	Li et al. (2016)
	Dailan bay	NA	1	Samples taken near aquaculture activity	Na et al. (2013)
Italy	River basins, Italy	ND - 1	NA	Freshwater trout farm	Lalumera et al. (2004)
Chile (Patagonia)	Calbuco Archipelago	Trace	ND	Samples collected near aquaculture farms	Buschmann et al. (2012)
	Puyuhuapi Fjord	ND	ND	Surface sediments- six month after medications	This study

The sensitivity of pollutant concentrations to advective influence computed by fugacity-based models is a well-known feature (Wang et al., 2020), especially for refractory pollutants. For instance, 80% of the decrease in benzo[a]pyrene concentration in Lake Michigan is due to advective fluxes (Huang and Batterman, 2014). However, in rural catchments of the Meijiang River watershed in China, the modelled degradation of tetracycline antibiotics contributed more to their decay than did advective fluxes (Chen et al., 2019). In contrast to this recent watershed model, the level III model that we tailored to Puyuhuapi Fjord showed that advection and water column processes (water depth) were responsible for the greatest losses of antibiotics (Fig. S1). This difference between models could result from high residence times of waters of the catchment in China compared to the waters of Puyuhuapi Fjord in which surface current velocities range from 3 to 10 cm s^{-1} (Schneider et al., 2014). Overall, in both studies, the models predicted almost total degradation of florfenicol and flumequine in the water column prior to settling in sediments (>99%).

Multimedia fugacity models have been used to predict concentrations of various pollutant in rivers, lakes, urban areas, and coastal waters. However, comparisons between modelled and measured concentrations have shown variable degrees of agreement. For PAH (polycyclic aromatic hydrocarbons) in continental water and sediment in the Shanghai area, for PCB (polychlorinated biphenyls) in the Ontario lake, and for the antibiotics oxytetracycline and chlortetracycline in Meijiang river sediments, measured concentrations were 2 to 10-fold higher than predicted values (Xu et al., 2013; Jung et al., 2014; Sun et al., 2018; Chen et al., 2019; Huang et al., 2019). In contrast, in Meijiang river sediments, tetracycline and doxycycline measurements were 3-times lower than predicted values (Chen et al., 2019). In the coastal waters of Boseong Bay, Korea, predicted concentrations of oxytetracycline were close to measured concentrations, but could be underestimated by up to 20-fold, depending upon model configuration (Kim et al., 2017).

In our study, samples were collected six months after the last medication, and in ~80% samples, antibiotics were undetectable, both in sediments and in the water column, thus matching the low values predicted by our model ca. 180 day after administration of florfenicol, and ca. 365 days after treatment by flumequine.

4.3. *Timescale of persistence of antibiotics in the fjord*

Our modelling approach considered microbial degradation of antibiotics excreted, and/or unconsumed, by salmon following daily additions of florfenicol for 10 days, and flumequine for 15 days. This resulted in a period of 104 days during which high levels of antibiotics were predicted in the water column, and surface sediments (Fig. 3). An important question is whether this timescale has relevance to the selection of bacteria resistant to antibiotics? Regarding concentrations of 0.1–0.7 ng L^{-1} florfenicol or 0.2–6 ng L^{-1} flumequine (Fig. 3A and B) predicted for the water column, these concentrations are much lower than the sub-MIC levels (~2 μg mL^{-1}, e.g. Cairns et al., 2018) shown to be associated with horizontal transfer of resistance genes.

In sediments, however, from day 10–50, flumequine contents between 2 and 8 μg gdw^{-1} were predicted, corresponding approximately to 2 μg flumequine per cm^3 sediment (Fig. 3D). This is within the order of magnitude of the sub-MIC concentration of 2 μg mL^{-1} (~4000 ng gdw^{-1} sediment) and could therefore promote selection of antibiotic-resistance bacteria. There is evidence of accumulation of antibiotic resistomes of bacteria in sediments beneath salmon cages that can be transferred from fish feces (e.g., Muziasari et al., 2017), and we hypothesize that the concentrations predicted in sediments by our study have the potential to cause emergence of microbial resistance, even at the end of the sanitary rest period. Although we did not detect antibiotics in sediments at sites located 2 km away from the cages, the potential reservoir of the antibiotic resistance genes area would nevertheless encompass the ~9% of Puyuhuapi Fjord (700 km^2) that is currently occupied by salmon cages.

There is growing evidence that antibiotic resistance genes can be enriched locally in sediments beneath cages (e.g., Ndi and Barton, 2011), and this question merits more focused research attention. There is clearly a potential risk for spread of genes to surrounding environments, as evidenced by the documented presence of resistance-genes for florfenicol, oxalic acid, oxytetracycline and quinolones in the Chiloé archipelago in northern Patagonia (Miranda and Zemelman, 2002; Miranda and Rojas, 2007; Buschmann et al., 2012; Tomova et al., 2015).

5. Conclusions

In a large Patagonian fjord impacted by intensive salmon culture, low levels of antibiotics were detected ca. 180 days after concerted medication with florfenicol, and ca. 360 days after treatment with flumequine at sampling sites located between 2 and 23 km from the nearest farm. The low concentrations measured in suspended particles were consistent with our predictions from a fugacity-based model.

Our modelling approach predicted that elevated contents of flumequine can remain within sediments for up to 2 months before degrading completely. Coastal sites influenced by antibiotic inputs can therefore become potential sites for accumulation of antibiotic resistomes of bacteria, and for the transfer of genetic material among microbial resistant groups. Because of the complex roles that bacteria play in marine ecosystems, any such potential perturbation is an issue of concern that should be addressed with further field data.

Credit author statement

Bibiana Jara, PhD candidate in the Doctorate Program in Oceanography at both University of Concepción and Sorbonne Université. She planned the study in conjunction with her supervisors **Silvio Pantoja-Gutiérrez** and **Laurence Mejanelle**. Set-up and improvement of the analytical method for determination of florfenicol and flumequine was designed by **Benjamín M Srain**. Field sampling strategy was designed by **Bibiana Jara, Camila Fernández** and **Silvio Pantoja-Gutiérrez Field** campaigns were conducted by **Bibiana Jara** and **Benjamín M Srain**, and sampling strategy was validated by **Silvio Pantoja-Gutiérrez** and **Camila Fernández**. Laboratory analyses were conducted by **Bibiana Jara** and **Benjamín M Srain**. QC was validated by **Silvio Pantoja-Gutiérrez** and **Mario Aranda**, and identification of low levels of antibiotics was confirmed by HPLC-MS-MS in **Mario Arandás** laboratory. Design and set-up of the model fugacity-based model was leaded by **Felipe Tucca**, with participation of **Bibiana Jara** and **Laurence Mejanelle**. Theoretical set-up of the model was agreed among **Camila Fernández, Mario Aranda** and **Silvio Pantoja-Gutiérrez**. Analyses of oceanographic data and chemical data was conducted by **Bibiana Jara** and **Benjamín M Srain**. The initial version of the paper was written by **Bibiana Jara, Felipe Tucca** and **Silvio Pantoja-Gutiérrez. Laurence Mejanelle, Camila Fernández, Mario Aranda**, and **Benjamín M Srain** contributed to writing of the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Supplementary Material

Antibiotics florfenicol and flumequine in the water column and sediments of Puyuhuapi Fjord, Chilean Patagonia

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Table S1. Location and characterization of sampling sites in Puyuhuapi Fjord during August 2016

Sampling site / Water depth	Latitude (S)	Longitude (W)	Sampling depths (m)	Temperature (°C)	Salinity (psu)	Oxygen (mL L^{-1})
1 / 280m	44° 31.607'	72° 42.312'	2, 10, 50, 140	10.9 – 9.4	33.8 – 27.1	5.9 – 1.4
2 / 140m	44° 33.854'	72° 42.127'	2, 100, surface sediment	10.7 – 9.2	33.0 – 25.8	6.0 – 3.1
3 / 270m	44° 35.309'	72° 43.587'	2, 10, 70, 140, surface sediment	10.7 – 9.5	33.8 – 30.4	5.5 – 1.4
4 / 190m	44° 35.906'	72° 42.721'	2, 10, 70, 140, surface sediment	10.7 – 9.4	33.8 – 29.6	5.7 – 1.4
5 / 130m	44° 43.341'	72° 45.866'	2, 10, 50, 100, surface sediment	10.8 – 9.3	33.8 – 29.1	5.9 – 1.4
6 / 230m	44° 43.330'	72° 43.896'	2, 10, 50, 160	10.8 – 9.0	33.4 – 25.8	6.1 – 2.2
7 / 0.5m	44°46.483'	72°42.000'	Cisnes River; surface water	No data	No data	No data
8 / 230m	44° 55.132'	73° 07.553'	2, 10, 100	10.8 – 9.1	33.5 – 24.1	6.4 – 2.3
9 / 145m	44° 55.067'	73° 05.318'	2, 10, 50, 150	10.4 – 8.5	33.9 – 17.1	7.2 – 1.6

Table S2. Physical and chemical properties of the antibiotics florfenicol and flumequine used in our modelling.

	Florfenicol	**Flumequine**
CAS	73231-34-2	42835-25-6
Chemical formula	$C_{12}H_{14}Cl_2FNO_4S$	$C_{12}H_{14}FNO_3$
Molecular weight (g mol^{-1})	358.21[a]	261.25[a]
Solubility in water (mg L^{-1})	423 to 5946[a,b]	14 to 2187[a]
Vapor pressure (Pa)	5.60 x10^{-14} to 7.33 x10^{-6}[a]	5.36 x10^{-7} to 2.48 x10^{-6}[a]
Henry's Law Constant (Pa m^3 mol^{-1})	3.37 x10^{-15} to 5.25 x10^{-6}[a]	6.04 x10^{-8} to 2.07 x10^{-4}[a]
Log Kow (L kg^{-1})	-0.12[b] to 0.19[a]	1.38 to 2.70[a]
Log Koc (L kg^{-1})	-0.19 to -0.51[c]	0.99 to 2.99[c]
Degradation half-life in water (d)	1.28[d]	4.77[a,f]
Degradation half-life in sediment (d)	1.06[d] to 1.70[e]	60[e] to 155[g]
Degradation half-life in fish (d)	0.59[a]	0.32[a]

[a]Predicted ranges from USEPA (https://comptox.epa.gov/dashboard). Henry's Law Constant (HLC) ranges were estimated through equation *HLC* = Vapor Pressure (Pa) / Water solubility (mol m^{-3})

[b]Kołodziejska et al. (2013)

[c]*Koc* = 0.41 *Kow* (Karickhoff, 1981). *Koc* is the organic carbon-water partition coefficient and *Kow* is the octanol-water partition coefficient

[d]Sun et al. (2012). Half-life estimated in microcosm experiments

[e]Hektoen et al. (1995). Half-life estimated for the top 1 cm of sediment

[f]Pouliquen et al. (2007). Half-life estimated for hydrolysis and photolysis in seawater. A total half-life ($t_{1/2}$) was calculated, adding $t_{1/2}$ for biodegradation (taken from USEPA[a]).

[g]Hansen et al. (1992)

Table S3. Environmental and salmon culture parameters for Puyuhuapi Fjord.

Parameter	Average (range)	Source
Salmon farms		
Number of salmon farms in Puyuhuapi Fjord	25	National Fisheries and Aquaculture Service of Chile (Sernapesca, www.sernapesca.cl)
Volume of salmon cages (m^3)	13,500	National Fisheries and Aquaculture Service of Chile (Sernapesca, www.sernapesca.cl)
Salmon wet biomass per cage (kg m^{-3})	14 (11–17)	National Fisheries and Aquaculture Service of Chile (Sernapesca, www.sernapesca.cl)
Mortality (%)	15	National Fisheries and Aquaculture Service of Chile (Sernapesca, www.sernapesca.cl)
Number of salmonids per cage	37,800–57,375	National Fisheries and Aquaculture Service of Chile (Sernapesca, www.sernapesca.cl)
Water column and sediment		
Water volume of Puyuhuapi Fjord (km^3)	40	100 km length, 8000 m width, 50 m depth
&Current velocity (cm s^{-1}), Gw	6 (3–10)	Subpesca (2017)
Water column depth (m)	50 (20–80)	Schneider et al. (2014)
Suspended particle concentration (mg L^{-1})	4.7 (2.4–7.0)	Measured
Suspended particle fraction, f_p	3.16 x10^{-6}	Particle concentration/particle density
Sediment volume of the Fjord (km^3)	0.04	Section 0–5 cm
Organic carbon fraction, f_{CO}	0.54 (0.44–0.64)	Measured
Deposition rate (m^3 m^{-2} d^{-1}), G_D	1.02 x10^{-6}	Chen et al. (1999) (~6 mm pellets)
*Resuspension rate (m^3 m^{-2} d^{-1}), G_R	2.64 x10^{-7}	Mackay (2001)
*Burial sediment rate (m^3 m^{-2} d^{-1}), G_B	8.16 x10^{-7}	Mackay (2001)
*Sediment-water mass transfer coefficient (MTC, m d^{-1})	0.24	Mackay (2001)
Salmon		

Harvest wet biomass (kg)	4.0 (3.0–5.0)	National Fisheries and Aquaculture Service of Chile (Sernapesca, www.sernapesca.cl)
Salmon lipid fraction, fl	0.16	Hamilton et al. (2005)
Pellet lipid fraction (%), f_{food}	20–30	Chen et al. (1999)
Dose florfenicol (mg kg^{-1} Fish), $E_{florfenicol}$	10 (for 10 consecutive days)	Agricultural and Livestock Service of Chile (SAG, www.sag.gob.cl)
Metabolization rate constant flumequine (d^{-1}), $m_{etabolization\ -florfenicol}$	1.17	Estimated from half-life in fish
Excretion rate constant florfenicol (d^{-1}), $e_{xcretion-florfenicol}$	1.13	Horsberg et al. (1996)
Dose flumequine (mg kg^{-1}), $E_{flumequine}$	30 (for 15 consecutive days)	Agricultural and Livestock Service of Chile (SAG, www.sag.gob.cl)
Metabolization rate constant flumequine (d^{-1}), $m_{etabolization-flumequine}$	2.17	Estimated from half-life in fish
Excretion rate constant flumequine (d^{-1}), $e_{xcretion-flumequine}$	0.42	Elema et al. (1995)
Growth rate constant (d^{-1}), g_{rowth}	0.004	Folkestad et al. (2008)

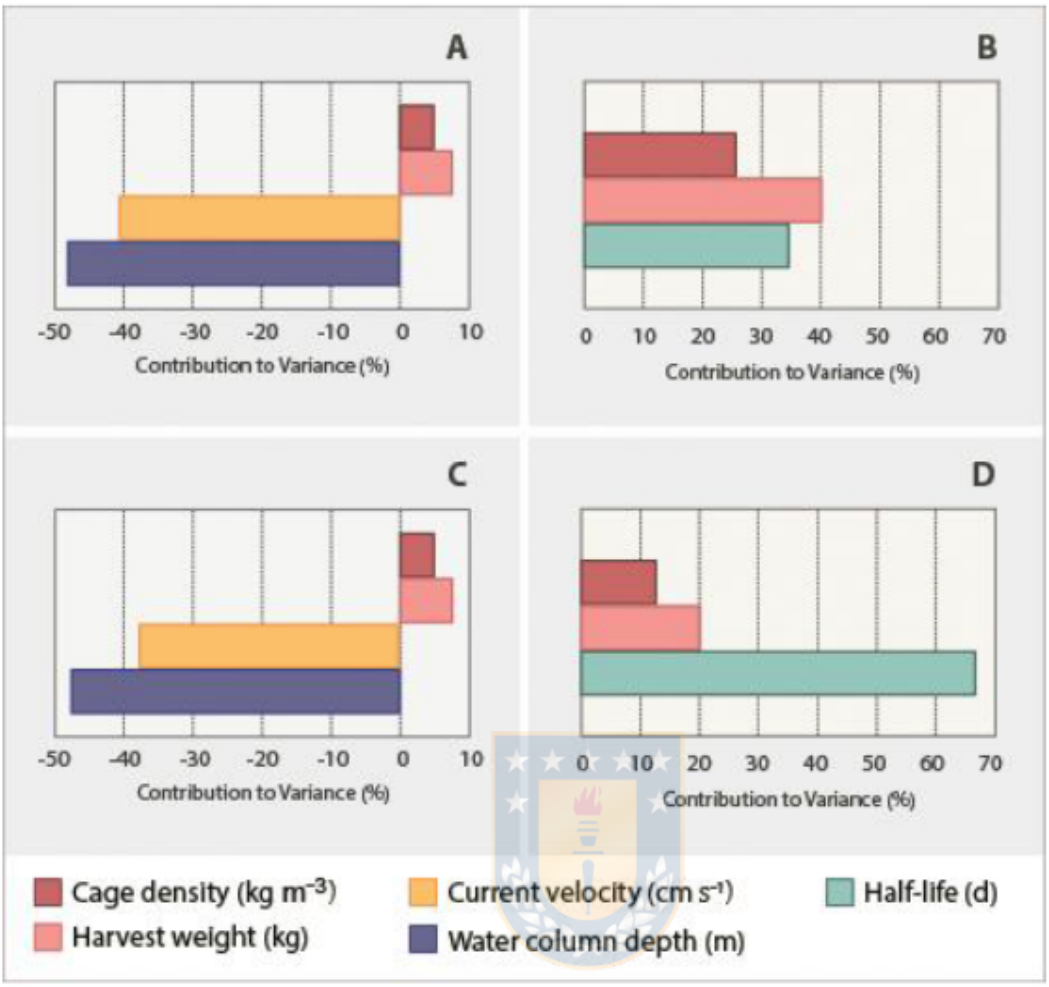
[&] average current velocity in the upper 15 m.

[*] When data were not available, we used values proposed in Mackay (2001)

Table S4. Model description (Z)

Compartment	Z value	Abbreviation
Water	$Z_w = 1/H$ or S/P	H = Henry's Law constant (Pa m^3 mol^{-1}) S = water solubility (mol m^{-3}) P = vapor pressure (Pa)
Sediment	$Z_s = Z_w * f_{co} * K_{OC} * (p^s/1000)$	f_{co} = organic carbon fraction K_{OC} = organic carbon-water partition coefficient p^s = density of sediment (1500 kg m^{-3})
Fish	$Z_f = Z_w * f_L * K_{OW} * (p^f/1000)$	f_L = volume fraction of lipid content in fish K_{OW} = octanol-water partition coefficient p^f = density of fish biomass in cages (1000 kg m^{-3})
Suspended particles	$Z_p = Z_w * f_p * K_{OC} * (p^p/1000)$	f_p = volume fraction particles in water p^s = density of suspended particles (1500 kg m^{-3})

Figure S1. Model sensitivity analysis for florfenicol (A, B) and flumequine (C, D) in seawater (left panels) and sediment (right panels) according to their physico-chemical properties and environmental variables: Log K_{OW}= octanol-water partition coefficient, HLC = Henry´s Law constant (Pa m^3 mol^{-1}), Log K_{OC}= carbon-water partition coefficient, S_W= solubility in water (mg L^{-1}), P_V= vapor pressure (Pa), P_S= half-life in sediment (d), Depth= water column depth (m), V_C= current velocity (cm s^{-1}), Weight= salmon weight per individual (kg), Density of salmon weight in cages (kg m^{-3}), SS= suspended solids (mg L^{-1}), f_{CO}= fraction of organic carbon in sediment .



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3.2 Chapter II: Batch experiment study of water-sediment partition of flumequine and florfenicol, two antibiotics used in salmon aquaculture in Chile

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Abstract

The water-sediment partitioning of flumequine and florfenicol, two antibiotics used in salmon aquaculture in Chile, was studied by batch experiments, conducted using either pure water or seawater, with or without sediment, and at two temperatures. For florfenicol in seawater, Log K_d (partition between water and sediment) varied from 0.71 ± 0.91 to 0.69 ± 0.69, and Log K_{OC} (partition between water and organic fraction of sediment) from 2.23 ± 2.44 to 2.21 ± 0.21. Higher values of Log K_d (0.85 ± 0.08 to 1.38 ± 0.66) and Log K_{OC} (from 1.50 ± 1.25 to 2.60 ± 2.85) characterized the greater affinity of flumequine to particles. Difference between K_{OC} and the octanol-water partition constant (K_{OW}) showed that for florfenicol, adsorption onto the surface of particles was a more significant process than the absorption driven by hydrophobicity. In contrast, for flumequine, hydrophobic absorption was a major driver of sorption to sediments

Keywords:

Emergent pollutant, antibiotic sorption, florfenicol, flumequine, K_d and K_{oc}.

Water-sediment partitioning of flumequine and florfenicol, two antibiotics used in salmon aquaculture in Chile

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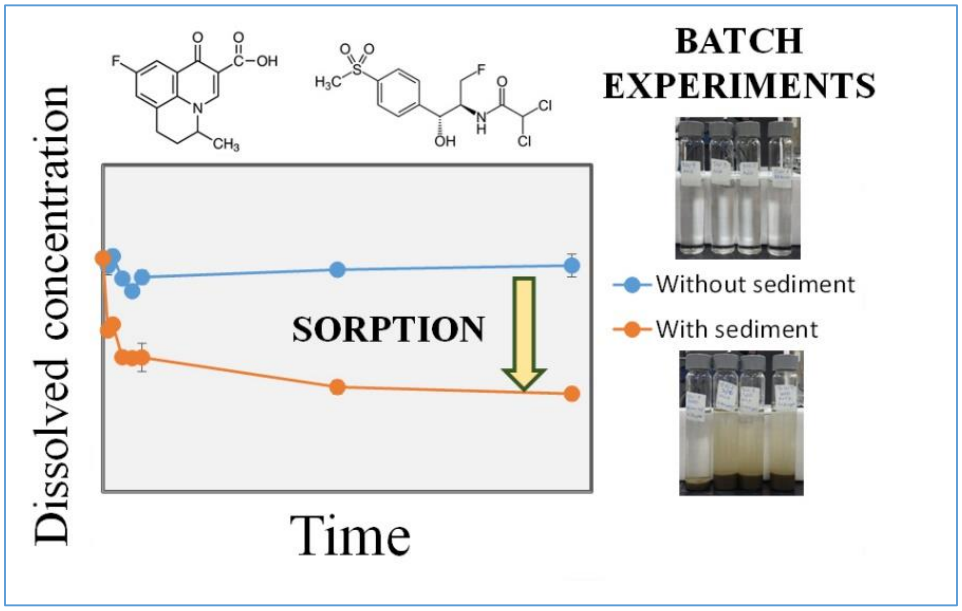
Highlights

- In seawater, 11 ± 5% of florfenicol and 24 ± 7% of flumequine are sorbed to particles.
- Flumequine has a greater affinity to particles than florfenicol.
- Log K_{OC} values of florfenicol are much lower than Log K_{OW} suggesting that surface-driven processes control the sorption of this compound.
- Log K_{OC} values of flumequine are close to Log K_{OW}.
- The formation of complexes with seawater ions lowers florfenicol concentration by 13 ± 8% with respect to Milli-Q water.
- Flumequine may form complexes with ions leached out from sediment.

Graphical abstract



Introduction

Chile is the second most important salmon producer in the world, after Norway (FAO, 2018), and is a major consumer of antibiotics per biomass of salmon produced (antibiotic consumption index of 0.05%, or wight antibiotics/harvested salmon, Miranda *et al*., 2018). Our understanding of the impact of these emerging pollutants used by aquaculture in coastal waters is limited to a few studies addressing their presence (Buschmann *et al*., 2012; Jara *et al*., 2021) and the development of bacterial resistance genes (Miranda and Zemelman, 2002; Miranda and Rojas 2007; Cabello *et al*., 2016). Two of the major antibiotics used are florfenicol and flumequine whose treatments are administrated to salmon through incorporation into food pellets. However, approximately 5% of unconsumed pellets can be deposited from aquaculture activities into sediments (Cabello *et al*. 2013; Miranda *et al*. 2018).

The impact of antibiotics depends on their mode of release into the environment, and on subsequent partition between the dissolved phase (truly dissolved, complexed, absorbed to dissolved organic carbon) and particles (suspended, sinking and sedimentary). Surface processes (adsorption) such as ion exchange, cation and hydrogen binding all govern reverse exchanges between water and particles for those antibiotics with ionic charges, whilst diffusive exchanges such as absorption to the organic phase drive the sorption of neutral antibiotics (Cao *et al*. 2015). The dissolved-particle

partition constant (K_d) and the octanol-water partition constant (K_{OW}) provide information on the tendency of chemicals to be affected by the aforementioned processes, but these constants are not presently available for all antibiotics. For instance, for flumequine and florfenicol, K_d values are only available for soils and not for marine sediments. For florfenicol, Log K_{OW} values reported in the literature vary over a range from -0.17 to 1.63. Predictions of the fate of these antibiotics in marine ecosystems housing salmon aquaculture activities are hindered by this lack of fundamental information.

In soils, the dependence of the sorbed fraction of antibiotics to their water concentration is described either by linear relationships (K_d, $C_s = K_f C_w$) or by Freundlich isotherms (K_f, $C_s = K_f C_w$ 1/n). In the concentration range up to 10 mg/ L, the Freundlich isotherm fitted the sorption of norfloxacin to marine sediments, whilst sorption was also well described by the linear relationship with R^2 values of between 0.94 and 0.98 (Cao *et al*., 2015). As the non-linear empirical variation applies to a higher concentration range, the sorption of florfenicol and flumequine in the sub mg/L range is assumed here to be linear, and described by K_d.

In the present study, batch experiments mimicking sorption-desorption between water and sediments were carried out at the sub-ppm level, over a range of conditions (temperature and salinity) typical of Chilean fjords (e.g. Schneider *et al*., 2014). The objectives were to provide experimental data on partition constants of florfenicol and flumequine between water and sediment particles (K_d), and between water and the organic matter associated with particles (K_{OC}).

Results

All experiments were characterized by a steep decrease of dissolved antibiotic concentration during the initial 3 hours of the experiment (Figure 1), followed by little variation between 4 and 48 hours. However, the concentration of florfenicol increased at the final sampling time under all conditions. We assumed that partition equilibrium was reached at 4 h, and therefore replicates at 4 h, 24 h, and 48h were used to calculate partition coefficients.

The Kruskal Wallis statistical tests performed on these equilibrium concentrations showed that temperature, matrix, and antibiotic type contributed to the observed variances, producing significative differences between the concentration in each treatment, for each factor (Kruskal Wallis; $p < 0.05$).

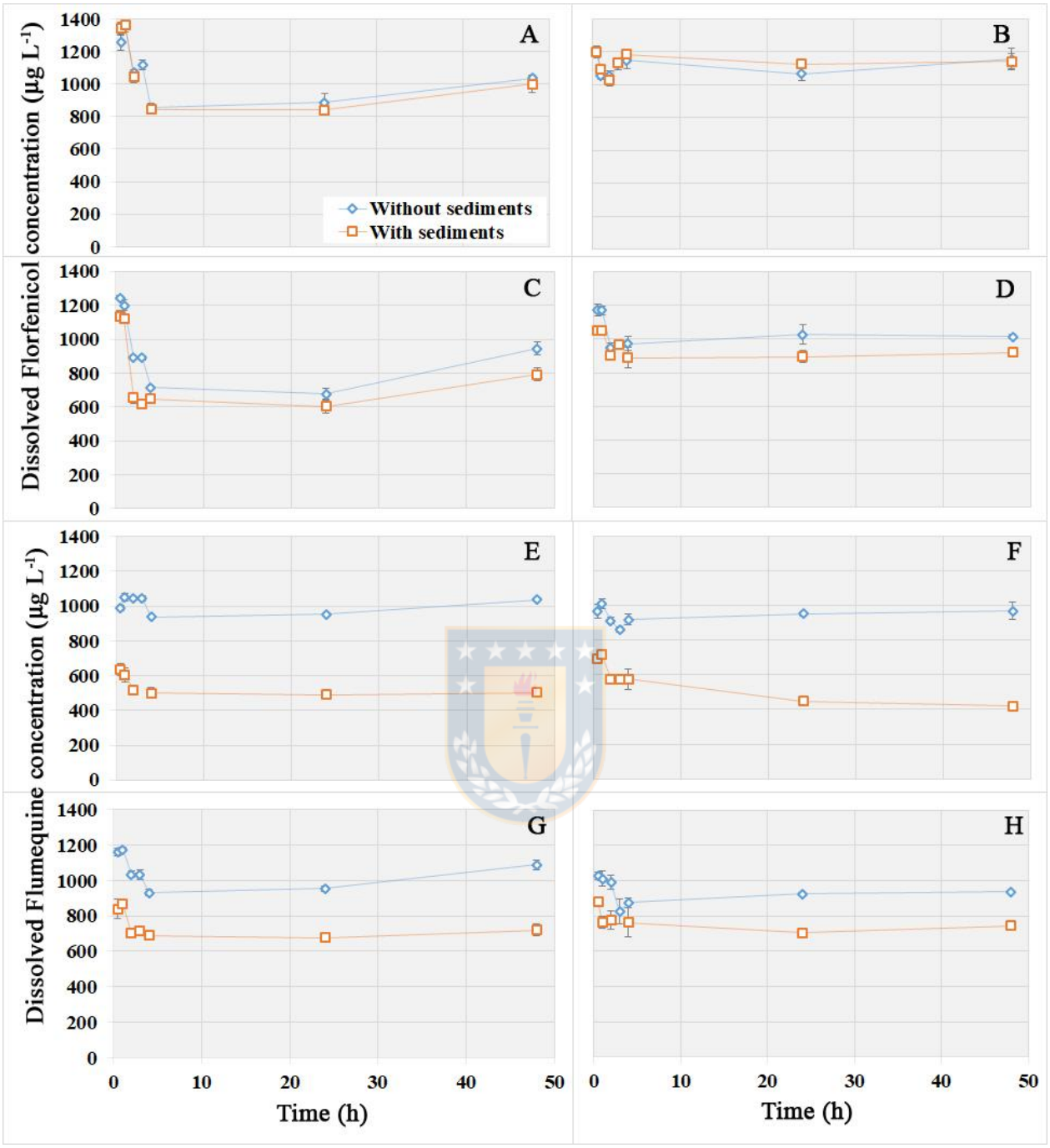


Figure 1. Florfenicol and flumequine dissolved concentrations (average ± SD) in batch experiments with (orange square) and without (blue diamond) added marine sediments: A) and E) pure water at 8°C; B) and F) pure water at 15°C; C) and G) Seawater at 8°C and D) and H) Seawater 15°C.

Florfenicol did not deccrease in concentration in pure water + sed compared to pure water alone (Figure 1) thus partition constants were not calculated for these conditions. In contrast, florfenicol showed significant sorption to the sediments in seawater treatments. In addition, whether sediment was present or not, florfenicol concentration decreased significantly by 20 to 40% at 8°C and by 4 to 15% at 15°C (Table 1).

For all experimental treatments, flumequine concentrations were significantly lower in the presence of sediment (Table 1). Flumequine showed greater decreases in concentration than florfenicol, suggesting a higher sorption tendency. After stable conditions were reached, 49 ± 10% of flumequine was sorbed to sediments in pure water, and 19 ± 6% in saline water, whilst the percentage of adsorbed florfenicol in saline water was less than 10 ± 2% (Figure 1). The influence of temperature on sorption was complex, with non-significant effects on dissolved florfenicol and flumequine in pure water without sediment. In contrast, flumequine concentrations decreased significantly at 15°C compared to 8°C in seawater treatments (Table 1). For florfenicol, the initial drop in concentration appeared to be more pronounced at 8°C and similar both with and without sediment. These observations are suggestive of enhanced adsorption onto vial walls at 8°C compared to 15°C. The proportion of flumequine adsorbed onto sediments was lower at the summer temperature, and in seawater compared to pure water (Table 1).

The sorption coefficient K_d describes the reversible tendency of compounds to adsorb onto particles. In seawater, Log K_d for florfenicol varied between 0.71 ± 0.91 and 0.69 ± 0.69 (Table 2), while previous K_d from freshwater environments are reported from -1.15 to 0.37 (Endris, 2004; 2013) and 2.9 for seawater (Na *et al*., 2013). K_{OC} is the organic carbon-normalized sorption coefficient and indicates the tendency of the compounds to sorb on the organic fraction of the particles. The range of Log K_{OC} measured for florfenicol in this study was 2.21 ± 0.21 to 2.23 ± 2.44.

Table 1. Wilcoxon test results of florfenicol (FLO) and flumequine (FLU), significant "p level" p < 0.05. PW: Pure water; SW: Seawater; SED: Sediments; *: significant differences.

Z values "p level"		Temperature 8° C								Temperature 15°C							
		FLU PW	FLU SW	FLU PW+ SED	FLU SW+ SED	FLO PW	FLO SW	FLO PW- SED	FLO SW- SED	FLU PW	FLU SW	FLU PW+ SED	FLU SW+ SED	FLO PW	FLO SW	FLO PW+ SED	FLO SW+ SED
Temperature 8° C	FLU PW																
	FLU SW	1.24 0.21															
	FLU PW+ SED	2.67 * 0.008	2.67 * 0.008														
	FLU SW+ SED	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008													
	FLO PW	2.19 * 0.03	2.67 * 0.008	2.55 * 0.011	2.67 * 0.008												
	FLO SW	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	1.84 0.07	2.67 * 0.008											
	FLO PW+ SED	2.55 * 0.011	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	1.33 0.18	2.55 * 0.011										
	FLO SW+SED	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	0.77 0.44	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008									
Temperature 15° C	FLU PW	1.33 0.18	0.67 0.051	2.67 * 0.008	2.67 * 0.008												
	FLU SW	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008					2.00 * 0.045							
	FLU PW+ SED	2.67 * 0.008	2.67 * 0.008	0.66 0.051	2.67 * 0.008					2.67 * 0.008	2.67 * 0.008						
	FLU SW+ SED	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008					2.67 * 0.008	2.67 * 0.008	2.67 * 0.008					
	FLO PW	2.67 * 0.008	2.55 * 0.011	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	0.66 0.051	2.67 * 0.008				
	FLO SW	1.01 0.314	0.30 0.77	2.67 * 0.008	2.67 * 0.008	1.95 0.05	2.67 * 0.008	2.43 * 0.02	2.67 * 0.008	1.33 0.18	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.00 * 0.045			
	FLO PW+ SED	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 00.08	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	0.67 0.051	2.67 * 0.008		
	FLO SW+SED	2.55 * 0.011	2.43 * 0.02	2.67 * 0.008	2.67 * 0.008	0.77 0.44	2.00 * 0.045	2.67 * 0.08	2.67 * 0.08	2.19 * 0.03	1.13 0.26	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	2.67 * 0.008	

Log K_d calculated for flumequine varied between 0.85 ± 0.08 and 1.38 ± 0.66 with lower values observed in seawater (Table 2), while K_d values of flumequine were only available for the humic fraction of soils in the literature, and correspond to Log K_d 3.4 to 4.0 (Tolls, 2001). Log K_{OC} values of flumequine calculated for pure water and seawater ranged between 1.50 ± 1.25 and 2.60 ± 2.85, while Log K_{OC} values representing flumequine sorption to humic acids in soils were higher, from 3.4 to 4.4 (Tolls 2001).

Table 2. Empiric values of the particle partition coefficient (K_d, L of water/ kg of dry sediment) and organic carbon (K_{OC}, L of water/ kg of organic carbon) for florfenicol and flumequine, in the different temperature and ionic conditions of batch experiments.

	Temperature	**Florfenicol**		**Flumequine**	
		8° C	15° C	8° C	15° C
Log K_d	Pure Water	-	-	0.85 ± 0.08	1.38 ± 0.66
	Seawater	0.71 ± 0.91	0.69 ± 0.69	1.08 ± 0.53	0.94 ± 0.55
Log K_{OC}	Pure Water	-	-	2.33 ± 1.44	1.66 ± 1.33
	Seawater	2.23 ± 2.44	2.21 ± 0.21	2.60 ± 2.85	1.50± 1.25

Discussion

The half-life of florfenicol and flumequine is one week and ~ 150 days, respectively (Hektoen *et al*., 1995; Halling- Sorenson *et al*., 1998), suggesting that the initial decreases in concentration observed in the present experiments were not related to degradation. The initial concentration decreases cannot be attributed to complexation because they occur in all conditions, even in pure water devoid of ions, and instead likely reflect adsorption of a proportion of the antibiotics to the walls of experimental vessels. Equilibrium times in our experiments (*ca* 4 h) are in good agreement with previous observations (>2 h, Guaita *et al*., 2011; within 6 h, Cao *et al*., 2015. The partition constants K_d and K_{OC} point to a sorption capacity of flumequine that is double that of florfenicol, thus implying potential storage of a greater fraction of flumequine in sediments in the vicinity of salmon farming activities. Sorption could be related to cation availability and complexation, ion strength changes, pH, dissolved organic matter (MacKay and Seremet, 2008; Guaita *et al*., 2011; Jia *et al*., 2013; Na *et al*., 2013). Various studies addressing the sorption of veterinary antibiotics to soils have shown that surface processes play a more important role than organic carbon absorption driven by hydrophobicity (Tolls 2001). However, some data from the marine environment contrast with this conclusion. In the Bohai and Yellow Seas, for example, the

formation of cation bridges between oxolinic acid and calcium and magnesium explained the adsorption of oxolinic acid to intertidal sediments, but failed to explain the adsorption of other quinolones (Lang *et al*., 2018) which were also not explained by sediment types, pH, or organic carbon content (Lang *et al*., 2018). When sorption of a compound is driven by hydrophobic diffusive exchange (*id est* absorption), environmental Log K_{OC} approaches the theoretical partition constant Log Kow. Florfenicol Log K_{OC} calculated from the present study was 2 to 3 units higher than other published values for Log K_{OC} (0.37 in Endris, 2004; 2013). Either hydrophobic absorption contributes to only a low percentage of the florfenicol associated with particles, while the major florfenicol-particle interaction is due to surface-driven processes, or published values of Log K_{OW} of florfenicol are underestimated.

Log K_{OC} of flumequine estimated in our batch experiments are also higher than published values, although by only more or less one unit on the Log scale. Our data contribute to a better definition of partition constants of antibiotics in the marine environment, but further research is required to better clarify estimates and to understand the processes underlying their variability. Comparing the partition in pure water with that in seawater provides evidence for the formation of complexes with marine major ions for florfenicol (significant Wilcoxon tests), however this was not observed for flumequine (non-significant Wilcoxon tests). The pKa for florfenicol's is 6.3, and therefore the basic ionized form dominates both in pure water (pH=7.2) and in seawater (pH=8.2, pH=8.7 in sediment slurries). The decrease in concentration of this basic, negatively charged form of florfenicol in seawater is likely due to the formation of complexes with major marine cations. Variable difference between pure and seawater concentration suggests that 16 ± 8% and 10 ± 8% of the florfenicol was complexed in winter and summer, respectively. Sulfamethazine also forms complexes which increase its affinity to particles (Wegst-Uhrich *et al*., 2014). Similarly, in the present batch experiments, florfenicol in seawater appears to bind to particles and form complexes. The pKa of flumequine is 9.3, and its acid neutral form dominates under all experimental conditions. The non-significant difference between flumequine concentrations in pure- and sea-water conditions argues against the complexation of flumequine. However, the complexation of flumequine with Cu (II) has been reported during experiments in soils (Guaita *et al*., 2011). Marine sediments may leach cations that are not present in seawater such as Cu^{2+}, and the potential complexation of flumequine cannot be ruled out in the presence of sediment. Further research is

also needed to explain why flumequine sorption to sediment is lower in seawater (19 ± 6%) than in pure water (49 ± 10%) (Figure 2).

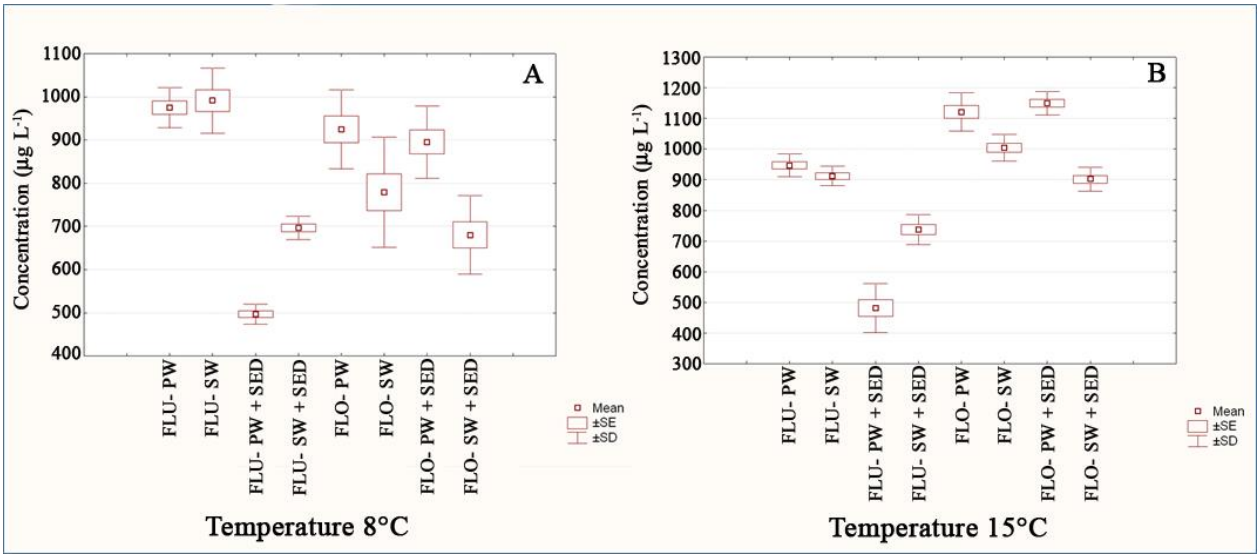


Figure 2. Box plot showing the means, standard errors, and standard deviations for dissolved concentration of flumequine and florfenicol in each treatment. A) 8°C (winter conditions) and B) 15°C (summer). FLU: Flumequine, FLO: Florfenicol, PW: pure water (pure water), SW: Seawater, and SED: with wet sediment added to the tube.

The role of temperature on antibiotic behavior was explored for the first time to our knowledge. Dissolved concentrations of florfenicol were significantly different at 8°C and 15°C in all treatments (Table 1). Flumequine dissolved concentrations showed no significant differences at both temperatures in pure water treatments, but were significantly different in marine conditions, either with or without sediments (Table 1). Moreover, the proportion of sorbed flumequine increased from 19 ± 6% under summer temperatures to 30 ± 6% under temperature conditions typical of Chilean fjords in winter. The underlying processes driving these novel findings are presently unknown, but there are clear seasonal implications for the retention of flumequine in the vicinity of aquaculture activities.

Conclusions

Analytical difficulties limit our understanding of the environmental impact of emerging pollutants, and specifically those antibiotics used by salmon farming in Chilean fjords (Jara *et al.*, 2021). Further in situ studies are clearly required, while batch experiments offer an alternative to

empirically describe the sorption behavior of these antibiotics in the coastal environment. The present study has shown flumequine to have a higher sorption tendency than florfenicol, and the fate of flumequine will therefore be more associated with processes such as particle transport and deposition onto the seafloor. In contrast, florfenicol shows a lower tendency to bind to particles, and the fate of this antibiotic will be to a greater extent related to hydrodynamic processes such as dispersion and water mass transport by currents. The discrepancy between K_{OC} and K_{OW} indicates that reversive absorption to organic carbon is not the dominant process driving sorption of florfenicol to sediment particles; other surface-driven processes (ion exchange, cation and hydrogen binding, and complex formation) are likely to drive partition processes (Tolls 2001). The present study provides experimental partition constants for flumequine showing that diffusive absorption to organic carbon is an important driver of the association of this compound with sediments.

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3.3 Chapter III: Pesticide fate and occurrence in non-target organisms

3.3.1 Fate of pyrethroids in freshwater and marine environments

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Abstract

As a consequence of their increasing use, pyrethroid insecticides are recognized as a threat for nontarget species and ecosystem health. The present chapter gives a state-of-art overview of individual pyrethroid occurrence in waters and sediments worldwide, together with recent reports of their quantification in the atmospheric gas and aerosol phases. Degradation rates, transport processes, and partitioning of pyrethroids between environmental phases are reviewed. River flow efficiently transports pyrethroids to river mouths and estuaries, while pyrethroid impact on the marine environment remains difficult to appraise due to lack of comprehensive studies. Nevertheless, aquaculture arises as an important but poorly understood environmental burden. Owing to their large organic carbon pool, sediments may act as a sink for pyrethroids and impair nontarget aquatic species. Partitioning potential of pyrethroids is compared to that of other well-known legacy pollutants in the light of their position in the phase space defined by key physicochemical properties (K_{OW} and H'). The transport and partition of pyrethroids away from their source are strongly dependent on their half-life, but their quasi constant emissions in urban and agricultural area may compensate for their degradation, therefore sustaining the occurrence and behavior of some individual pyrethroids as "quasi persistent organic pollutants."

1.0 Introduction

In the last 20 years synthetic pyrethroids have gradually replaced other pesticides. These pyrethroids has been widely used in agriculture and aquaculture (Aznar-Alemany and Eljarrat, 2020a) and also extensively used in urban and industrial areas and livestock farms to control pests such as mosquitoes, lice, and wood-destroying dwellers. The major advantage of pyrethroids are low cost, low mammalian toxicity, and shorter persistence in the environment than other classes of pesticides (Wolfram et al., 2018).

Pyrethroids treatments has been applied against insects and crustaceans. The distribution of bifenthrin and three other pyrethroids was only a few percents in the freely-dissolved portion of several samples while the major fraction was associated to DOM and solid phases (Bondarenko *et al.*, 2006). Once released into the environment pyrethroids tend to sorb on organic particles and sediments (log K_{OW} from 4.8 to 7.0). When these compound are sorbed on particles, the carrier particles may be consumed by filter feeders and transfer pyrethroids to higher trophic levels, or alternatively, particles may consist in a reservoir for these pollutants, probably reducing their biodegradability in natural waters. As a result of biomagnification at high trophic levels, negative impact of pyrethroids has been suggested to cause immunity and estrogenic disruption to mammalians (Aznar-Alemany and Eljarrat, 2020b).

Distribution and fate of pyrethroids depend of their properties such as air-water or water-sediment partition behavior, degradation processes (biological, hydrolytic and photolythic), transport processes (diffusive and advective), organic content and transference to sediments, fluxes and biota interactions (Ernst *et al.*, 2014). Bioavailability of pesticides have a direct relationship first with diffusive processes such as water-particle partitioning, like air-water exchange, water-sediment partition, gas-aerosol partition, while advective transport consists in the movement or flux of the phase itself, transporting the pesticides which it contains (Tucca *et al.*, 2017; Urbina *et al.*, 2019).

The major impacts of pyrethroids are the effects on non-target organism (Mazzola and Sarà, 2001; Mugni *et al.*, 2013; Norambuena-Subibabre *et al.*, 2016; Gebauer *et al.*, 2017; Parsons *et al.*, 2020), which can have severe consequence at the ecological level (Friberg-Jensen et al., 2003; Van Geest et al., 2014a). The high persistence of pyrethroids (Hamaotene *et al.*, 2018; Hamoutene and Salvo, 2020) can increase the exposure of non-target organisms increasing the likelihood of being bioconcentrated and bioaccumulated and eventually biomagnified (Mazzola and Sarà, 2001; Xue

et al., 2005; Alonso *et al.*, 2012; Van Geest *et al.*, 2014a, b) and eventually be transferred to humans through food consumption. (e.g., Burridge *et al.*, 2010).

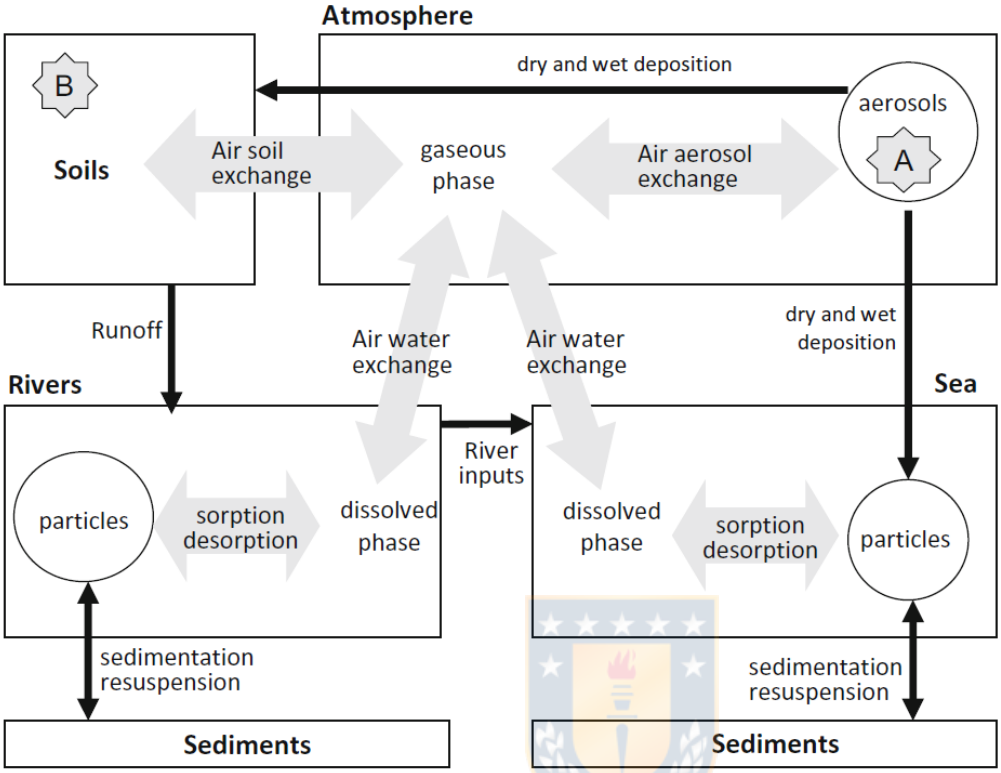

Figure 1. Scheme of the geochemical cycle of pyrethroids in the environment. Boxes represent the environmental phases. The soil box represents both the solid phase of soils (plants and soil particles) and the soil porous water. Arrows represent the fluxes between phases, thin black arrows stand for fluxes of key transport (advective) processes and large gray arrow show key partition (diffusive) fluxes. Gray stars symbolize pyrethroid direct emissions to the environment; A is the emission that remains as aerosol during spray application, mostly to cropland; B is the emission that is deposited on soils and plant during spray application.

3.3.2 Occurrence of pyrethroids in marine benthic filter-feeders in the Puyuhuapi fjord (44°57'S; 73°21'W), Chilean Patagonia

Abstract

Pesticides deltamethrin and cypermethrin, introduced in Chile in 2007 and 2009, respectively, have been used to control the outbreaks of ectoparasitic infection in marine salmon farming. Once these pyrethroids are released to the water column, and due to the high affinity of organic particles and lipid content, they are deposited into the marine sediment and bioconcentrated in benthic organisms. Sponges and bivalves have been described as good bioindicators due to with high filter capacity and bioconcentrate organic and inorganic pollutants. This study aimed to evaluate the occurrence of deltamethrin and cypermethrin, in suspended particles and benthic filter-feeding organisms collected in the Puyuhuapi fjord, an area with active aquaculture. Deltamethrin was applied in Puyuhuapi fjord in January and April 2016, while cypermethrin was never used. Deltamethrin was detected in suspended particles at very low concentrations with values of 0.01 and 0.05 ng L^{-1} (stations 1 and 7), suggesting possible resuspension from surface sediments. Cypermethrin concentrations were detected in most analyzed benthic filter-feeding organisms with maximum values of 1.76 ng lipid dw^{-1} for sponges and 1.04 ng g lipid dw^{-1} for bivalves. Our cypermethrin concentration values (0.04 to 0.05 ng g^{-1}, average all stations) were comparable to those reported in wild salmon (0.04 ng g^{-1}), which suggests a possible indirect exposure to cypermehrin that should be investigated.

1.0 Introduction

Pesticides are mainly used to control the outbreaks of ectoparasitic infection in freshwater and marine salmon farming (*e.g.* Bravo, 2003; Johnson *et al.*, 2004; Burridge *et al.*, 2010; Lhorente *et al.*, 2014; Dresdner *et al.*, 2019). Regardless of pesticide treatment, food (*e.g.*, emamectin benzoate), or baths (pyrethroids), these compounds and their secondary metabolites can be deposited in the marine sediments and, eventually, bioconcentrated in organisms (*e.g.*, Xue *et al.*, 2005; Alonso *et al.*, 2012; Van Geest *et al.*, 2014a, b). Deltamethrin and cypermethrin (second-generation of pyrethroids) represent more than 25% of the world's market pesticides (Cycoń *et al.*, 2016), which have been introduced in Chile for the salmon industry since 2007 and 2009, respectively (Bravo *et al.*, 2008, 2010). After bath treatments, these compounds are released into the environment where, according to their physicochemical properties (Table 1), they can be absorbed by organic particles (Méjanelle *et al.*, 2020). Once these compounds are absorbed, they undergo horizontal transport, degradation processes and are deposited in the marine sediments (Erst *et al.*, 2014; Méjanelle *et al.*, 2020). However, their high affinity with organic particles can result in major protection of biological degradation and, as a consequence, an increase in bioavailability for benthic filter-feeding organisms (Tucca *et al.*, 2017; Urbina *et al.*, 2019; Méjanelle *et al.*, 2020). Several studies have reported noxious effects of pyrethroids in non-target organisms during the dissolved phase in the water column (Mugni *et al.*, 2013; Gebauer *et al.*2017; Parsons *et al.*, 2020) and particulate phase in marine sediment (*e.g.*, Mazzola and Sarà, 2001; Norambuena-Subibabre *et al.*, 2016). The effect of lethal or sublethal concentrations in non-target crustaceans in the water column and marine sediments could have severe ecological implications (Friberg-Jensen *et al.*, 2003; Van Geest *et al.*, 2014a). Due to their high affinity with lipids (Log Kow 5 or 6), these compounds can be bioaccumulated into the non-target invertebrates (bivalves, sponges, coral, etc.) and biomagnification for the vertebrate organisms can occur (*e.g.*, Mazzola and Sarà, 2001; Alonso *et al.*, 2012; Azmar-Alemany *et al.*, 2017a).

Considering the high affinity of deltamethrin and cypermethrin for organic particles, the subsequence sinking into the sediments, and incorporation into non-target organisms, this study aimed to evaluate the occurrence of deltamethrin and cypermethrin in suspended particles and benthic filter-feeding organisms collected in the Puyuhuapi fjord, an area with active aquaculture.

Table 1. Physicochemical properties and biological effects of antibiotics and pesticides used in Chilean aquaculture industries.

Compounds		**Cypermethrin**	**Deltamethrin**
Chemical formule[a]		($C_{22}H_{19}Cl_2NO_3$)	($C_{22}H_{19}Br_2NO_3$)
Molecular weight (g mol^{-1})[a]		416.3	505.2
Octanol/ water partition (Log Kow; L kg^{-1})[c]		6.6	6.2
Organic carbon partition (Log Koc; L kg^{-1})		5.5[g]	5.8[h]
Water solubility (mg L^{-1})[c]		0.004	>0.002
No Observed Effect Concentration (NOEC; mg L^{-1})[e]	Algae	1.3	nd
	Invertebrate	0.00004	0.0000041
	Fish	0.00003	>0.000032
Lethal Concentration (LC_{50}, mg L^{-1})[e]	Algae	nd	nd/
	Invertebrate	0.0128	nd
	Fish	0.0028	nd
Half maximal Effective Concentration (EC_{50}, mg L^{-1})[e]	Algae	>0.1	9,1
	Invertebrate	0.0003	0.00056
	Fish	nd	0.00026
No-Observed Ecosystem Adverse-Effect Concentration (NOEAEC, mg L^{-1})[&, e]		0.00005	0.0032
Bioconcentratio Factor (BCF, L kg^{-1})[e]		1204	1400
Half-life (days)[e]	Water	22.1 (pH 8)	17 to 48
	Sediment	30[d] to > 730[i]	65 to 285[b]
	Biota	0,8 to 10[f]	nd

&: Mesocosmos study data; nd: No data. References: a: https://sitem.herts.ac.uk/aeru/ppdb/en/index.htm; b: Benskin *et al.*(2016); c: Oros and Werner (2005); d: Mackay *et al.*, (2006); e: http://sitem.herts.ac.uk/aeru/vsdb/index.htm; f: USEPA (1989); g: Maund *et al.*(2002); h: K_{OC} = 0.41 K_{OW} (Karickhoff, 1981); i: flocculated marine sediments (Hamaotene *et al.*, 2018)

2.0 Results

2.1 Total lipids in benthic filter-feeding organisms

A total of 26 benthic organisms, distributed in seven sponge species and two bivalve species, were processed for lipid measurement. The values have shown a range of 133.8 to 20.7 mg chol gdw^{-1} and 142.3 to 30.4 mg chol. gdw^{-1} for sponge's and bivalve's, respectively (Figure 2). Patagonian oyster (*C. patagonica*) was ~ 2.5 times lower than *M. chilensis* with average values of 38.9 ± 12.1 (two stations) and 98.9 ± 38.9 mg chol. gdw^{-1} (four stations), respectively. A wide range of lipid concentration was observed in sponges with average values between 33.4 (*Biemna sp*, one station) and 102.2 ± 27.6 mg chol. gdw^{-1} (*Tedania spinata*, three stations). Only sponges *Axintella crinite* and *Cliona chilensis* were present in most sampling stations. The average values were 39.5 ± 16.9 mg chol gdw^{-1} (six stations) and 56.9 ± 13.5 mg chol. gdw^{-1} (seven stations) for *C. chil*ensis and *A. crinite*, respectively. Figure 2 showed that *C. chilensis* have an increased

tendency at station 4 and a lower decrease at station 6. A similar trend was observed in *A. crinite* with a major value in station 3 and a small decrease at stations 5 (near Jacaf fjord) and 6 (Figure 1).

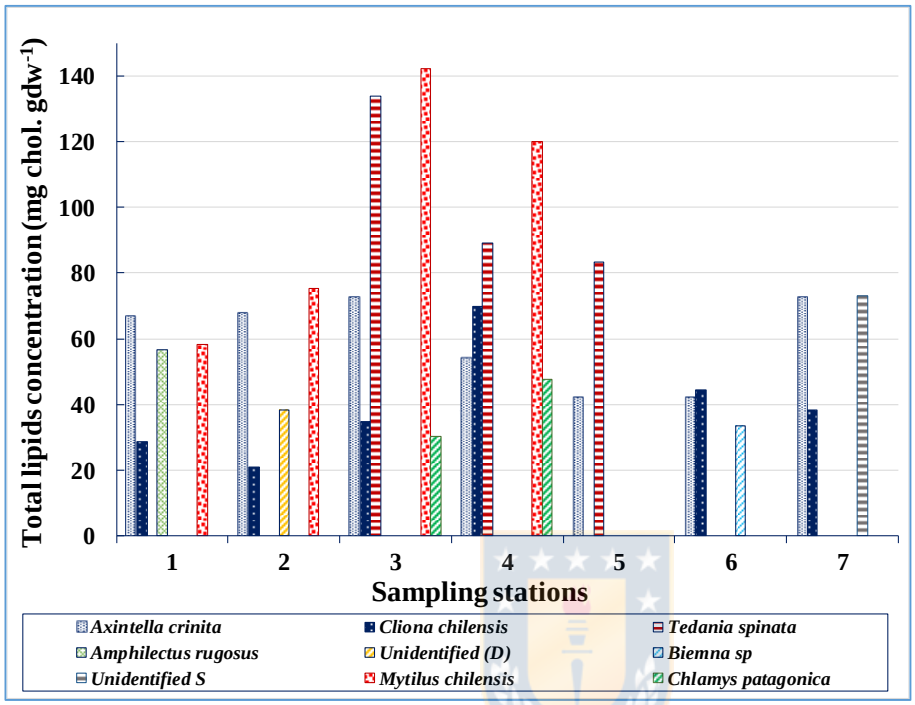

Figure 1. Total lipids concentration (mg chol. gdw^{-1}) in benthic filter-feeder's samples obtained in Puyuhuapi fjord in March 2017.

2.2 Pesticides in Puyuhuapi fjord

Only cypermethrin and deltamethrin were detected in organisms and suspended particles, respectively. Cypermethrin was found in 65% of organisms analyzed (Figure 3). The highest values were found in site 3 in sponges (*C. chilensis* and *T. spinata*) with values of 1.8 and 1.3 ng g lipids dw^{-1} (respectively) and site 4 in oysters and sponges (*C. patagonica* and *C. chilensis*) with values of 1.04 and 0.96 ng g lipids dw^{-1}, respectively. The lowest contents of cypermethrin were found at Station 1 with values of 0.12 and 0.09 ng g lipids dw^{-1} (*C. chilensis* and *A. rugosus*, respectively), while in site 7 was not detected. Deltamethrin in suspended solid samples was detected at stations 1 and 7 (Figure 4, Table 2).

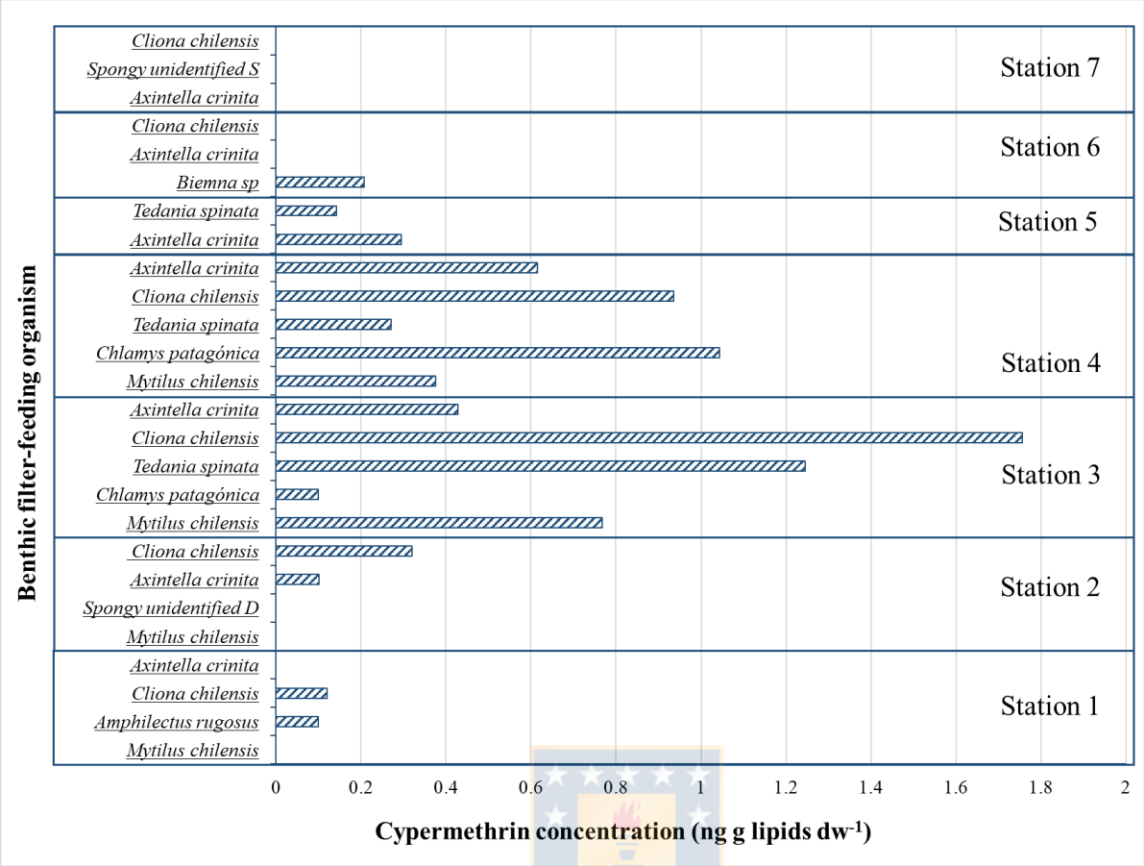

Figure 2. Cypermethrin concentration (ng g lipid dw^{-1}) in benthic organisms collected in the Puyuhuapi fjord.

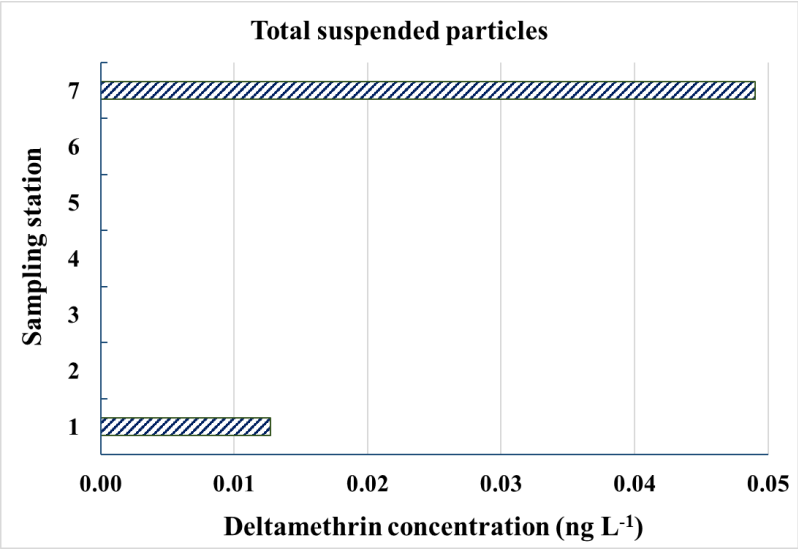

Figure 3. Deltamethrin concentration (ng L^{-1}) in particulate matter collected near localities where organisms were collected.

3.0 Discussion

3.1 Comparison of pesticide concentrations in organisms and the environment

Table 4 compares concentrations of deltamethrin and cypermethrin from suspended solids and in organisms reported in different studies. Concentrations of deltamethrin in total suspended particles were very low with a range of 0.01 to 0.05 ng L^{-1}, and located at less than 1 km (0.74 km) south of the salmon farming center (stations 1 and 7, Figure 1). The last medication was applied almost one year before (Sernapesca, 2016b) but we do not know which center applied the medication. Several studies have reported the highest toxicity of deltamethrin in non-target crustacean groups in the water column and sediments (Van Geest *et al.*, 2014a, b; Urbina *et al.*, 2019; Frantzen *et al.*, 2020) and additionally, this pesticide has a high tendency to be accumulated in bivalves, but also they have a high depuration rate (Brooks *et al.*, 2019).

Our values were two orders of magnitude lower than values reported in Monterrey Bay, during a storm event, with a concentration of 1.8 ng L^{-1} (Ng *et al.*, 2012). Seawater samples collected in South Africa estuary with a concentration of 253 ng L^{-1} (Wolfand *et al.*, 2019), were four orders of magnitude higher than our values. A similar situation was observed for samples collected near aquaculture centers in New Brunswick (Canada) with a deltamethrin concentration of 400 ng L^{-1} (Ernst *et al.*, 2014).

Table 2. Comparison of cypermethrin and deltamethrin concentration in total suspended solids and organisms in freshwater and seawater environments. The range values consider all stations and samples measure. LOQ= Quantify limit, nd= not detected, na= not analyzed.

Localities	**Conditions**	**Cypermethrin**	**Deltamethrin**	**Reference**
Total suspended solids (ng L^{-1})				
Salinas river and Monterey Bay	Storm event	nd- 23.4	nd- 1.8	Ng *et al.*, 2012
San Diego River	During storm events	nd- 492	nd- 253	Wolfand *et al.*, 2019
South African estuary	Seawater samples collected seasonally during 2002 and autumn 2003	0.33- 2.78	na	Bollmohr *et al.*, 2007
New Brunswick, Canada	Near aquaculture centers	na	nd- 400	Ernst *et al.*, 2014

Puyuhuapi Fjord, Chile	A total of seven stations were sampled during an active salmon culture.	nd	0.013- 0.049	This study
Organism				
River Basin, Spain: Guadalquivir river, Júcar river, Ebro river, Llobregart river *(ng g lipid w^{-1})*	Fish samples: Were collected specimens as barbel (*Barbus guiraonis and Luciobarbus sclateri*), carp (*Cyprinus carpio*) and trout (*Salmon trutta*)	3.82 - 1520	LOQ - 96.2	Corcellas *et al.*, 2015
River Basin, Spain: Guadalquivir river, Júcar river, Ebro river, Llobregart river *(ng gdw^{-1})*	Fish samples: A total of 59 specimens were distributed in 13 species (barbell, carp and trout). These range values consider all stations.	nd- 92	nd- 21	Pico *et al.*, 2019.
North-western (NW) Portuguese Atlantic coast *(ng g lipid gdw^{-1})*	Sea urchin (*Paracentrotus lividus*): These range values consider six-station with a total of 120 specimens	nd- 3.7	nd- 1.8	Rocha *et al.*, 2018.
Salmon farming samples come from Norway, Chile, Spain, Denmark, and Scotland. Wild salmon samples come from Alaska and the Pacific Ocean. *(ng gww^{-1})*	A total of 39 salmon farming samples (*Oncorhynchus mykiss* and *Salmo salar*).	nd- 4.42	nd- 2.21	Aznar-Alemany *et al* 2017a.
	A total of 12 wild salmon samples (*O. gorbuscha, O. keta, O. kisutch,* and *O. nerka*).	nd- 0.04	nd	
Puyuhuapi fjord, Aysen Region, Chile. *(ng g lipid dw^{-1})*	A total of 20 sponges specimen samples were distributed in 7 species (*Cliona chilensis, Axintella crinite, Amphilectus rugosus, Tedania spinata, Biemna sp, Unidentified D and Unidentified S*).	nd- 1.76	nd	This study

	A total of 6 bivalves specimen samples were distributed in 2 species (*Mytilus chilensis*, *Chlamys patagonica*).	nd- 1.04	nd	
Mediterranean coast of Andalusia (Alboran Sea, Spain) *(ng g lipid dw^{-1})*	Dolphin (*Stenella coeruleoalba*): A total of 27 male and 10 female stranded animals liver samples were collected with different maturity states (calves, juvenile and adult)	nd	nd- 78	Aznar-Alemany *et al.*, 2017b

Deltamethrin concentration in Puyuhuapi fjord (0.01 to 0.05 ng L^{-1}), were two orders of magnitude lower than the No Observed Effect Concentration (NOEC) value for invertebrates and three orders of magnitude lower for fish with values of 4.1 ng L^{-1} and >32 ng L^{-1}, respectively (Table 1). While, half-maximal Effective Concentration (EC_{50}) values were five orders of magnitude higher than our deltamethrin concentration, with values of 560 ng L^{-1} and 260 ng L^{-1} invertebrates and fish, respectively. These results suggested that measured deltamethrin concentration in Puyuhuapi fjord did not have an effect on the non-target organisms and neither at the ecological level where deltamethrin concentrations of no-observed ecosystem adverse-effect concentration value (NOEAEC) reaches a value of 3200 ng L^{-1} (Table 1). The presence of deltamethrin concentrations in our study area suggests i) the possible incorporation in the water column from marine sediment by resuspension processes, where this compound can be accumulated; ii) a possible particle transport from the adjacent fjord during their medication processes applied in December 2016.

Cypermethrin was not used in our study area, however, we found it in the majority of the benthic filter-feeding organisms collected in march 2017, ranging from no detected to 1.8 ng g lipid dw^{-1} in sponges and 1.0 ng g lipid dw^{-1} in bivalves, where the major concentrations were observed in sites 3 and 4 (Figure 3). Our values were from two to three orders of magnitude lower (3.8 to 1520 ng g lipid dw^{-1}, respectively) than those reported by Corcellas *et al.* (2015) in fish collected in four rivers in Spain and almost two times lower than in sea urchin collected at the Portuguese Atlantic coast with a value of 3.7 ng g lipid dw^{-1} (Rocha *et al.*, 2018) (Table 4).

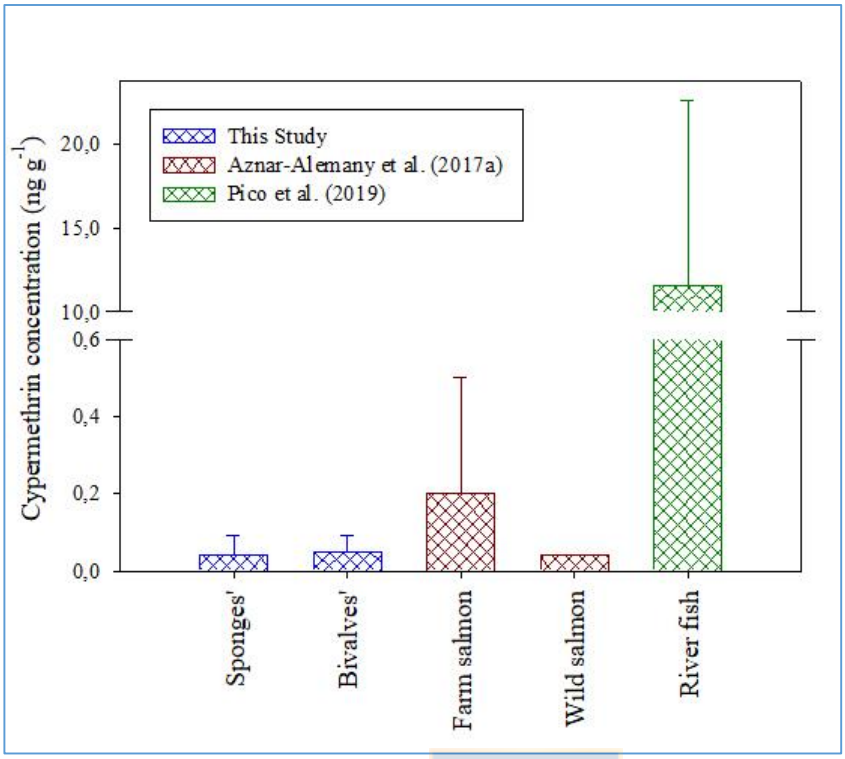

Figure 4. Comparison of cypermethrin concentration (ng g^{-1}) of invertebrates (sponges' and bivalves'), River fish (from Spain), and salmon for human consumption (several countries). The values correspond to the average of all data reported by the authors.

A comparison of the average of cypermethrin concentrations in ng g^{-1} (Figure 5), considering all organism and sampling stations, shows that our values were two orders of magnitude lower than those reported from river fish collected in Spain (Pico *et al.*, 2019) and one order of magnitude lower than farmed salmon approved for human consumption, collected from supermarkets and markets (Aznar- Alemany *et al.*2017a) with several countries as sources (*e.g.*, Norway, Chile, Spain and others). However, our average cypermethrin values (0.04 and 0.05 ng g^{-1}, sponges and bivalves respectively) were similar to those obtained from wild salmon (0.04 ng g^{-1}) collected in Alaska and the Pacific Ocean (Aznar- Alemany *et al.*2017a). The similar concentration of cypermethrin between wild fish and our organisms suggests that our samples have had an indirect exposition.

Several studies have indicated that sponges and bivalves are appropriate bioindicators of contamination by organic and inorganic compounds. (*e.g.*, Khazri *et al.*, 2015; Brooks *et al.*, 2019; Girard *et al.*, 2021). Gowland *et al.*, (2002) have demonstrated that mussels (*M. edulis*) can

bioconcentrate cypermethrin under laboratory conditions, but they suggested that is a low probability of this process occurring under natural conditions.

Our results suggest that Puyuhuapi fjord has an indirect exposition to cypermethrin considering that; i) this compound was not used in Puyuhuapi fjord according to official reports of the Undersecretariat of Fisheries and Aquaculture (SERNAPESCA); ii) a possible input of organic particles derived from adjacent localities connected to our study area (Figure 1) that applied cypermethrin in December 2016, considering the high affinity with organic particles; iii) and, eventuality, that cypermethrin was applied without informing the authorities in charge.

3.2 Input of pesticides to Puyuhuapi Fjord

The Puyuhuapi fjord has massive and extensive salmon farms since 2001 (Sernapesca, 2016c) and these activities have been considered one of the major sources of pesticide inputs in this zone. The Chilean government maintains specific annual monitoring of salmon infections caused by ectoparasitic *C. rogercresseyi* in the austral zone, through the Risk Disease Monitoring Program (Sernapesca, 2016a; 2017). These sanitary reports indicate that 20% of active farms in the Puyuhuapi fjord received pesticide treatments during 2016 and 67% in 2017, while in the Jacaf fjord, 71% of active farms were treated with pesticides in 2016 and 40% in 2017 (Figure 1). According to government information, the pesticides azamethiphos, emamectin benzoate and deltamethrin have been used as treatments in the Puyuhuapi fjord during 2015 and 2016 (Sernapesca, 2016b). Similar compounds were reported for treatments in the Jacaf fjord during 2015, however, during 2016, cypermethrin was added to the other treatments used so far (Sernapesca, 2019).

A total of 0.3 kg of the active ingredient in deltamethrin was applied (bath treatments) in April and May 2016 at the Puyuhuapi fjord, while 0.16 kg were applied during September and October 2017, after our samples were collected (March 2017). The dissolved phase of this compound has a half-life time of 48 d (Table 1) and dispersion of 39 km^2 (Parson *et al.*, 2020), but this compound (as well as cypermethrin) has a high affinity for particles and tends to be deposited into marine sediments. Cypermethrin was not used at the Puyuhuapi fjord, according to official reports from Sernapesca, but was reported that a total of 1.5 kg of its active ingredient was applied in the adjacent fjord during April and May 2016. However, the amount of cypermethrin applied in

the adjacent fjord, by itself, is not sufficient to explain the presence of this compound in our study area.

3.3 Residence time of pyrethroids in the marine environment

The persistence of these pesticides after being released in the water column should correspond to at least 22 days for cypermethrin and 48 days for deltamethrin (Table 1) and having a dispersion rate of almost 39 km^2 (Parson *et al.*, 2020), having an important impact on non-target organisms around the treated salmon farms (Urbina *et al.*, 2019). Besides, these compounds have a high affinity with organic particles which increases the protection against degradation processes (Urbina *et al.*, 2019; Méjanelle *et al.*, 2019), and therefore it increases their residence time especially in sediments (Hamaotene *et al.*, 2018; Hamoutene and Salvo, 2020). Degradation processes depend on factors such as light, temperature, pH, organic matter content, and oxygen concentrations (Farghaly *et al.*, 2013; Meyer *et al.*, 2013; Benskin *et al.*, 2016). Under conditions of high organic carbon content and low dissolved oxygen concentrations, the residence time in sediments can be extended at least by 9 months for deltamethrin (Benskin *et al.*, 2014) and more than two years for cypermethrin (Hamaotene *et al.*, 2018). The Puyuhuipi fjord has a high primary production with an average value of 1.4 $gC\ m^{-2}\ d^{-1}$ (Daneri *et al.*, 2012) and limitation on ventilation due to the presence of the Jacaf and Puyuhuapi sills (Figure 1), which yielded hypoxia conditions below 120 m (Schneider *et al.*, 2014; Silva and Vargas 2014). These characteristics of our study area can promote the persistence of cypermethrin and deltamethrin, considering for example that the last deltamethrin treatment was applied at least a year before. While the presence of cypermethrin can be associated with a major persistence in sediments and a major bioavailability to the benthic filter-feeding organisms.

The trophic transference of contaminants has been a major concern, especially when marine organisms were used for human consumption (*e.g.*, Burridge *et al.*, 2010). Mussels, oysters, and sponges can bioconcentrate contaminants, due to their high filtering capacity and, by depredation, they can transfer these contaminants to high trophic levels (*e.g.*, Wulff, 2006; Brooks *et al.*, 2019; Rosado and Otero, 2020; Almeida *et al.*, 2021). Despite that sponges have not a direct human concern (*i.e.*, to be consumed), we can not deny the ecological level importance in different ecosystems (e.g, tropical, temperate, and polar; Bell *et al.*, 2019). Several studies on sponges have described the capacity of accumulation of trace metals (*e.g.*, Rosado and Otero, 2020) and organic

contaminants like polychlorobiphenyl (PCBs' 38.2 ng L^{-1}), polycyclic aromatic hydrocarbons (Perez *et al.*, 2003; Batista *et al.*, 2013), but have no reported bioconcentration of pyrethroids. The effect of these contaminants in sponges was not clear, but some studies suggested an eventual impact on the endosymbiotic microorganisms and fungi sponges (*e.g.*, Yarden *et al.*, 2014; Thomas *et al.*, 2016; Rosado and Otero, 2020; Konstantinou *et al.*, 2021) and finally to the sponge itself.

4.0 Conclusion

The pyrethroids cypermethrin and deltamethrin (applied by bath treatments) tend to be deposited into the marine sediments and bioconcentrated into non-target benthic organisms due to a high affinity with organic particles and lipid content, respectively. Very low deltamethrin concentration values (0.01 to 0.05 ng L^{-1}) suggested that this compound does not have an effect on non-target organisms (NOEC, LC_{50} and EC_{50}; Table 1) and neither at the ecological level according to the value of the concentration of no-observed ecosystem adverse-effect concentration value (NOEAEC; 3200 ng L^{-1}) at the Puyuhuapi fjord. The last deltamethrin bath treatment in the Puyuhuapi fjord was applied in April and May 2016, therefore the presence of deltamethrin in the water column can be associated with the incorporation in the water column from marine sediment by resuspension processes or a possible external input from the adjacent fjord.

Cypermethrin was not used as sea lice treatment in our study area, however, low concentrations of it were observed in sponges and bivalves collected in March 2017. Our results suggest an indirect exposition of cypermethrin considering that; i) this compound was not used in Puyuhuapi fjord according to official reports of the Undersecretariat of Fisheries and Aquaculture (SERNAPESCA); ii) a possible input of organic particles derived from adjacent localities connected to our study area (Figure 1) that applied cypermethrin in December 2016, considering the high affinity with organic particles; iii) and, eventuality, that cypermethrin was applied without informing the authorities in charge. Our results were the first reports of pyrethroids in marine sponges, suggesting that this group was an appropriate bioindicator of compounds used in aquaculture activities.

5.0 References

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3.4 Chapter IV: The impact of antibiotics and pyrethroids used in aquaculture activities on marine community respiration

In this chapter, we show preliminary results of respiration experiments in the water column and marine sediments conducted at LECOB laboratory. This study aimed to establish the effects of antibiotics (Florfenicol, Flumequine, and Oxytetracycline) and pesticides (Cypermethrin and Deltamethrin) on community respiration in the water column and marine sediments. Sediments from regions where there is some aquaculture activity may recede microorganisms with resistance genes against antibiotics. As our target was the response of benthic ecosystems in a more general way, we targeted the response of ecosystems with no previous aquaculture activity. This is why samples were collected in Banyuls bay (France), an area without aquaculture activity.

Our preliminary results suggest some impact on carbon cycling in both water column and sediments experiments because significant differences in dissolved oxygen concentration were observed between treatments. Along with this, we also observed differences in parameters such as dissolved organic carbon, nutrients, and ammonium, both in the water column and in the sediment supernatant. These differences may be associated mainly with the microbial component, since there are no significant differences in the meiofauna (ANOSIM, p= 0.055), which will be corroborated once the results of the DNA studies in both experiments are obtained.

3.4.1 Respiration experiments in marine sediment microcosms

Fifteen sediment cores were collected in the MESO sampling station to conduct respiration experiments, according to section 2.2. Three cores were processed to retrieve baseline information. Three cores were used for each treatment, adding a final concentration of 500 ng L^{-1} of each compound as described in Figure 8 (section 2.2). Dissolved oxygen concentration was measured during the experiment with a microelectrode. Overlying water was collected for nutrients and dissolved organic carbon (DOC) measurements. The top 3 cm of sediments were collected for analysis of abundance and diversity of meiofauna and microbial communities, and elemental analysis (CHN).

3.4.1.1 Dissolved oxygen in sediment cores

The depletion of dissolved oxygen in the microcosm was observed in the first 24 h after adding the compounds (antibiotics and pesticides) with values ranging from 157 to 139 μM for Pesticides and Antibiotics + Pesticides, respectively (Figure 1). Anova test (p= 0.0001) results suggest significant differences in oxygen concentration values, while the Tukey test showed significant differences between treatments at 24 h, 120 h and 144 h. During the first 24 h, highly significant differences were observed between control (solvent only) and the treatments, while a minor significant difference was observed between pesticide and antibiotics + pesticides treatments. As the experiment progresses, and after supplying oxygen during 72 h, oxygen concentration decreased after 5 and 6 days (120 and 144 h) with no significant differences between treatments. However, on days 7 (168 h) and 8 (192 h) of the experiment, significant differences could be observed between treatments. On day 7 (168 h), a highly significant difference was observed between control solvent and antibiotics + pesticides treatments, while less significant differences were observed between control solvent and pesticides treatments, and between antibiotics and antibiotics + pesticides. On day 8 (192 h), highly significant differences were observed between control solvents and antibiotic + pesticides treatments, and between antibiotics and antibiotics + pesticides treatments. A less significant difference was observed between pesticides and antibiotics + pesticides.

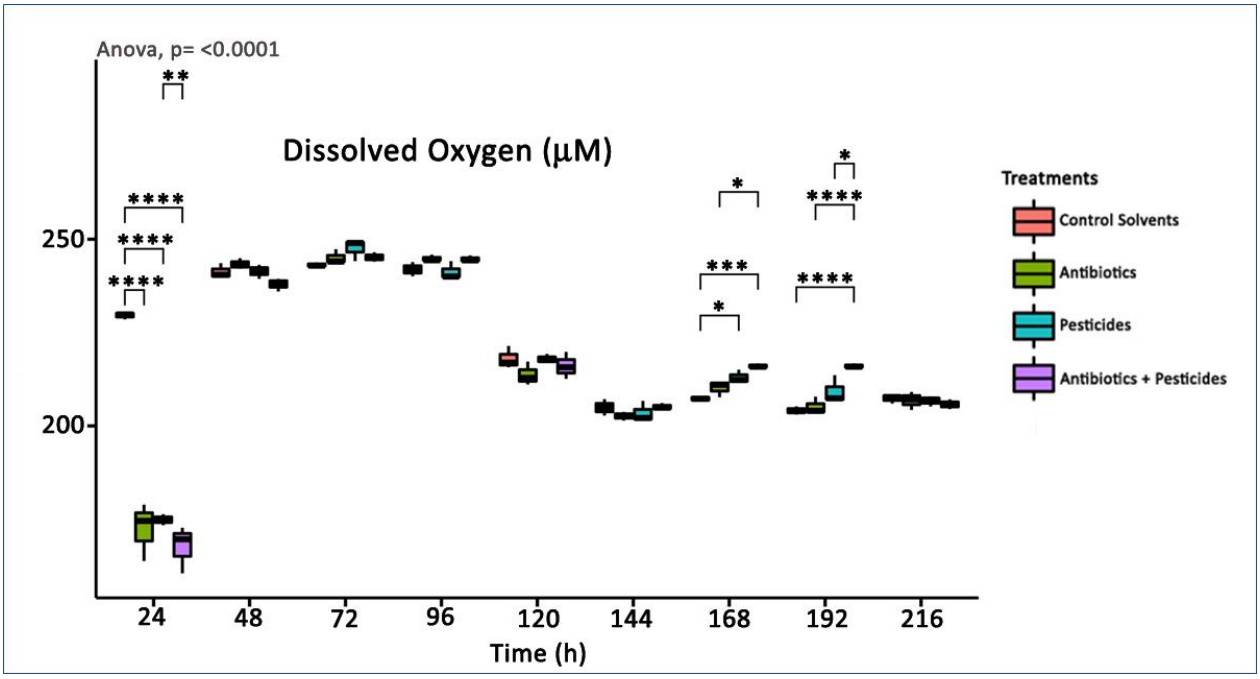

Figure 1. Dissolved oxygen concentration (average ± standard deviation; µM) in overlying water (1 cm over the sediment surface) and the sediment (every 0.5 cm) at the beginning (baseline) and the end of the respiration experiment in sediment microcosms.

3.1.1.1 Nutrients and DOC in bottom water

Overlying water was collected at the beginning and the end of the experiments (Figure 2). Dissolved organic carbon shows that the values of pesticide treatment (217 ± 55 µM) were near baseline values (170 ± 9), while the concentration in control solvents (1573 ± 208 µM), antibiotics (1213 ± 337 µM), and antibiotics + pesticides (1323 ± 310 µM) treatments were 825, 614 and 678% higher than baseline values. Anova tests with a p= < 0.0001 suggest very high significant differences in our experiments. Turkey tests results showed highly significant differences have been observed between pesticides and the control solvents, and between pesticides and antibiotics + pesticides treatments. Minor but significant differences were observed between antibiotics and pesticides' treatments. In contrast, no significant difference was observed between baseline and pesticide treatment.

Phosphate concentration decreased from 81 to 88 % between baseline (0.35 ± 0.04 µM) and treatment values (range of 0.04 ± 0.002 µM to 0.07 ± 0.01 µM). Similar concentrations of

phosphate were observed between control (0.04 ± 0.002 μM) and pesticide treatments (0.04 ± 0.02 μM), followed by antibiotic treatments with a value of 0.06 ± 0.004 μM, while the highest concentrations were observed in the antibiotics + pesticides treatment (0.07 ± 0.01 μM). Very high significant difference in our experiment according to Anova test (p= <0.0001), where we can assume were produced by differences between baseline and the treatments. However, Turkey test suggests no significant differences between treatments (Figure 2, Phosphate).

Nitrogen components (nitrate, nitrite, and ammonium) showed a major variation in concentration between treatments and baseline. Ammonium was almost depleted in the control solvents and antibiotic treatments (near the detection limit of 9 μM), decreasing by ~99% compared with baseline values (762 ± 261 μM), while in the pesticides (4227 ± 3061 μM) and in the antibiotics + pesticides (4012 ± 3818 μM) treatments ammonium concentration increased by 400%. Despite of depletion and increase of ammonium concentration, Anova test suggests no significant differences in our experiment with p= 0.081 (Figure 2).

Nitrate concentration decreased by 77% in the pesticide treatments with a value of 0.8 ± 0.4 μM compared to baseline (3.4 ± 0.2 μM), while in the control and the antibiotic and Antibiotics + pesticides treatment showed a very low concentration (~ 0.04 ± 0.03 μM). According to Anova test (p= <0.0001) our experiment present a very high significant difference. A comparison between treatments (Turley test) suggest low significant differences between pesticides and the other treatments (Figure 2)

Similar tendencies were observed in nitrite concentration between treatments and baseline where the values decreased on 20, 54, and 65% for antibiotics + pesticides, antibiotics, and control treatments (0.113 ± 0.016, 0.077 ± 0.014, 0.059 ± 0.026, μM, respectively) compared to baseline (0.167 ± 0.006). While pesticide treatments were increased by 4% with a value of 0.174 ± 0.06 μM. Statistical analysis suggests significant differences in our experiment (Anova p= 0.003). A low significant difference was observed between pesticides and antibiotics treatments, while a middle significant difference has been observed between antibiotics and pesticides treatments (Figure 2).

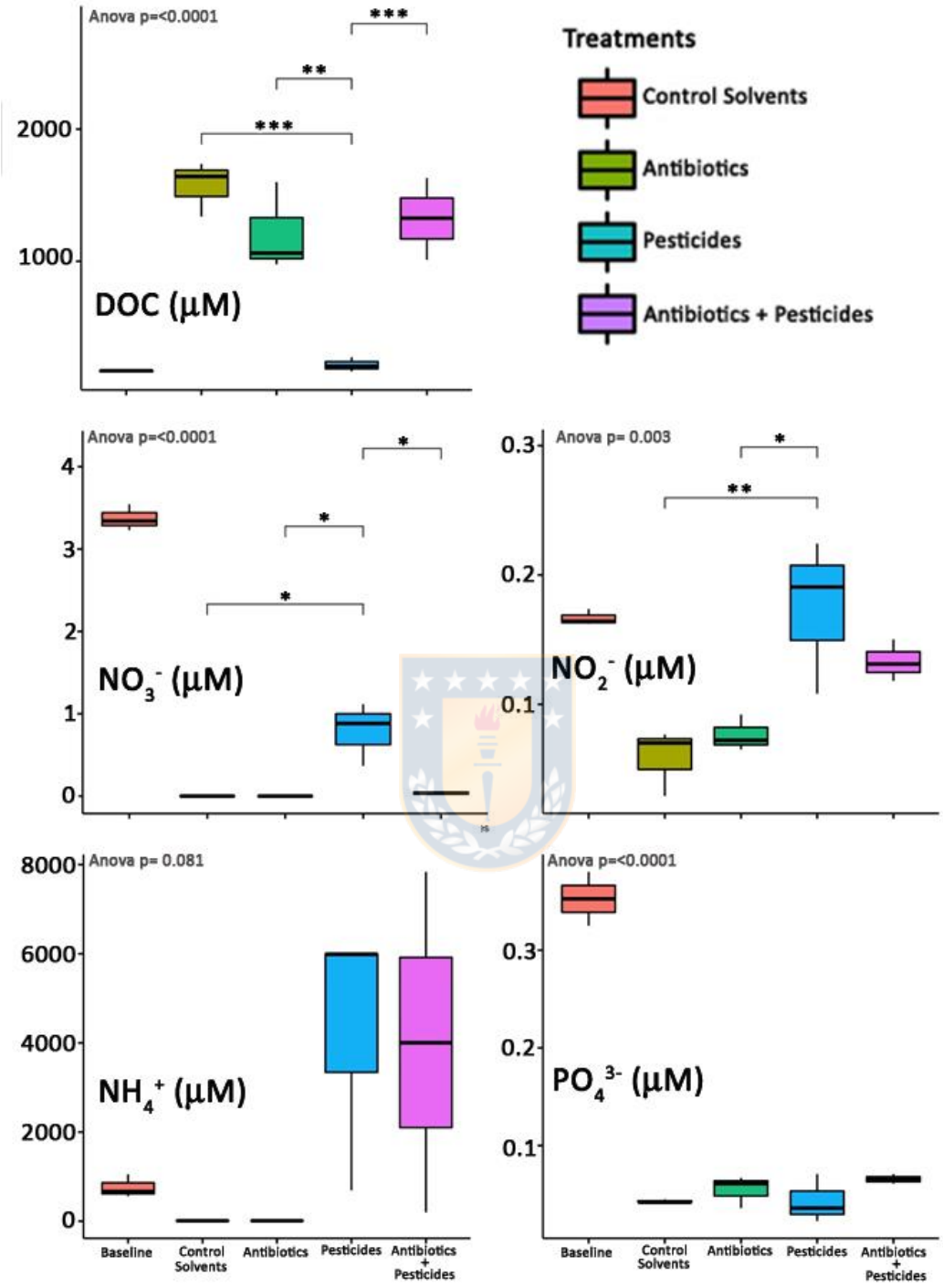

Figure 2. Dissolved organic carbon and nutrient concentration (average ± standard deviation; µM) in the overlying water at the beginning (baseline) and the final respiration experiment in the sediment microcosm.

3.4.1.2 Organic carbon and total nitrogen in sediments cores

Carbon and nitrogen were analyzed in sediment cores by elemental analysis (CHN) at baseline and the end of the respiration experiments in the sediment microcosms (Appendix 2, Figure 1). No significant differences were observed in the experiments according to Anova test, although it is possible to observe some differences in the percentage of nitrogen and carbon. Organic carbon showed a small decrease when total carbon is considered, while organic carbon shows an increase in the antibiotic and pesticide treatments compared to the control treatment. A similar condition is observed with organic nitrogen, while total nitrogen shows an increase in the control and antibiotic + pesticide treatments compared to baseline.

3.4.1.3 Meiofauna abundance and diversity in sediment cores

The abundance and diversity of meiofauna were obtained from the top 3 cm of sediment cores at the beginning (baseline) and the final respiration experiment. Abundance (Appendix 2, Figure 2) showed no significant differences according to Anosim test (R= 0.2193; p= 0.055). However, antibiotic treatments (390 ± 45 indv. $10cm^{-2}$) were less abundant than the control and the pesticide treatments (508 ± 182 indiv. $10cm^{-2}$, 511 ± 205 indiv. $10cm^{-2}$). Antibiotics + pesticides treatments (617 ± 77 indiv. $10cm^{-2}$) had a similar abundance value at baseline (604 ± 88 indiv. $10cm^{-2}$).

Nematodes, copepods, and polychaetes were the most abundant groups while kinorhynkes and ostracodes were less abundant (Appendix 2, Figure 3). More than 60% of meiofauna corresponded to the nematode group, followed by copepods (range 12 to 23%) and polychaetes (range 13 to 20%). A similar presence of groups was observed between baseline and pesticide treatments, as it was between control and antibiotics + pesticides treatments, while in antibiotic treatments were observed a minor presence of copepods, an increase of polychaetes group, and an absence of the kinorhynkes.

3.4.2 Respiration experiments in water column microcosms

Dissolved oxygen concentration was measured during the experiment with a microelectrode. Water subsamples were collected for nutrients, dissolved organic carbon (DOC), flow cytometry analyses (phytoplankton and microorganisms), and microbial diversity by DNA analysis. Samples were collected at the beginning (baseline) and final respiration experiments.

3.4.2.1 Dissolved oxygen measurements

Figure 3 shows the dissolved oxygen concentration in the water column microcosms during six days of experiments. During the first 24 h, the values of dissolved oxygen showed a slight increase in all the treatments (range of 219 ± 0. 6 to 222 ± 0.9 μM) compared to baseline (212 μM). Dissolved oxygen concentration decreases slowly between 24 and 120 h with a range of ~ 220 to ~118 μM. According to Anova test (p= <0.0001) very high significant differences were observed, but only the last measurement at 144 h shows significant differences between treatments (Turkey test).

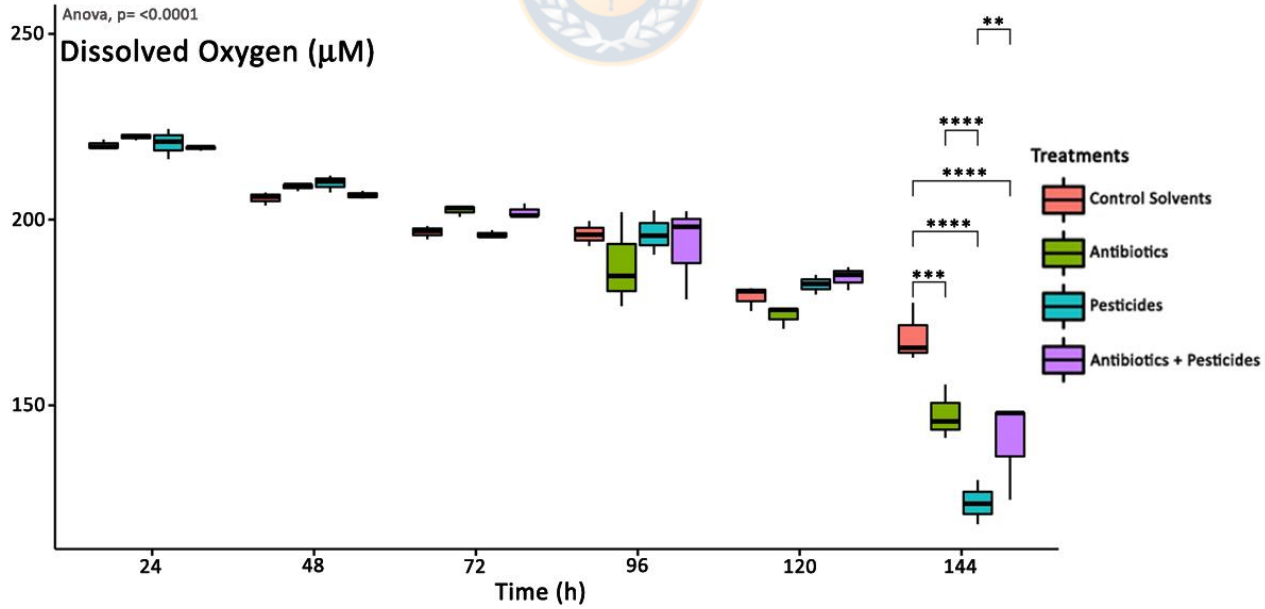

Figure 3. Dissolved oxygen concentration (average ± standard deviation, μM) in the water column at the beginning (baseline) and the final respiration experiments in the water column microcosm.

The lowest oxygen value was observed in the pesticide treatment (124 ± 6 μM), followed by the antibiotic + pesticide treatment (140 ± 13.1 μM) and finally the antibiotic treatment (148 ± 7.4 μM), while the highest concentration was observed in the control treatment with a value of 169 ± 7.4 μM. If we consider the respiration rate of the microcosms, estimated from the slope of the linear regression curve, it is possible to point out that the highest respiration rate was observed in the pesticide treatment with a value of 0.69 μM h^{-1} (R_2 = 0.81), followed by the antibiotic and antibiotic + pesticide treatments (0.59 μM h^{-1}, R_2 = 0.97, and 0.57 μM h^{-1}, R_2 = 0.81, respectively). The lowest respiration rate was observed in the control treatment with a value of 0.41 μM (R_2 = 0.96).

3.4.2.2 Nutrients and dissolved organic carbon (DOC) measurements

Dissolved organic carbon and nutrient concentrations were measured at the beginning (Baseline) and the final respiration experiment (Figure 4), according to section 2.2. The DOC concentration in treatments increased by 362 to 900% compared to the baseline concentration. The highest concentrations of DOC were observed in the control and antibiotic + pesticide treatments with values between 2729 ± 67 μM and 2771 ± 37 μM (respectively), followed by the pesticide treatment with a value of 1666 ± 106 μM, and the antibiotic treatment with a concentration of 1278 ± 47 μM. Very high significant difference was observed in our experiments according to Anova test (p=< 0.0001). A comparison between treatments shows a very high significant difference between control solvents and Antibiotics treatments; control solvents and pesticides treatments; Antibiotics and Antibiotics + Pesticides treatments; Pesticides and Antibiotics + Pesticides treatments. Less significant differences were observed between Antibiotics and Pesticides treatments.

Phosphate concentration was very low and similar between treatments; however, we observed a decrease of 86 and 89 % between baseline (0.074 ± 0.014 μM), and treatments ranged from 0.08 ± 0.003 μM to 0.01 ± 0.005 μM. According to Anova test (p=< 0.0001) very high significant differences were observed in our experiments, but no significant differences were observed between treatment, only between baseline and the treatments. While in the nitrogen components (nitrate, nitrite, and ammonium) was possible to observe a major variation in

concentration between treatments and baseline, with an increment in ammonium and a decrease in nitrite and nitrate. No significant differences have been observed according to Anova test for ammonium ($p= 0.48$), Nitrate ($p= 0.56$), while, nitrite show a significant difference with $p= 0.042$, however, no significant differences were observed between treatments.

The ammonium concentration shows an increase of ~905 to ~1550 % compared with baseline (58 ± 84 μM). However, the difference between treatments is not clear, according to standard deviation, where the highest value was observed in pesticide treatments (956 ± 713 μM), followed by antibiotics + pesticides treatment (749 ± 84 μM), and finally by antibiotic treatments (582 ± 220 μM).

Nitrate concentration decreased between 26 to 65% compared with the baseline which reaches a concentration value of 0.34 ± 0. 021 μM. Similar concentrations were observed in control and pesticide treatments with values of 0.12 ± 0.13 μM and 0.13 ± 0.16 μM (respectively), and antibiotics and antibiotics + pesticides treatments with values of 0.22 ± 0.17 μM and 0.24 ± 0.20 μM, respectively. Nitrite concentration decrease from ~22 to 48% compared with a baseline which concentration value was 0.052 ± 0.007 μM. Control and antibiotic treatments were similar with values of 0.040 ± 0.003 μM and 0.041 ± 0.015 μM (respectively), followed by pesticide treatment (0.032 ± 0.008 μM) and finally, antibiotics + pesticides treatments with a lower value (0.027 ± 0.006 μM).

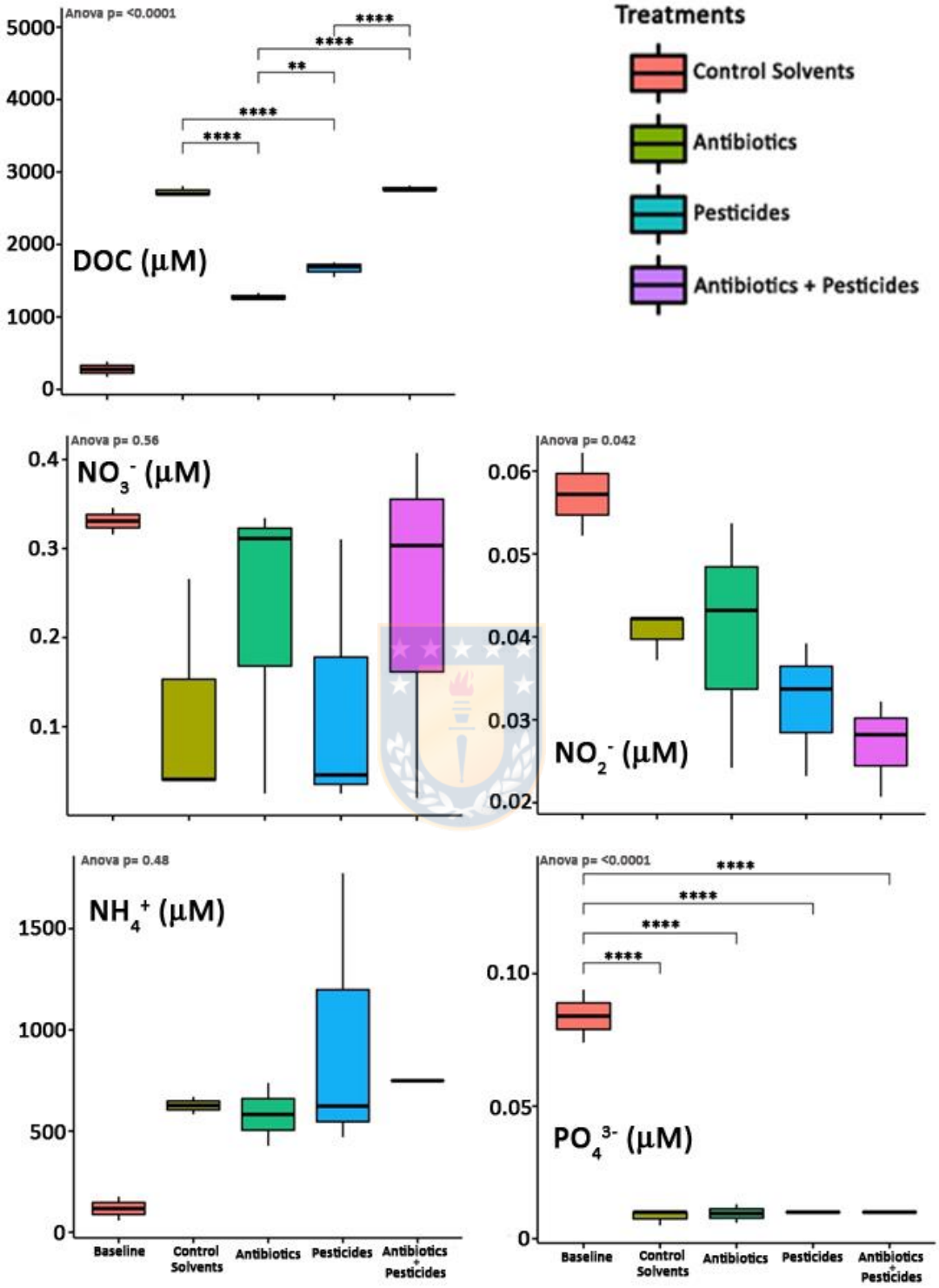

Figure 4. Nutrients and DOC concentration (average ± standard deviation, µM) at the beginning (Baseline) and the end of respiration experiments in the water column microcosms.

3.4.2.2 Phytoplankton and bacterial abundance measurements

The abundance of phytoplankton and bacteria (cells mL^{-1}) was measured by flow cytometry at the beginning (baseline) and the final respiration experiment. Anosim test suggest no significarive differences (R= 0.1156, p= 0.217).

Phytoplankton abundance decreased between 93 to 95 % when we compared the baseline and treatments, which were conducted under dark conditions (Appendix 2, Figure 4). The highest abundance, without considering the baseline, was observed in the control treatment with a value of 839 ± 90 cells mL^{-1}, while a lower value was observed in antibiotic treatments with a value of 605 ± 86 cells mL^{-1}. The largest abundance decrease was observed in *Synechococcus* and Nanoplankton with ≥ 94%, followed by Picoplankton with a range of 84 to 91%, and finally, Cryptophyceas with a range of 52 to 72%.

Synechococcus showed a lower abundance in antibiotic treatments (417 ± 70 cells mL^{-1}), while the higher abundance was observed in the control and pesticides treatments (567 ± 70 cells mL^{-1} and 579 ± 33 cells mL^{-1}, respectively). Nanoplankton abundance was highest in control treatments with a value of 109 ± 7.5 cells mL^{-1}, while similar but lower values were observed in the other treatments with a range of 77 ± 2.2 to 82 ± 5.6 cells mL^{-1}. A similar tendency has been observed in Picoplankton abundance where control treatment showed the highest value (159 ± 15.6 cells mL^{-1}), while the lower abundance was observed in antibiotics + pesticides treatment with a value of 95 ± 2.4 cells mL^{-1}. Cryptophyceas abundance showed the highest abundance in the control treatment with a value of 8.2 ± 3.9 cells mL^{-1}, while the lower value was observed 2.3 ± 2.1 cells mL^{-1}.

The major contribution to total phytoplankton abundance in baseline and treatments was *Synechococcus*, Nanoplankton, and Picoplankton, while the presence of Cryptophyceas was negligible (Appendix 2, Figure 5).

Total bacterial abundance decreased between ~24 to 54% compared with baseline abundance with a value of 350 ± 92 10^3 cells mL^{-1} (Appendix 2, Figure 6). Control, antibiotic, and pesticide treatments showed similar values with a range of 219 ± 24 to 223 ± 34 10^3 cells mL^{-1}, while a lower value was observed in antibiotics + pesticides treatments with a value of 161 ± 29 10^3 cells mL^{-1}. The abundance of high acid nucleoid content (HNA), with a major contribution to

total bacterial abundance, decreased between 37 to 56 % compared with baseline (253 ± 28 10^3 cells mL^{-1}). The lower value was observed in antibiotics + pesticides treatments (155 ± 27 10^3 cells mL^{-1}), while the control, antibiotics, and pesticides treatments were similar with a range of 214 ± 25 to 222 ± 14 10^3 cells mL^{-1}. Despite the minor contribution (~33%) to the total bacterial abundance of the low acid nucleoid content (LNA), they decreased ~98% compared to baseline with the treatments. The LNA abundance was similar between treatments and not superior to 7 ± 2 10^3 cells mL^{-1}.

4.0 DISCUSSION

In the last decades, aquaculture activities have supported high food fish demands, because natural fishing has reached a limit at *ca.* 90 million tons since the early '90s (Ottinger *et al.*, 2016; FAO, 2020). The extensive and massive salmon and trout production have long been known to generate local impacts on the water column and marine sediments (*e.g.* Cromey *et al.*, 2002; Buschmann *et al.*, 2006), where occurrence, fate, and impact of antibiotics and pesticides (released into the environment), have been poorly studied and understood in areas such as the Patagonia fjords. Some studies on antibiotics and pesticides used in marine aquaculture suggest that they may provoke local negative consequences on the environment (Neori *et al.*, 2004; Nash *et al.*, 2005; Willis *et al.*, 2005; Gaw *et al.*, 2014; Price *et al.*, 2015), however, few studies attempt to understand the consequences along to areas with high pressure from aquaculture activities (*e.g.*, Kim *et al.*, 2017; Chen *et al.*, 2019).

Understanding the impact of aquaculture at the scale of the fjord is not trivial because besides toxicological effects, other impacts may exist, at low levels. (Rain et al., Valentina). The way to estimate such impacts does not exist at date. Therefore, we set up an approach on the basis (mixing) three strategies (Figure 3, chapter I). The first one is to assess the occurrence of antibiotics and pesticides in the environment, during the rest period and at the onset of aquaculture activity (when treatments have not started yet). Second, a model was developed to predict the fate of antibiotics. Third, experiments were carried out to assess specific missing understanding, like the partition of antibiotics on one hand, and the change in mineralization on the other.

In this work, we have focused on the occurrence and fate of antibiotics in the Puyuhuapi fjord (chapter I, section 3.1), and their partitional behavior through an experimental approach (chapter II, section 3.2). Besides, we evaluated pesticide occurrence in the water column and sessile filter-feeding organisms, which results were discussed in chapter III-B (section 3.3.2). Finally, we showed preliminary results from community respiration experiments, in which we sought to evaluate the impact of antibiotics and pesticides on the sediment biota and the remineralization function of this ecosystem (chapter IV, section 3.3.4).

In chapters 3.1 and 3.2 we showed the results of a study of the occurrence and fate of antibiotics in the Puyuhupi fjord and their coefficient partitional constant (K_d and K_{OC}) under laboratory conditions simulating fjord conditions of temperature and salinity. First, we know that

the occurrence, fate, and persistence of antibiotics in coastal areas, derived from aquaculture activities, are a growing concern due to their possible impacts, especially on bacterial populations, because of their capacity to develop antibiotic resistance and, eventually, to be transferred to humans (Burridge *et al.*, 2010; Yang *et al.*, 2013; Miranda *et al.*, 2018). Second, several studies clearly showed local impacts of antibiotics mainly through their occurrence and the presence of resistant genes in marine sediments (Björklund *et al.*, 1991; Herwing *et al.*, 1997; Schmidt *et al.*, 2000; Miranda and Zemelman, 2002; Miranda and Rojas, 2007; Buschmann *et al.*, 2012; Tomova *et al.*, 2015). Finally, few studies have focused on understanding the dynamic and fate of antibiotics along the coastal area with high aquaculture pressure (*e.g.*, Kim *et al.*, 2017). The Puyuhaupi fjord is an area under aquaculture pressure since 2001 (Sernapesca, 2016a), where the use of ca. 20 tons of florfenicol was reported during 2015, and *ca.* 4 tons during 2016 (Sernapesca, 2016c), as a treatment for an outbreak of *Piscirickettsiosis* in 2016 (Rozas and Enríquez, 2014, Sernapesca, 2017a). In consideration of the above, several questions arose that are described and answered below.

First, the rest period aims at the recovery of background fjord conditions. Is it possible to detect antibiotics in the fjord after several months without treatments, even far away from culture centers? Our results showed that florfenicol was detected only in the particulate phase (trace to 23.1 ng $L^{-1)}$, while flumequine was present in one sample at trace concentration. These very low concentrations were detected ca. 180 days after concerted medication with florfenicol, and ca. 360 days after treatment with flumequine at sampling sites located between 2 and 23 km from the nearest farm. Despite being detected in a relatively small proportion of samples, they are in general agreement with previously published studies in a range of marine environments under human influence around the world (Table 2, section 3.1). Second, what concentration would you expect six months after the last treatments? In what compartment would we find it? and what concentration would you expect six months after the last treatments? For to respond to these questions we use the fugacity model (Level III) developed to determine the fate and concentration of antibiotics after one day of medication. Thus, our results suggested that >90% of the antibiotics (florfenicol and flumequine) may be lost in the water column by advective flow, and antibiotics deposited in the surface sediments below the cages may undergo near-total degradation. (Fig. 2, section 3.1). Third, how long are the antibiotics florfenicol and flumequine in sub-Minimal Inhibiting Concentrations (sub-MIC)? Our result, using this model, also predicted that flumequine should theoretically

remain in surface sediments at sub-minimal inhibitory concentrations (sub-MIC) that have previously been shown to promote the selection of antibiotic resistance in bacteria, which can become a risk for human health through the consumption of marine products, such as described for florfenicol, oxalic acid, oxytetracycline, and quinolones in the Chiloé archipelago in northern Patagonia (Miranda and Zemelman, 2002; Miranda and Rojas, 2007; Buschmann *et al.*, 2012; Tomova *et al.*, 2015).

The partitioning behavior of antibiotics can help understand their fate because sorption processes are driven by physicochemical characteristics and particle composition (Lang et al., 2018; Vasudevan et al., 2009; Feng et al., 2016). These parameters, partition constants coefficient (K_d and K_{OC}), have a key role in fugacity models but their published values suffer large differences (Kołodziejska et al., 2013). Establishing its value in the environmental conditions of the Puyuhuapi Fjord would be useful to better predict the fate of antibiotics. The partitioning behavior of antibiotics was estimated through bath experiments under temperature conditions similar to those reported in the Puyuhuapi Fjord (chapter II; section 3.2). Partition constants K_d and K_{OC} derived from batch experiments point to a sorption capacity of flumequine twice higher than that of florfenicol (Table 2, section 3.2), suggesting that flumequine has a greater tendency to sorb to sediments than florfenicol. Besides being mostly associated with the dissolved phase, dissolved florfenicol may in part be complexed to cations, as shown by concentration differences between seawater and pure water treatments, and similarly to what was observed for sulfamethazine (Wegst-Uhrich et al., 2014). In contrast, dissolved flumequine showed no tendency to form a complex (similar concentrations in seawater and pure water). Under the experimental conditions, the protonated form of flumequine tends to dominate, which may not favor complex formation. When sediments were added, flumequine concentrations were significantly different in pure and seawater, suggesting that a significant portion of flumequine was complex. Under saline conditions, the sorption seemed lower than in freshwater treatments suggesting that some cations prone to complex formation, were not present in seawater, and were released by sediments. This hypothesis in line with observations in soils, where flumequine is sorbed to copper ions (II) and the proportion of sorbed flumequine was up to 70% (Gaita et al., 2011).

In this work, the occurrence of pesticides used in aquaculture in the Puyuhuapi fjord after the rest period was assessed in benthic filter feeder organisms, and in particles, sampled along the fjord, and not in the vicinity of aquaculture cages, as previously assessed. Along with the use of

antibiotics in the salmon farming industry, the use of pesticides is also necessary to control outbreaks of ectoparasitic infections caused by *C. rogercresseyi*, which can generate severe skin damage and increase salmon susceptibility to suffering a bacterial and viral infection (Bravo, 2003; Johnson *et al.*, 2004; Lhorente *et al.*, 2014; Dresdner *et al.*, 2019) and, as a consequence, severely reduced salmon production (FAO, 2020). Once pesticides have been released in the water column, and because of their high affinity for particles and their lipids, they can be deposited in marine sediments and eventually incorporated by non-target benthic organisms (Tucca *et al.*, 2017; Urbina *et al.*, 2019; Méjanelle *et al.*, 2020). Bivalves and sponges have been reported to be suitable pollution bioindicators for organic and inorganic compounds (*e.g.*, Khazri *et al.*, 2015; Brooks *et al.*, 2019; Rosado and Otero, 2020; Girard *et al.*, 2021), due to their high filtration capacity and, in turn, an eventual transfer of these contaminants to high trophic levels (*e.g.*, Wulff, 2006; Brooks *et al.*, 2019; Rosado and Otero, 2020; Almeida *et al.*, 2021). When we considered these evidence, we wonder if pyrethroids, used in aquaculture, could be detected in the sessile filter-feeding organism in the Puyuhuapi Fjord? As an answer, we evaluate the occurrence of cypermethrin and deltamethrin in suspended particles and benthic filter-feeding organisms in the Puyuhuapi Fjord (see chapter 3.3.2).

Deltamethrin was detected in total suspended solids with a concentration ranging from 0.01 to 0.05 ng L^{-1}. These values were lower than NOEC, $LC_{50,}$ and EC_{50} (Table 1, section 3.2.2) suggesting no effect on non-target organisms. They were also below the ecological level according to NOEAEC concentration value (3200 ng L^{-1}). The presence of deltamethrin concentrations in our study area, after more than 12 months since the last medication, suggested possible incorporation from resuspension of marine sediment where deltamethrin would be stored, or from allochthonous particles, coming from the adjacent fjord. The adjacent fjord has not the same periodicity of culture and rest periods.

Despite the non-use of cypermethrin in our study area, very low concentrations were observed in almost all of our benthic filter-feeding organisms, with a maximum value of 1.8 ng g lipid dw^{-1} for sponges and 1.0 ng g lipid dw^{-1} for bivalves (Table 2, section 3.3.2). Average values (considering all stations) were similar to those reported by Azar-Alenmany *et al.* (2017) in wild salmon without direct exposure to cypermethrin (Figure 5; section 3.3.2). These results suggest that organisms of Puyuhuapi have indirect exposure to cypermethrin, probably from external input from adjacent locations with active salmon cultures or resuspension of sediments with

cypermethrin accumulated by treatments applied and not reported officially. The Puyuhuapi fjord, with hypoxia characteristics and high primary production, has the favorable conditions to allow long residence time of deltamethrin in marine sediments at least for 9 months (Benskin *et al.*, 2014) and more than two years for cypermethrin (Hamaotene *et al.*, 2018).

Exposure of bivalves to pesticides may be of immediate concern because they are used for human consumption, unlike sponges which are ecologically important in different ecosystems (e.g, tropical, temperate, and polar; Bell *et al.*, 2020). Sponges capacity to accumulate trace metals (*e.g.*, Rosado and Otero, 2020) and organic contaminants such as polychlorobiphenyl (PCBs') and polycyclic aromatic hydrocarbons (Perez *et al.*, 2003; Batista *et al.*, 2013) was well known, but our study was the first to report pyrethroids' accumulation. Our study suggests that sponge groups can be an appropriate bioindicator of pesticides used in aquaculture activities. In terms of aquaculture impacts, it shows that the exposure expected by current knowledge (pyrethroid residence time, rest period set-up) fails to explain the occurrence of deltamethrin and of cypermethrin. Even though the levels are of no environmental concern, more research is needed to reconcile *in-situ* observations with scientific knowledge and official information.

An important impact to take into account in the salmon industry is the possible impact of antibiotics and pesticides on biogeochemical cycles, due to their possible effect on microorganisms and non-target organisms that play a key role in these cycles. We know that organic matter degradation processes are key steps in marine biogeochemical cycles, where meiofauna and microbial populations play fundamental roles in both the water column and marine sediments (*e.g.* Azam *et al.*, 1983; Azam and Malfatti, 2007; Nascimiento *et al.*, 2012; Bonaglia *et al.*, 2014). Aquaculture not only increases the organic matter content in the water column and sediments near the salmon cage (Fodelianakis *et al.*, 2015; Kamjunke *et al.*, 2017), but may also affect their degradation processes, due to the impact on biological communities when antibiotics and pesticides have been applied (*e.g.*, Friberg-Jensen *et al.*, 2003; Van Geest *et al.*, 2014a, Valdés-Castro and Fernández, 2021). Those effects are observed at a concentration of antibiotics and pesticides much lower than concentrations causing toxicological responses, and the impacts targetted here are different. Several studies have reported changes in the taxonomic diversity, composition, and function of bacterial communities in sediments in different areas with active aquaculture. (*e.g.*, Christensen *et al.*, 2000; Holmer *et al.*, 2003; Bissett *et al.*, 2007, 2009; Castine *et al.*, 2009; Hornick and Buchmann, 2018).

Antibiotics released into the environment can affect the degradation of organic matter, inhibiting some bacterial biochemical processes (*e.g.*, Chellosi *et al.*, 2003; Marti *et al.*, 2014) or causing changes in microbial communities, which promotes the presence of genetic resistance (*e.g.*, Dang *et al.*, 2007; Nogales *et al.*, 2011; Tomova *et al.*, 2015). The impact of pesticides in the water column and marine sediments has been poorly understood because not only does it have noxious effects in non-target crustaceous communities (Mazzola and Sara, 2001; Van Geest *et al.*, 2014a, b; Norambuena- Subibabre *et al.*, 2016) but rather in bacterial activities as photo and chemoautotrophic carbon fixation (Rain-Franco *et al.*, 2018) and as chemo and photoautotrophic ammonium uptake (Valdes-Castro and Fernández, 2021). The close relationship between meiofauna and the microbial community has significant relevance in biogeochemical cycles because their interaction can stimulate the degradation of organic matter (Bonaglia *et al.*, 2014) and also compete for the consumption of organic matter (*e.g.*, Nascimento, 2010). Copepods (arthropods), which are the second most abundant group in the meiofauna (*e.g.*, Coull, 1999; Sajan *et al.*, 2010; El-Serehy *et al.*, 2015) and the preferred prey of invertebrates and fish (Coull, 1999), could also be affected by pesticides which in turn, would affect both the degradation process and the trophic structure of the sediment. Based on these antecedents we wonder if is it possible that antibiotics and pesticides at a concentration well below NOEC have an impact on the carbon cycle? To answer this question, we seek to establish the effects of antibiotics and pesticides on community respiration, through community experiments with the exposition of antibiotics and pesticides in seawater and sediments microcosms. The exposure concentrations were 500 ng L^{-1}, which corresponds to the treatment situation, close to aquaculture centers during acitive medication periods (section 2.4).

Chapter 3.4 were described our preliminary results suggesting that exposure to pesticides (cypermethrin and deltamethrin) and antibiotics (oxytetracycline, florfenicol, and flumequine) produce changes in mineralization processes in both sediments and the water column, in an area without aquaculture activity (Banyuls Bay, France). Significant differences in dissolved oxygen values were observed during community respiration experiments 24 h after to inoculate antibiotics and pesticides and after 168 h and 192 h of the respiration to and times on sediments (Figure 1, section 3.4.1) and at the end of water column experiments (Figure 3, section 3.4.2).

Significant differences in DOC concentration (+ 900% in the water column experiments +antibiotics + pesticides, whilst it was +600% in the control) suggested that aquaculture related

treatments may have an impact on mineralization close to culture centers, and during the treatment period. Ammonium, nitrate, and nitrite concentrations suggested that this effect also concerned the nitrogen cycle in sediments (~ + 450% in the pesticide and antibiotic + pesticide treatments, while ammonium almost disappeared in the control and antibiotic treatments). Nitrate

The concentrations of nitrate were not detected in the control and antibiotic treatments and have a very low concentration in the antibiotic + pesticide treatment, while in the pesticide treatment it shows the highest concentration among the treatments. While the nitrite concentration does not show changes in the pesticide treatment, with a value similar to starting time, it shows its lowest concentration in the control and the antibiotic treatments. In addition, impacts observed in the seawater differed from the impacts observed in the sediment mesocosms.

The impact on nitrogen compounds and DOC in the sediment experiments, coupled to the small difference between meiofaunal abundance, suggests that exposure to antibiotics and pesticides during salmon treatments may affect the mineralization by the microbial community. This will be further evaluated by microbial diversity analyses in the sediments, once pandemic conditions allow it. The same conclusion can be drawn for the water column experiments. A significant difference in total bacterial abundance and HNA bacteria (103 cells mL^{-1}) was observed in the antibiotic + pesticide treatment. Bacterial diversity will help relate it to the changes in nitrogenous species' concentrations. The abundance of phytoplankton decreases by at least 93% where *Synechococcus*, with the highest contribution to the total abundance, seems to be more sensitive to antibiotics than to pesticides (Figure 4, Appendix 2).

4.1 Perspectives for future research.

Future research would gain in including the study of the fjords and canals adjacents to our study area, with and without aquaculture activity. This will allow the authorities to better evaluate the sanitary rests considering the interconnection of farming neighborhoods. It is also suggested to use both sponges and bivalves to evaluate the environmental conditions of an area, with or adjacent to aquaculture activity. On the other hand, it is necessary to make modifications to the fugacity model used in our study, incorporating the presence of at least two layers in the water column.

5.0 CONCLUSION

This thesis work concludes that the Puyuhuapi fjord shows the occurrence of antibiotics (florfenicol and flumequine) and pesticides (deltamethrin and cypermethrin) derived from aquaculture activities in particulate phases and benthic filter-feeding organisms during the sanitary rest period. Experimental studies mimicking the treatment period evidenced no toxicological impacts, however supporting changes in the mineralization functions of the ecosystem. Specific evidence and conclusions were:

1. Low florfenicol and flumequine levels were detected about 180 days and 360 days after concerted medication, respectively, at sampling locations between 2 and 23 km from the nearest farm. The results of the fugacity model predicted that high flumequine contents may remain in sediments for up to 2 months before being completely degraded to a sub-Minimum Inhibitory Concentration (sub-MIC), which may promote the selection of bacteria with antibiotic resistance and eventually become a risk to human health through the consumption of seafood products.
2. Flumequine has a higher sorption tendency than florfenicol, and therefore flumequine fate will be more associated with processes like particle transport and deposition to the seafloor. In contrast, a smaller portion of florfenicol bounds to particles, and the fate of this antibiotic is to a higher extend related to hydrodynamic processes like dilution and transport by currents. The discrepancy between K_{OC} and K_{OW} shows that absorption into the organic carbon phase is not the dominant process driving sorption of florfenicol and that other surface-driven processes, like ion exchange, cation and hydrogen binding, and complex formation also mitigate its partition (Tolls 2001). The present study provides experimental partition constants of flumequine showing that absorption by diffusive processes to hydrophobic organic carbon is an important driver of this compound association to sediment.
3. Low deltamethrin concentration values (0.01 to 0.05 ng L^{-1}) in total suspended solids, found in our study, did not affect non-target organisms and or had any effects on an ecological level (NOEC, LC_{50}, EC_{50}, NOEAEC; Table 1). In addition, considering that the last treatments were applied more than a year ago, the presence of deltamethrin in the water

column could be associated with sediment resuspension processes or a possible external input from the adjacent fjord with active aquaculture.

4. Despite cypermethrin not being used as sea lice treatment in our study area, low concentrations were observed in sponges and bivalves (1.8 and 1.0 ng g lipid dw^{-1} for sponges and bivalves, respectively). Our results, as total average values (0.04 and 0.05 ng g^{-1}), were similar to those reported in wild salmon without direct exposure to cypermethrin (Figure 5; section 3.3.2), suggesting a possible external input to our study area and/or by resuspension of cypermethrin accumulated in the sediments from non-reported treatments applied, considering that the Puiyuhuapi fjord may promote its persistence. Besides, our study is the first to report the presence of pyrethroids in marine sponges, suggesting that this group is an appropriate bioindicator to evaluate compounds used in aquaculture activities.
5. Despite that preliminary result not showing clear differences in dissolved oxygen concentrations during community respiration experiments, we can observe some differences between treatments in DOC and nitrogen components (ammonium, nitrate, and nitrite) concentrations, suggesting some changes in biological components, however, we must wait for DNA analysis to determine variations in bacterial diversity.

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7.0 APPENDIX

Appendix Content

7.1 Appendix 1

1.0.- Fate of Pyrethroids in Freshwater and Marine Environments

Fate of Pyrethroids in Freshwater and Marine Environments

Laurence Méjanelle, Bibiana Jara, and Jordi Dachs

Contents

Abstract As a consequence of their increasing use, pyrethroid insecticides are recognized as a threat for nontarget species and ecosystem health. The present chapter gives a state-of-art overview of individual pyrethroid occurrence in waters and sediments worldwide, together with recent reports of their quantification in the atmospheric gas and aerosol phases. Degradation rates, transport processes, and partitioning of pyrethroids between environmental phases are reviewed. River flow efficiently transports pyrethroids to river mouths and estuaries, while pyrethroid

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impact on the marine environment remains difficult to appraise due to lack of comprehensive studies. Nevertheless, aquaculture arises as an important but poorly understood environmental burden. Owing to their large organic carbon pool, sediments may act as a sink for pyrethroids and impair nontarget aquatic species. Partitioning potential of pyrethroids is compared to that of other well-known legacy pollutants in the light of their position in the phase space defined by key physico-chemical properties (K_{OW} and H'). The transport and partition of pyrethroids away from their source are strongly dependent on their half-life, but their quasi constant emissions in urban and agricultural area may compensate for their degradation, therefore sustaining the occurrence and behavior of some individual pyrethroids as "quasi persistent organic pollutants."

Keywords Air, Freshwater, Marine, Partition, Pyrethroids, Sediment, Transport, Water

1 Introduction

A major change in the use of pesticides over the last 20 years has been the gradual replacement of organophosphate and organochlorine pesticides by synthetic pyrethroids. The regulation and the ban of formerly used active agents have been followed by an increased use of a wide variety of current-use pesticides such as pyrethroids in agriculture and aquaculture [1]. Pyrethroids are also extensively used in urban and industrial areas and livestock farms to control pests such as mosquitoes, lice, and wood-destroying dwellers. In addition, synthetic pyrethroids have the advantage of low cost, low mammalian toxicity, and shorter persistence in the environment than other classes of pesticides [2].

The exposure mechanism leading to acute neuronal toxicity to insects and crustaceans is through dissolved water in the water column and through pore water in the sediments [3]. Other impacts have been reported and are related to trophic transfer in food webs. Even though pyrethroids are degraded faster than other pesticides, they have been shown to occur in water bodies, allowing their transfer to the aquatic food webs [4]. Pyrethroids have hydrophobicities in the same range as legacy organochlorine pesticides (log K_{OW} from 4.8 to 7.0) and thus tend to sorb on organic particles and sediments. Insecticides sorbed in particles may be consumed by filter feeders and be transferred to higher trophic levels, or alternatively, particles may consist in a reservoir for these pollutants, probably reducing their biodegradability in natural waters. As a result of biomagnification at high trophic levels, negative impact of pyrethroids has been suggested causing immunity and estrogenic disruption to mammalians [4].

The impact of pyrethroids is the result of both the exposure to dissolved pyrethroids and to particle-associated ones. A comprehensive understanding of pyrethroid impact to nontarget species starts with the understanding of pyrethroid occurrence in the various environmental phases: dissolved water phase, particles, and sediments. This chapter reviews the current knowledge on the occurrence of

pyrethroids in water, particles and sediments of freshwater and marine environments, and the underlying partition and transport processes between those phases. Pyrethroids are often applied to water bodies, and after introduction to the dissolved phase, they partition between the different environmental compartments, being subjected to a number of sinks, particularly degradation. The elucidation of the occurrence, partition, and sinks of pyrethroids will allow to identify research lines that would help to better constraint the environmental risk associated to pyrethroids and to orientate protection measures.

2 Pyrethroid Sources and Emissions in Surface and Marine Water Bodies

Because of their wide spectrum of targets, pyrethroids are used in a variety of applications; agriculture and urban householding pest control compose two of the major market shares. Accurate estimates of their use are made difficult because nonprofessional uses are often not reported and by off the counter sales. The use of pyrethroids by aquaculture activities leads to important amounts of pyrethroids directly released to the marine environment, which can be important in specific marine areas [5, 6]. Overall, pyrethroids represent more than one third of the insecticide market, with a worldwide annual use of active ingredients around 7,000 tons per year between 1990 and 2013 (with peaks above 12,000 tons in 1997 and 2012) [7].

Structural and householding usages constitute an important part of the pyrethroid market. Several studies report that these compounds are not completely eliminated in conventional wastewater treatment plants (WWTPs) [8, 9], and thus they can be introduced into the environment through WWTPs effluents. Pyrethroids from urban sources were identified as the cause of toxicity in 80% of river sediments in the vicinity of the city of Salinas in Southern California [10].

3 Occurrence and Composition of Various Pyrethroids in Water Ecosystems

In order to estimate the potential impact of pyrethroids on aquatic environments, research projects and monitoring programs have surveyed pyrethroid occurrence mostly in the vicinity of agricultural and urban areas concerned by pyrethroid primary use. California is the world location from which more data are available as a result of numerous monitoring programs setup at the municipal to state level [11]. As a result of their affinity for organic matter, pyrethroids have been detected both in the water phase and in the sediments. Table 1 reviews water concentrations of pyrethroid in the current literature, and Table 2 reports their levels in sediments.

Table 1 Non-exhaustive selection of concentrations ranges, in ng L^{-1}, of individual pyrethroids in water from different locations worldwide

	Year	Sample type	Individual pyrethroids								References
			Bifenthrin	Fenpropathrin	λ-cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Fenvalerate	Deltamethrin	
Freshwater											
Northern California											
American River, flood events	2009–2010	WS	nd–106	nd	nd	nd–111	nd–26.6	nd–9.4	nd	nd	[12]
San Francisco Bay, drains sampled after storm	2014	WS	nd–9.9								[13]
Central California											
Creeks and drains in the San Joaquin watershed	2007	Diss	nd–15.8	nd–2.6	nd–19.8	nd–1.1	nd–2.9	nd–5.7	nd–5.1		[14]
Creeks and drains in the San Joaquin watershed	2007	P	nd–9.6	nd	nd–11.1	nd–1.1	nd	nd	nd–5.1		[14]
Puerto Creek channel into San Joaquim Rivers	2007	WS				nq–93					[15]
Wadworth channel into Sacramento River	2003	WS				nd–94					[15]
Sacramento River	2008–2009	Diss	nd–24	nd–8.5	nd	nd	nd	nd	nd	nd	[16]
Del Puerto and Oreshimba creek	2007–2008	WS?	nd–5	nd	nd–16	nd	nd–21	nd	nd	nd–6.28	[17]
Salinas River and Monterey Bay, storm events	2008–2009	P	0–21.6		0–7.6	0–36.0		0–23.4	0–35.6	0–1.8	[18]
Creeks and drain on the Salinas and Santa Maria River watershed	2014–2015	WS?	nd–11.4		nd–447	nd–17.1	nd	nd	nd–39.7		[19]
Southern California											
Los Angeles and San Gabriel Rivers low flow conditions	2011	WS	nd–9	nd	nd	nd–18	nd	nd	nd	nd	[20]
San Diego River during storm events	2017	Diss	1–20.4	nd–24.9	nd–30.3	nd–55.9	nd–50.2	nd–55.4	nd–102	nd–62.2	[21]
San Diego River during storm events	2017	P	1–347	nd–63.2	nd–96.9	x–367	nd–205	nd–492	nd–56.9	nd–253	[21]

Asia											
Leyte island, Philippine rice agriculture	2010	Diss									[22]
Rivers passsing through large Vietnamese cities	2011–2012	WS				x–4,390					[23]
Beijing GuanTin Reservoir, China	2003–2004	Diss						nd–1.89		nd–6.28	[24]
Urban stream, Guangzhou, China		Diss	0.28 ± 0.25[a]		0.52 ± 0.59[a]	3.7 ± 3.1[a]		5.0 ± 3.3[a]			[25]
Urban stream, Guangzhou, China		P	0.84 ± 0.48[a]		4.3 ± 4.4[a]	9.0 ± 7.2[a]		20.0 ± 14.5[a]			[25]
Chenab River, Pakistan		Diss	nd–92			nd–103		nd–97		nd–108	[26]
Europe											
Rivers in the Humber catchment, UK	1996–1997	P				nd–3,500					[27]
Ebro River delta, Spain	2008–2009	Diss	nd	nd	nd	nd	nd	0.73–57.2	nd	2–58.8	[28]
Ebro River delta, Spain	2008	Diss						5–30			[29]
Valencia paddy field, surface water and groundwater, Spain		Diss									[30]
Streams, Central Germany	2009	Diss			nd–55			nd–86	nd		[31]
Streams, Central Germany	2009	P			nd–88			nd–180	nd		[31]
Seawater											
Estuarine catchment sites, NE Australia	2016–2017	WS?	nd–20.6 mg L^{-1}			nd		nd			[32]
NW Portugal Coast	2016–2017	Diss	nd		nd	nd	nd	nd–31	nd	nd	[33]
South African estuary	2002–2003	P						0.33–2.78	0.03–0.79		[34]
Pearl River estuary, urban creek at Guanzhou, China	–	Diss	0.3 ± 0.3		0.5 ± 0.6	4 ± 3		5 ± 3			[25]
Pearl River estuary, urban creek at Guanzhou, China	–	P	0.8 ± 0.6		4 ± 4	9 ± 7		20 ± 14			[25]

(continued)

Table 1 (continued)

	Year	Sample type	Individual pyrethroids								References
			Bifenthrin	Fenpropathrin	λ-cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Fenvalerate	Deltamethrin	
In seawater concerned by salmon aquaculture											
Close to salmon cages, Southern Chile	–	Diss						4.4 ± 0.7			[35]
Close to the shore, Southern Chile	–	Diss						2.1 ± 0.8			[35]
1–2 weeks after treatment, Norway	2014	Diss						nd		nd	[36]
Near aquaculture centers, New Brunswick, Canada	2010	Diss								nd–40	[5]
Near aquaculture centers, New Brunswick, Canada	2010	P								nd–400	[5]

The first part of the table reviews concentration from freshwater bodies, and the second part reviews data from marine environments. Sample type is referred to as follows: *WS* refers to Whole Samples (dissolved phase + particles), *Diss* stands for dissolved phase analyzed after prefiltration, *P* stands for for particles suspended in the water and collected on a filter. When the method description does not describe in detail if the water is prefiltred before extraction, its is assumed that the data concerns whole sample, and the sample type is indicated as WP?
nd not detected, *nq* below quantification limits, *x* minimum value not reported
[a]When the concentration range is not available in the reference, the average and standard deviation is reported instead

Table 2 Non-exhaustive selection of individual pyrethroid levels, in ng g^{-1}, in sediments from different locations worldwide

	Year	Sample type	Individual pyrethroids													
			Allethrin	Resmethrin	Bifenthrin	Fenpropathrin	Tetramethryn	Amethryn	Prometryn	λ-cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Deltamethrin	Fenvalerate	Delta
Freshwater sediments																
Northern California																
Del Puerto Creek	2005–2006	SED			0–286					43.3	23.1	0-21			*1.5–2.2	
Central California																
Orcutt Creek, Santa Maria River watershed	2002–2003	SED								nd–59.4	nd–107				nd–32.6	
Lower Santa Maria River	2003	SED									1.54				0.56	
Creeks and drains in the San Joaquin watershed,	2007	SED		nd–aq	nd–15.8	nd–2.6				nd–19.8	nd–14.5	nd-6.9	nd–5.7		nd–2.9	
Southern California																
Orange county		SED			21–487					nd–79.7	nd–165.2	nd-66.7	nd–34.4		nd–4.7	nd–2
Alamo River and New River, Salton Sea	2010	SED			nd–1.90	nd				nd	nd–17.56	nd	nd		nd	nd
Salton Lake sediments Southern California	–												nd–183			
San Diego River during storm events	2017															
Other sites from the USA																
Metropolitan streams, nationwise survey in the USA	–			nd–38.3	nd–11.2					nd–3.0	nd-9.3		nd–8.9			
Minnesota stormwater ponds	2009			nd	nd–37.2		nd			nd	nd–41.9	nd	nd		nd	nd
Argentina																
Carnaval creek, Buenos Aires, Argentina	2015–2016				nd					1.8–649	nd		4.2–34.8			nd
Australia																
Queensland rivers and drains	1998				0–29			0–130	0–45							
Asia																
Vietnamise Rivers	2011										nd–7,850					nd–5
Rice cultivation in Leyte island, Philippines	2010							59		nd–29			nd–1,400			nd–4

Table 2 (continued)

	Year	Sample type	Individual pyrethroids													
			Allethrin	Resmethrin	Bifenthrin	Fenpropathrin	Tetramethryn	Ametryn	Prometheryn	λ-cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Deltamethrin	Fenvalerate	Deltamethrin
Pearl River sediments, South China	–				0.38–6.54	0.37–1.49				0.35–1.87	0.88–35.4	nd	0.22–20.4		nd	nd–1.29
Wetland, Beijing, China	2004–2006												nd–0.008		nd–0.047	nd–0.448
Beijing GuanTin Reservoir, China	2003–2004	Diss											nd–8.87	nd–26.3	nd–54.2	
Beijing GuanTin Reservoir, China	2003–2004	SED											nd–0.00877	0.0454–0.158	0.0786–0.301	
Urban creek in Guanghou, South China	–	SED			6 ± 1					11 ± 8	40 ± 56		68 ± 67			
Liaohe River, northeastern China	2014	SED	0.6–29		nd–0.33	nd–23	nd–1.7			nd–4.4			1.6–33		nd–4.6	nd–4.7
Chenab river, Pakistan	2015–2016	SED			nd–325						nd–291		nd–343			134–411
Europe																
River United Kingdom, river sediments	1996–1997	SED									50–300					
Ebro River delta sediments	Jun 2009	SED		nd	nd	nd	nd			nd	nd	nd	8.27–71.9		nd	nd
Ebro River delta sediments	Oct 2009	SED											0.13–2.92			
Marine sediments																
Southern California																
Creeks and estuary, Ballona creek,	2007–2008	SED			3–80[b]					nd–15[b]	5–150[b]	nd-25[b]	1–190[b]		nd–2[b]	
Southern California Bight	2008	SED			nd–64.8						nd–132					
Ports and bays, Monterey Bay	2008–2009	SED			2.80 ± 3.31[a]											
Europe																
North Western Portugal Coast	2016–2017	SED			nd		nd		nd	nd	nd	nd	nd		nd	nd
China																
Haibei creek, Guanghou, Southern China	–	SED			nd–18.8	nd–54.5				nd–32	nd–128	nd-2.5	nd–179		nd–5.4	
Pearl River estuary, China	2012	SED			5 ng g											

The first part of the table documents freshwater sediments and the second part reviews results obtained from marine sediments

Sample type is referred to as follows: *SED* refers to the solid phase of the sediment; *Diss* stands for pore water dissolved phase

nd not detected, *nq* non quantified, compound identified in concentrations below the limits of the calibration curve

[a]When the concentration range is not available in the reference, the average and standard deviation is reported instead

[b]For [53], numbers were graphically read on Fig. 4 in [35]

Many studies reported pyrethroid concentrations in total water samples: the water collected is directly adsorbed on a SPE cartridge or is directly solvent-extracted, without previous filtration [12, 17, 22, 30]. Therefore, in these reports, both dissolved and particle-bound pyrethroids are jointly extracted and reported. A filtration step before pre-concentration was the preferred approach in some studies [26, 28, 29, 31, 33, 48], and the concentrations reported herein are that of dissolved pyrethroids, which includes the truly dissolved form and the colloidal-associated pyrethroids as part of the dissolved organic carbon pool. Pollutants associated to dissolved organic carbon are also retained in the adsorbents designed for sampling truly dissolved pollutants, together with pollutants associated to colloids, as known to occur for other hydrophobic chemicals [56]. Distinguishing concentrations of dissolved active compounds from those of particulate ones is important because both modes of occurrence are affected by distinct processes of transport and degradation rates (see later), in turn shaping differently the ultimate fate of pesticides. A strong recommendation for futures studies is to analyze separately the dissolved and particulate phases [21], and in any case, to state clearly which phase is characterized. The first part of Table 1 reviews dissolved and particle-bound pyrethroid concentration ranges. Whereas dissolved pesticides are bioavailable, it is not clear if the sorbed pyrethroids are toxic through feeding intake or as a transient repository, being desorbed later on and supporting the dissolved phase levels [31].

Pyrethroids dissolved in fresh and marine waters have been measured in a number of studies worldwide with the objective to check whether their concentrations were below thresholds of water quality guidelines. The dissolved form of pesticides is the form that is bioavailable and represents a threat for arthropods and fish. Dissolved pyrethroids were detected in agricultural drains, creeks, streams, and also in their collecting large rivers downstream agricultural land (Table 1). For example, in seven counties of California, 65–153 metric tons of pyrethroids were sold for licensed use between 1999 and 2008 [52], and 422 tons for the whole California state in 2010 [18].

The occurrence of individual pyrethroids varies geographically and seasonally as a response to agricultural use [19], and the consequent emission to the water, but probably also to different seasonal and site degradation potential. In Hospital Creek, a tributary of the San Joaquin River (Central California), bifenthrin was responsible for the greatest part of the toxicity of particles, whereas cyhalothrin was the prominent toxicant of particles in Ingram Creek, another tributary located less than 50 km away from the former [14]. Esfenvalerate and permethrin occurred in some water samples of tributaries of the Sacramento River after storm events in 2003 [15]. In tributaries of the San Joaquin River, cyfluthrin and cyhalothrin were the most frequent pyrethroids detected after winter storms, whereas bifenthrin and cyhalothrin were only identified in samples collected in March [17]. In central California, several surveys also reported bifenthrin as the main pyrethroid detected, its occurrence being related to storm events [13, 14, 16], while cyhalothrin and esfenvalerate dominated in the San Joaquin watershed [16]. Another study in Southern California sampled San Diego River during storm events and showed that six pyrethroids were present for 80% of the particle samples: bifenthrin, λ-cyhalothrin, permethrin, deltamethrin,

cypermethrin, and cyfluthrin [21]. Even though the same compounds were also detected in the dissolved phase, their relative abundance differed from that of the particles. Comparison of the suspended/dissolved concentration ratio to the soil-water partition constant showed that bifenthrin was not at equilibrium and in excess in the particles [21]. In contrast, dissolved+particulate samples collected in two others rivers of Southern California during low flow period showed much lower concentrations, and only bifenthrin and permethrin were detected [20].

Generally, the past and on-going water survey programs setup in California have yielded an important and valuable amount of data on the occurrence of pyrethroids. These studies demonstrated that one or two pyrethroids were frequently present in whole water samples, and that the dominant active compound differed in space and time (both years and seasons), reflecting the distinct agricultural targets, shifts in usages, and emissions from urban pest control [11, 19]. A metadata analysis gave the integrated view that cyhalothrin and bifenthrin were the compounds most frequently exceeding Regulatory Threshold Levels in surface freshwater of the USA and reached higher maxima in concentration [2].

In developing countries, the impact of current-use pesticides on freshwater quality is a growing concern, and an increasing literature documents pyrethroids in Asian water bodies, whereas reports on Africa are still too scarce [34]. Together with hundreds of other micro-pollutants, two pyrethroids were monitored in rivers and canals flowing through Vietnamese large cities and showed occasionally very high permethrin concentrations [23]. Cypermethrin and permethrin also dominated in the dissolved phase and in suspended particles of an urban creek, close to Guangzhou (Southern China, [25]). In GuanTin reservoir close to Beijing, deltamethrin was the more frequently detected pyrethroid insecticide in spring [24]. In streams and rivers of a rice cultivation area in the Philippines, cyhalothrin, cypermethrin, and deltamethrin were frequently detected, at concentrations exceeding water quality thresholds in half of the samples [48]. In Pakistan, deltamethrin and permethrin were close to water quality threshold in winter samples [26].

In European Rivers, permethrin was detected in the UK [27], cyhalothrin and cypermethrin in dissolved water and suspended particles of seven streams of Central Germany, especially after rain events [31]. Cypermethrin was the most frequently detected pyrethroid in the dissolved phase of the Ebro Delta (Spain), where rice is cultivated [28, 29]. Cypermethrin and deltamethrin concentrations varied in space and time, with peaks in concentration at the end of May followed by an apparent removal within 3 weeks [28, 29]. This finding demonstrated, by in situ observations, the fast degradation of pyrethroids in freshwater. In another Spanish rice paddy area, cypermethrin, bifenthrin, esfenvalerate, and cyhalothrin were present in most surface and groundwater total water samples analyzed [30], with the number of pyrethroids detected and their concentrations exceeding those measured in the Ebro Delta. In addition to broadcast on paddy fields, urban emissions through waste water treatment plant emissaries were likely responsible for this contamination. Despite a more restricted literature on European waters than for American ones, pyrethroid residues occur in agricultural freshwater environments and their concentrations may exceed

threshold values especially in suspended particles after rain events (in 80% of the samples in Germany [31]).

Because pyrethroid pesticides have been quite often detected in streams, creeks, and receiving rivers, they should also reach marine coastal waters. However, research addressing the occurrence of pyrethroids in estuarine and marine environments is limited. Due to the dilution of river water into the sea, pesticides often fall below detection limits. For instance, in seawater off Portugal, only two of the nine targeted pyrethroids could be detected, and only one could be quantified, whereas five were present in oysters [33]. Analytical difficulties may be a reason for the scarcity of published data in seawater (Table 1).

A specific risk for the marine environment is associated with aquaculture treatment of salmon against ectoparasites [5, 57]. Formulations used in aquaculture contain deltamethrin or cypermethrin together with emulsifiers for bath treatment of caged fish. Once the treatment is over, the bath water is released into the seawater, where pyrethroids are diluted by currents. In a case study in Canada, the deltamethrin plume could be detected up to 5.5 h after emission and the plume extended a few km away from the cages [5]. In this study, deltamethrin was emitted as a dissolved pesticide, and it was monitored both in the dissolved phase and in the suspended particles. Interestingly, deltamethrin concentration in the particle phase was approximately three to four times greater than in the aqueous phase, which demonstrates the quick partition of pyrethroids to organic carbon in seawater and, thus, their affinity for particles [5]. Variable responses of natural marine microbial communities to the input of anti-lice pesticides have been evidenced in Southern Chile [58]. At some locations and season, deltamethrin inputs resulted in an increase of carbon fixation by photosynthesis, likely resulting from a decrease in arthropod grazing pressure; however increase in carbon fixation was also observed at other sites and seasons. The diverse responses observed evidenced complex relationships between environmental factors (nutrient levels, zooplankton abundance, etc.) and pesticide impacts. These responses of marine organisms, distinct from toxicity alone, need further research to understand the overall impact of aquaculture and, more generally, of pyrethroid emissions, on marine ecosystems. More detailed information on the effect of salmon industry in the marine environment is presented elsewhere [6].

However difficult it is to detect pyrethroids in the marine environment, this task should not be overlooked because marine crustaceans and fish have been reported to be more susceptible to pyrethroids than freshwater ones [29, 34, 48].

4 Occurrence and Composition of Pyrethroids in Sediments

Table 2 documents pyrethroid occurrence in sediments. The solid phase of sediments acts as a sorbent for pesticides and likely integrates over time water pyrethroid concentrations in the overflowing water and also the accumulation of sinking particles in sea and river beds. Because of their quick association to river sediment, pyrethroid contamination of riverbed sediment has emerged as an important environmental threat to benthic organisms, and the literature reporting sediment toxicity

of pyrethroids has developed in the recent decade. Sediment toxicities toward the benthic amphipod *Hyalella azteca*, toward the cladoceran *Ceriodaphnia dubia*, and toward the midge of the Diptera *Chironus dilutus* are common tools to survey environmental quality of freshwater sediments. When pesticides are also measured, it allows to identify which toxicant causes the observed impairment [11, 38, 49, 59].

Recent monitoring studies document the occurrence of several pyrethroids in riverbed sediments (Table 2) and have been reviewed at the global scale by Stehle and Schulz [60]. Their residual occurrence in sediments is presently recognized as a threat to diversity of sediment-dwelling invertebrates and also as the cause of a decrease of diversity in aquatic environments at a global scale. Table 2 reports sediment pyrethroid concentrations at sites covering several continents. In some studies, sediment pore water concentrations are also given together with solid phase sediment concentrations. The occurrence of pyrethroids in sediments evidences clearly the propensity of pyrethroids to sorb onto and into particles. Owing to the large organic carbon pool comprised in sediments, sediments have the potential to act as a sink for pyrethroids. Organic carbon content, silt, and clay fractions are sediment bulk characteristics that usually correlate with pesticide levels [11, 24].

The concern about pyrethroid sorption to sediments in Californian streams exposed to agricultural and urban emissions led to the development of monitoring programs addressing the benthic environment in addition to water-based surveys. The considerable amount of data generated by those programs points to bifenthrin being the most commonly found residues in the sediments (Table 2). In Del Puerto Creek, a northern California stream flowing through agricultural land, it was the main contributor to sediment toxicity, with a smaller contribution of cyhalothrin, esfenvalerate, and cyfluthrin [37]. In sediments from the Santa Maria River (central California), the pesticide chlorpyrifos was the main contributor to the toxicity to the benthic amphipod *Hyalella azteca*, while cyhalothrin and permethrin also contributed to sediment toxicity in some locations in June 2002, but not in May 2003 [38]. In sediments collected in California from 2008 to 2012, the most frequent pyrethroid detected was bifenthrin; the other active compounds cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, esfenvalerate/fenvalerate, fenpropathrin, or permethrin, occurred in one fifth to one third of the samples [11]. Bifenthrin was also the main pyrethroid in sediments of rivers alimenting Salton Sea in southern California [41]. In an urban estuary of southern California (Ballona Creek, Los Angeles), permethrin dominated over bifenthrin, while cypermethrin and cyfluthrin were next in abundances [52]. In Minnesota, permethrin and bifenthrin were at the top of pyrethroid sales, permethrin for animal care, structural applications, home and garden holding, while bifenthrin was mostly used as crop chemical [44]. In this state, 33% of sediments of stormwater ponds contained permethrin and 20% bifenthrin; this pattern was in line with results from other urban locations statewide as reviewed by Crane [44]. Another nationwide study addressed metropolitan streams in the USA and found bifenthrin detected in 47% of the bed sediments followed by cyhalothrin, while permethrin, resmethrin, and cypermethrin occurred with much lower frequency [43]. Recent observations in 99 streams across Midwest USA also found

bifenthrin responsible for most of the toxicity in half of sediments and also attributed urbanization rather than agriculture as responsible for its emission [59].

In Southern America (Argentina), cyhalothrin was the dominant pyrethroid in sediments of rivers flowing through large monocultural horticultural fields [45]. The percentage of detected herbicides and pesticides varied seasonally according to their application, while pyrethroid residues were consistently detected in sediments, attesting for an environmental risk for the benthic biota.

An increasing body of literature evidences pyrethroid occurrence in Asian riverbed sediments and shows the prevalence of cypermethrin at many sites (Table 2). In large cities of Vietnam, permethrin was the dominant pyrethroid, and its geographical repartition brings evidences that it is sourced by structural and householding uses and disease vector controls rather than agricultural spraying [47]. Deltamethrin was only detected once in this study but at very high levels from an undetermined source. In Southern China, cypermethrin, cyhalothrin, permethrin, and deltamethrin dominate over other pyrethroids in sediments of the Pearl River; their concentrations may reach notably high values in small creek sediments collected upstream in the river [49]. Cypermethrin and permethrin also dominate in sediments from an urban creek, close to Guangzhou (Southern China, [25, 61]). In Beijing GuanTin reservoir, fenvalerate and deltamethrin were the dominant pyrethroids [24]. In Pakistan deltamethrin and permethrin were the dominant pyrethroids, with deltamethrin present in all samples and reaching concentrations above environmental quality thresholds (namely, NOEC of *Hyalella azteca* [26]).

Australia's state Queensland has a low population and sugarcane and cotton cultivation dominate its agricultural activities. Ametryn and prometryn were the most frequent pyrethroids detected in sediments from irrigation drains and channels, reaching high concentration levels, while bifenthrin occurred in only one cotton production area [46].

In Europe, cyhalothrin and cypermethrin are ubiquitous at large river mouths, whereas riverbed sediment also showed frequent amounts of bifenthrin and tefluthrin, together with cypermethrin and cyfluthrin in some rivers of Italy and France [62]. In sediments of the Ebro Delta (Spain), cypermethrin was detected in some sediments, whereas deltamethrin, detected in the water, was below detection limits in the sediments [28]. In contrast, cypermethrin, cyfluthrin, and esfenvalerate were abundant in the paddy fields of Albufera de Valencia [30]. These paddy fields are filled with water coming from a lake receiving agricultural and urban effluents, and both surface water and groundwater contained high levels of dissolved phase pyrethroids.

Similarly to the reports of seawater concentrations, pyrethroid abundances in marine sediments are evaluated by a limited number of comprehensive studies. In an intensely urbanized estuary in Southern California, bifenthrin and cyfluthrin were the most frequently detected pyrethroids with their highest concentrations at 132 and 65 ng/g, respectively, at sites located near sources of runoff emissions from urban watersheds. They accounted for a part of the toxicity of the sediments to a standard amphipod *Eohaustorius estuarius*; however they were not the major toxicant at all the studied stations [52]. Samples with the highest concentrations of pyrethroids

were located in close proximity to river mouths and cities, whereas samples located more offshore showed lower concentrations, or pyrethroids were below detection limits. This distribution supports urban pyrethroid emissions. In another area of Southern California, sediments from the Monterey continental shelf were analyzed together with suspended solids in the three rivers flowing into this marine region. Whereas pyrethroids were found in almost all rivers particles (sampled after rain events), with bifenthrin and permethrin as the dominant pyrethroids, they could not be detected in the estuary nor in the deeper sediments of the Monterey canyons (from 100 to 300 m depth). A similar situation was observed in marine coastal waters off Portugal, whereas no pyrethroid could be detected in sediments, while cypermethrin was detected in the dissolved phase and tetramethrin, bifenthrin, cyhalothrin, fenvalerate, and permethrin occurred at low concentrations in some samples of oysters collected in the same area [33]. In marine sediments, contaminated river particles are diluted by the autochthonous marine particles and by older riverine particles in which pyrethroids have had the time to be degraded. As a consequence of dilution, pyrethroids are often below detection limits in marine sediments (Table 2).

A recent review documented the occurrence of pyrethroids in sediments worldwide and showed significant correlations between pyrethroid occurrence and sediment toxicity [7]. The good correlations obtained proved that pyrethroids were the main cause of toxicity and strongly suggested potential ecological risk to nontarget aquatic species. Nevertheless, at some locations, such as in sediments from the Pearl River Delta (China), other pollutants than pyrethroids likely contributed to the overall toxicity of sediments. The authors concluded that the frequent occurrence at high concentrations of pyrethroids in sediments from agricultural and residential areas constitute a threat to freshwater ecosystems [7].

5 Pyrethroid Degradation

A characteristic feature of pyrethroid contamination in water and benthic ecosystems is that a few compounds of the pyrethroid family may be present but not all the series, in concentrations generally under the 100 ng/L range for water samples or under the 100 ng/g range for sediments. Pyrethroid occurrence is highly variable in time and space, so that samples from a given area may show detectable amounts of one or several pyrethroids while others do not or comprise other active compounds. This feature is much different from other ubiquitous pesticides classes and is a consequence of their higher lability. The routes of degradation of pyrethroids may be abiotic (hydrolysis, photolysis, and oxidation) or mediated by bacteria and fungi. Pyrethroids degradation by microorganisms and fungi have been studied in soils [63, 64]. Various carboxylesterases may induce the degradation of pyrethroids; generally one gene exists in one pyrethroid-degrading microorganisms, with the exception of *Ochrobactrum anthropi*, that possesses two pyrethroids degrading genes [63]. Optimal conditions of pyrethroid biodegradation are between 30 and 35°C. Organic matter and clay content are also important parameters controlling

pyrethroid bioavailability to microorganisms. Half-lives of bifenthrin, cypermethrin, and permethrin in soils were 12–1,410, 14–106, and 5–55 days respectively, under temperature conditions between 25 and 30°C (Table 2 in [63]). The biodegradation rates in freshwater sediments have been seldom determined, and they are longer than in soils [18]. Depending on conditions, long persistence was observed for bifenthrin and permethrin. Under both aerobic and anaerobic conditions, and the half-life of bifenthrin in sediment of drainage channels ranged from 8 to 17 months at 20°C, while that of *cis* and *trans* permethrin varied between 2 to 13 months [65]. In liquid media, bacteria (*Bacillus*, *Brevibacillus*, *Ochrobactrum*, *Pseudomonas*, *Serratia*, and *Sphingobium*) and fungi (*Cladosporium*, *Candida*) degrade efficiently pyrethroids. At temperatures ranging from 27 to 38°C, most strains degraded pyrethroids within 5 days, with the fastest degradation observed for permethrin in 3 days [63]. However, the experimental conditions at which the experiments were carried out were not the same as natural field conditions, where lower temperatures and lower bacteria or fungi abundance can be expected to increase half-life of pyrethroids.

6 Pyrethroid Occurrence in the Atmosphere

Because of their relatively low vapor pressure, pyrethroids are assumed to have low tendency to volatilize during application, as well to revolatilize from soils or water bodies [7]. During application, 20–30% of the applied doses can be emitted as aerosols and drift away from their source by atmospheric transport [66]. Post-application emissions have also been reported to occur via volatilization [67]. For deltamethrin, having one of the lowest Henry's law constant values among pyrethroids, it was experimentally demonstrated that 70% of deltamethrin sprayed on the surface of the water was quickly emitted as aerosols [68]. Taken as a whole, these evidences point to likely atmospheric emissions of pyrethroids, at least during and shortly after application by spray broadcasting.

The widespread occurrence of pyrethroids in some areas also questions whether their volatilization to the gas phase is possible, ensuing a likely atmospheric transport to proximate or remote ecosystems (see Sect. 7). A few reports have recently evidenced that pyrethroids were present in the atmosphere, both as aerosols and as vapors in the gas phase. The particle-bound fraction is susceptible to be atmospherically deposited or to be washed out by rain or snow whereas gas-phase pyrethroids will be removed by photodegradation or air-soil, air-vegetation, or air-water diffusive exchange, probably resulting in longer atmospheric residence times [69]. Table 3 reviews the concentrations of pyrethroid insecticides bounds to aerosols or as vapors. The first report of pyrethroids in the gas phase of Brazilian alpine reserves showed that cypermethrin was the second pesticide in abundance, whereas gas phase concentrations of legacy pollutants, such as chlordane, chlorinated cyclodienes and hexachlorobenzene, were around background levels [70]. In aerosols and in the gas phase of Guangzhou (south China), eight pyrethroids were detected, and

cypermethrin was the dominant one [71]. Concentrations of aerosol-bound cypermethrin were comparable to those measured in a horticulture area in Malaysia [72]. Li et al. measured allethrin and tetramethrin in higher proportions in the gas phase whereas bifenthrin, cyhalothrin, permethrin, cyfluthrin, and cypermethrin were predominantly associated with the aerosols [71]. Bifenthrin was also detected in almost all samples of fine aerosols in Northern Brazil [73].

The recent recognition of pyrethroid occurrence in aerosols and in the gas phase opens a challenging view of their biogeochemical cycle and prompts further research to assess the relevance of atmospheric transport and occurrence of pyrethroid insecticides.

7 Key Physicochemical Properties of Pyrethroids, Transport Processes, and Modelling

Legacy pollutants like polychlorinated biphenyls (PCBs), chlorinated pesticides such as *p*,*p*′-dichlorodiphenyltrichloroethane (DDT), lindane, and organophosphate pesticides persist long enough in the environment to be transported by advective and diffusive processes and undergo long-range transport far away from their primary emission regions. Diffusive transport of pesticides results in an environmental partitioning of these pollutants among the different environmental matrices, such as water, particles, air, soils, biota, and sediments. For instance, water-particle partitioning is the result of a net quantity of pesticides transferred from the dissolved water phase to the organic part of the particles. Meanwhile the quantities of water, of particles, and of organic carbon do not change concurrently when pesticides partition among these phases. A change of any of these quantities would induce a re-partitioning of the chemical. Other relevant diffusive processes are air-water exchange, water-sediment partitioning, gas-aerosol partitioning, bioconcentration in organisms at different trophic levels, etc. Organic carbon occurrence in water stretches from truly dissolved organic carbon to particulate organic carbon, with a continuum in particle sizes. The division of dissolved and particle phase is operational, usually the dissolved phase refers to the pesticides passing through the filter cut-off size (e.g., 0.7 μm for a GF/F filter), but this dissolved phase can also include the colloidal phase. In Fig. 1 relevant diffusive (partitioning) processes for pyrethroids are represented by the wide gray arrows. Diffusive partitioning is always driven by a fugacity gradient among the two phases and is always a bidirectional process. In contrast to diffusive processes, an advective transport consists in the movement or flux of the phase itself, transporting the pesticides which it contains. Advective transport processes of pyrethroids in aquatic environments are represented by the thin black arrows in Fig. 1. For example, the transfer of atmospheric pesticides to soils or aquatic ecosystems can be by air-water exchange (partitioning) or by wet and dry deposition, which are advection transport processes. In dry deposition there is a settling of aerosol-bound pesticides, while in wet

Table 3 Selection of individual pyrethroid concentration in the atmospheric gas phase, in ng m^{-3}, and in aerosols in ng g^{-1} from different locations worldwide

	Year	Sample type	Individual pyrethroids							
			Allethrin	Bifenthrin	Tetramethryn	λ-cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	References
Gas in pg m^{-3}										
Brazilian alpine mountains, nationa parks	2013–2015	G					nd–40		nd–881	[70]
Guangzhou, urban area, South China	2011–2012	G	nd–66	nd–48	nd–8	nd–nq	nd–37	nd–nq	nd–16	[71]
Floriculture region Malaysia	2004	G							142–2,740	[72]
Aerosols in pg m^{-3}										
Guangzhou, urban area, South China	2011–2012	A	nd–139	nd–54	nd–28	nd–51	nd–88	nq–17	17.1–1,380	[71]
Todos os Santos, Northern Brazil	2010	A		14–72			62–945			[73]

nd not detected, *nq* under quantification limits, *G* atmospheric gas phase, *A* atmospheric aerosols

deposition by rain or snow, there is a scavenging of gas and aerosol phase pesticides by the rain drops or snowflakes. In terms of primary sources, after pesticide application on agriculture fields (rice, cotton, wineyard, etc.) by spraying, pyrethroids may reach surface aquatic environments through edge of field runoff, which is an advective soil to water input of irrigation water or rain water, entraining dissolved pyrethroids and also pesticides bound to particles or that have re-partitioned to the run-off water. Storm events after pesticide treatment have been shown to release high amount of pyrethroids into freshwater streams in the vicinity of fields [37]. Despite degradation and dilution processes, pyrethroids sorbed to river suspensions are effectively transported to the lower stretches of rivers [18, 63]. Particle vertical settling and sediment resuspension are advective processes transporting pyrethroids between water and sediment, which transport chemicals in parallel to the water-sediment diffusive partitioning. Nevertheless, the latter may only be effective for sediment pore water and benthic waters, while settling of organic carbon-bound pyrethroids is an advective flux affecting all the water column. Soils may act as transient repositories for pyrethroids that may gradually be desorbed into irrigation or rain water by leaching. In addition, sorption to soils, particle, and sediment may lower their degradability and thus increase their persistence in the environment [65]. Similarly to diffusive sediment-water exchange, particle-water exchange (or partitioning) continuously occurs, with a distribution of the chemical between organic carbon and the dissolved phase depending on temperature and quality of the organic matter.

The key condition for pyrethroids to be transported away from their source is that they persist long enough in the environment before being degraded. Their potential for being transported is also dictated by their physicochemical properties. The octanol-water partitioning coefficient, K_{OW}, characterize the potential of compounds for being absorbed into organic matter, either in sediments or in suspended particles. Even though, conceptually, it does not take into account surface adsorption, it is a common practice to use K_{OW} as a surrogate for adsorption/absorption, as experimentally it is very difficult to discern organic pollutants adsorbed or absorbed to particulate organic carbon. Henry's law constant (H) or the dimensionless Henry's law constant ($H' = K_{AW}$=H/RT) of a given pollutant characterizes its air-water diffusive partitioning and thus its potential to accumulate in water or being volatilized to the atmosphere facilitating their long-range transport. Each pyrethroid has specific values for these physicochemical constants. Figure 2 shows the phase space for organic chemicals and compares the values of both constants for pyrethroids to the values of these partitioning constants for other pollutant classes which behavior in the environment is better studied and understood. The phase space shown in Fig. 2 provides a simplified view of environmental partitioning and transport potential. Compounds in the upper area of the plot space have a higher potential to partition to the gas phase relatively to water than compounds on the bottom area of the plot. Similarly, compounds plotted on the right area of the plot have a greater potential to partition to organic carbon relatively to water than those plotted on the left side. Permethrin is plotted very close to PCB 101, thus have the similar partition characteristics than PCB101 and bifenthrin have an even higher K_{AW}. Therefore, both

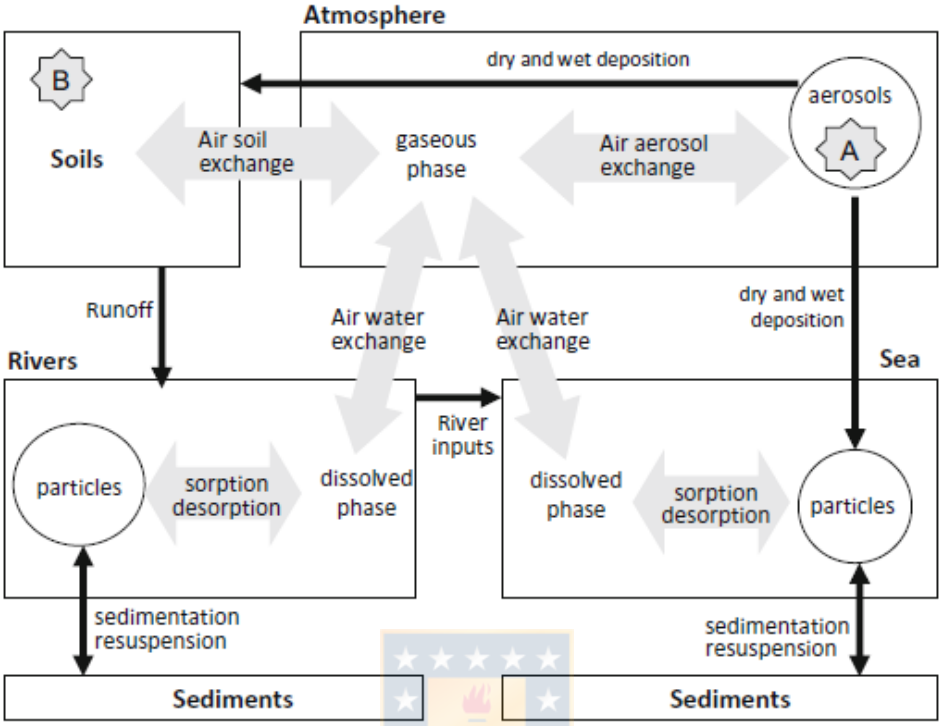

Fig. 1 Scheme of the geochemical cycle of pyrethroids in the environment. Boxes represent the environmental phases. The soil box represents both the solid phase of soils (plants and soil particles) and the soil porous water. Arrows represent the fluxes between phases, thin black arrows stands for fluxes of key transport (advective) processes and large gray arrow show key partition (diffusive) fluxes. Gray stars symbolize pyrethroid direct emissions to the environment; A is the emission that remains as aerosol during spray application, mostly to cropland; B is the emission that is deposited on soils and plant during spray application. See text in Sect. 7 for more explanation

compounds have a potential for long-range transport through grasshopping, that is, successive volatilization and deposition steps. In the case of pyrethroids, the potential for long range transport is limited by their potential degradation in the environment. It has to be underlined that in the case of cold environments with snow deposition events, even chemicals with high K_{AW} partition coefficients can be deposited due to the high sorption capacity of snow [74]. More importantly, the physicochemical characteristics of the other pyrethroids are similar to that of high molecular weight polycyclic aromatic hydrocarbons (PAHs), DDT and its degradation products (DDE and DDD), and hexachlorobenzene; therefore pyrethroids can be expected to have the same environmental behavior. In contrast, organophosphosphate pesticides have a greater solubility in water (lower K_{AW}) and will behave more as "swimmers," tending less to sorb on particles and with limited atmospheric transport [75].

In the case of legacy persistent organic pollutants (POPs), their important emissions combined to analytical progresses made it possible to quantify their

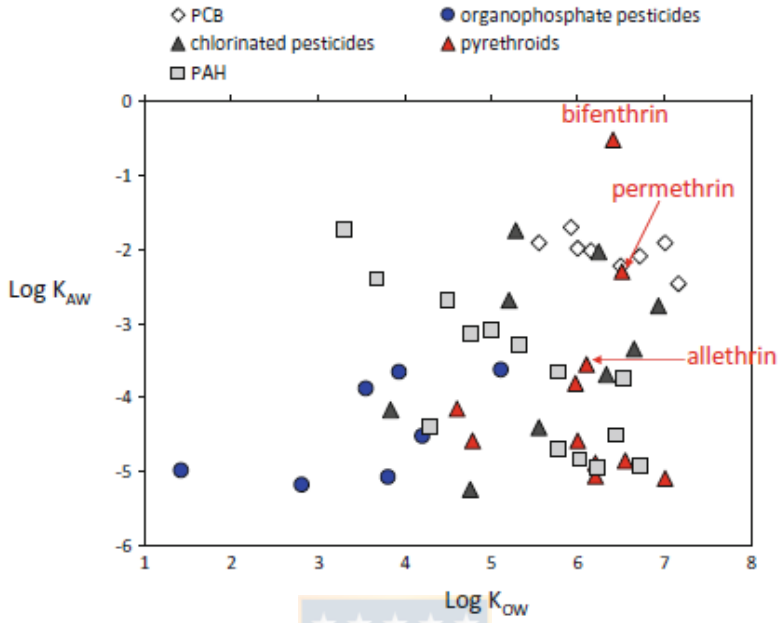

Fig. 2 Comparison of the partition behavior of current-use pyretroid insecticides and of other legacy pollutants. K_{AW} is the air-water partition coefficient, and K_{OW} is the octanol-water partition coefficient

abundances in water, suspended particles, sediments, atmospheric gas, and aerosols phases from regional to a global scales. Scientific efforts addressing pollutant detection in several environmental compartments brought quantitative appraisals and understanding of transport fluxes between air, seawater, soils, etc. This holds true for PCBs [76] and PAHs [77] but also for pesticides like lindane [78]. In contrast to legacy pollutants, pyrethroids are current-use pesticides, and they have been used and emitted to the environment for only the last few decades, and scientists have been able to quantify pyrethroids at environmental levels only for a decade [79]. As a consequence, the occurrence of pyrethroids in environmental phases relevant to the understanding of their biogeochemical cycle is still incompletely understood.

A comprehensive assessment of pyrethroid cycle in an urban area of Southern China used a fugacity-based model coupled to concentrations measured in different environment phases to calculate the diffusive and advective fluxes [25]. Sinking of suspended particles accounted for the higher fluxes, and resulted in water bed sediments fluxes 1 or 2 order of magnitude higher than air-water diffusive exchange. The higher fugacity of pyrethroid in water than in the gaseous atmosphere drove volatilization fluxes from the water to the air, permethrin, and cypermethrin having the higher fluxes. Despite this work, pyrethroids have received less attention in terms of their fate, transport, and biogeochemistry, and how these processes ought to be modelled. The comparison with other families of POPs with similar properties provide clues of their environmental fate and point to potential research efforts to be carried out in the future. Unless pyrethroids are efficiently degraded in the

atmosphere, some of them have the potential for long range transport as pentachorinated PCBs, 4–5 rings PAHs and DDD (Fig. 2). In comparison to those legacy pollutants and hydrocarbons, current-use pesticides such as pyrethroids are often reported in one environmental phase, chiefly dissolved freshwater phase or riverine sediments. Both dissolved phase and suspended particles [31] or suspended particles and sediments [14] or dissolved water phase and sediments [24, 27, 28, 33] are considered jointly in order to assess combined risks for the water ecosystem and for the benthic ecosystem. Future research efforts should address their multiphase partitioning, including the atmosphere, to elucidate their capacity to affect proximate or distant ecosystems from their primary sources. The advective transport of pyrethroids has been largely addressed only in relation to their dispersion by river flow notably during storm events. However, the partition between dissolved pyrethroids and particles is specifically addressed by one study, showing that for this particular site, a diffusive flux of bifenthrin existed from the particles toward the dissolved water [21].

8 Future Research Integration

Because of their rapid decay, pyrethroids are reported above detection levels in areas and at times closed to their point sources, and a global appraisal is still missing. It can be foreseen that pyrethroids might threaten biodiversity in some geographical areas where data is still lacking to date. Most croplands are indeed not studied for pyrethroids (Africa, Brasil, etc., see review [62]). In African market, esfenvalerate was the highest pesticide residue in fruits and vegetables, and allethrin was also detected, attesting for their use [80–83]. Ukraine, Pakistan, Turkey, Paraguay, and India registered the larger pyrethroid use while environmental informations on pyrethroid occurrence are mainly lacking for those countries [7, 26].

Pyrethroids are degraded in the environment so that they are not conspicuously detected, with the exception of some agricultural or urban areas. Their high degradation rates with respect to legacy pollutants support the belief that they are unlikely to persist in the environment. However, extension of cropland and of urbanized space will likely result into an increase in pyrethroid uses and emissions, because better alternatives to control pests are still lacking. In the case where the rate of inputs of pyrethroids would compensate for their degradation, pyrethroid occurrence may become more continuous and their behavior may then be assimilated to that of "quasi persistent organic pollutants", with secondary transport evading them away from their application area. In California, past and current monitorings have demonstrated that there is a persistent threat to aquatic ecosystems because of current-use pesticides, with an increasing share by pyrethroids [19].

In conclusion, the shift to current-use pesticides demands a better understanding of the occurrence of pyrethroids in developing countries where the market shares are the highest. The partition, transport, and degradation fluxes of pyrethroids need to be

better appraised locally, regionally, and globally, taking into account the so far underestimated importance of atmospheric transport.

River flow efficiently transports pyrethroids to river mouths and estuaries. It is difficult to detect pyrethroids in the marine environment because of dilution. However aquaculture is a locally direct source that likely constitutes an important environmental burden for seawater, which it is very poorly surveyed and comprehensively understood.

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7.2 Appendix 2**.**

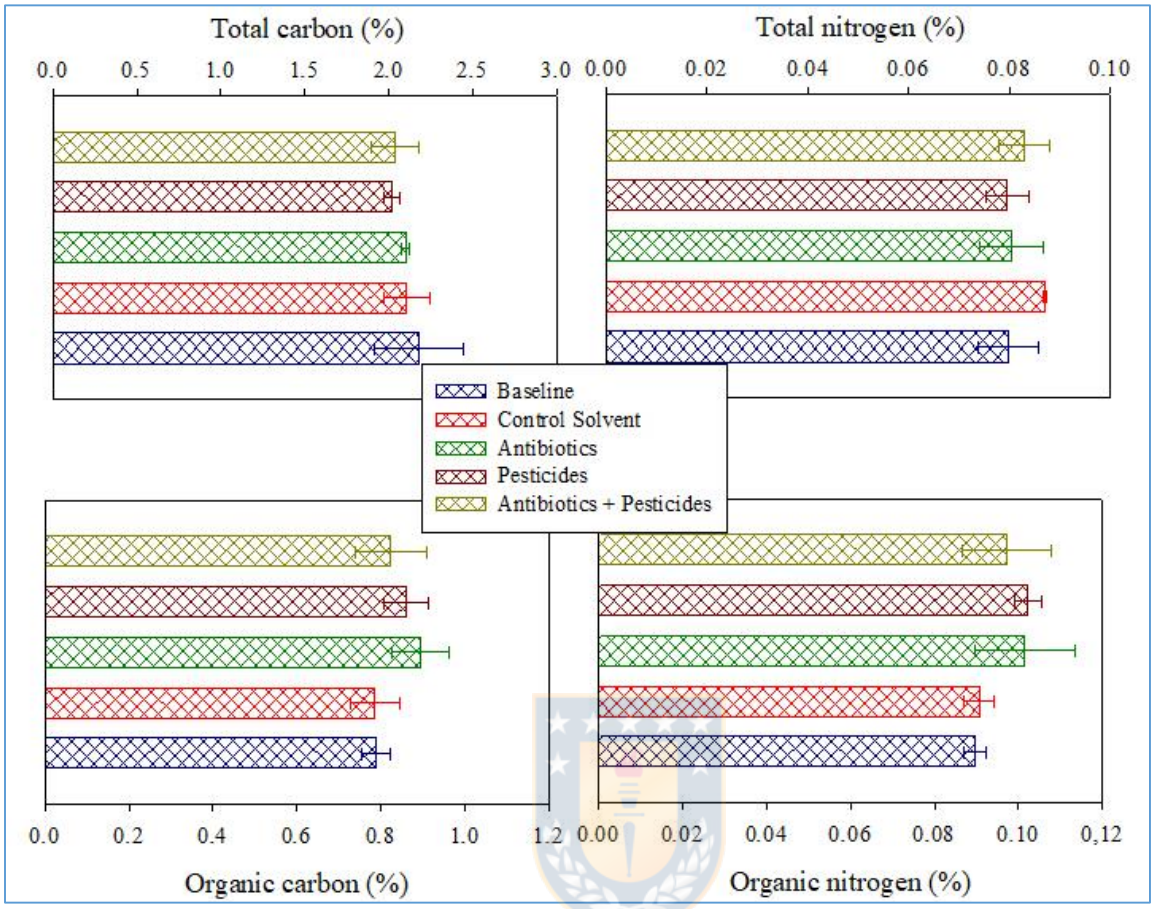

Figure 1. Total carbon, Total nitrogen, Organic carbon, and organic nitrogen (%) in the sediments at the beginning (baseline) and final respiration experiment in sediment microcosms.

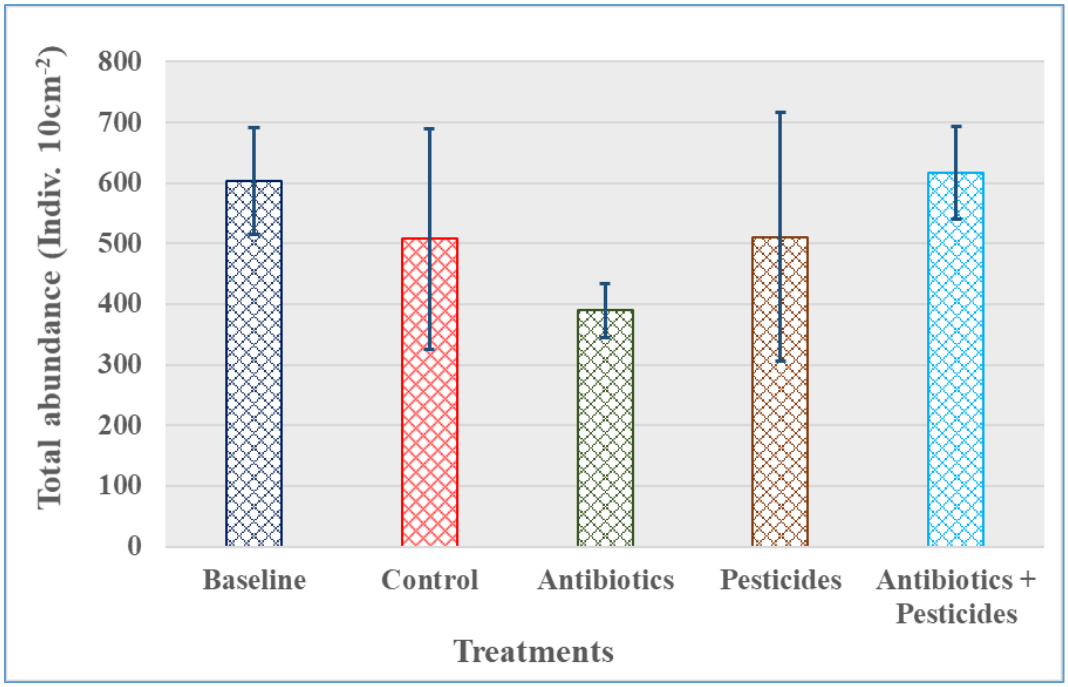

Figure 2. Total abundance of meiofauna (average ± standard deviation; Indiv. $10cm^{-2}$) in the top 3 cm of the sediments, at the beginning (baseline) and the final experiment, in sediment microcosms.

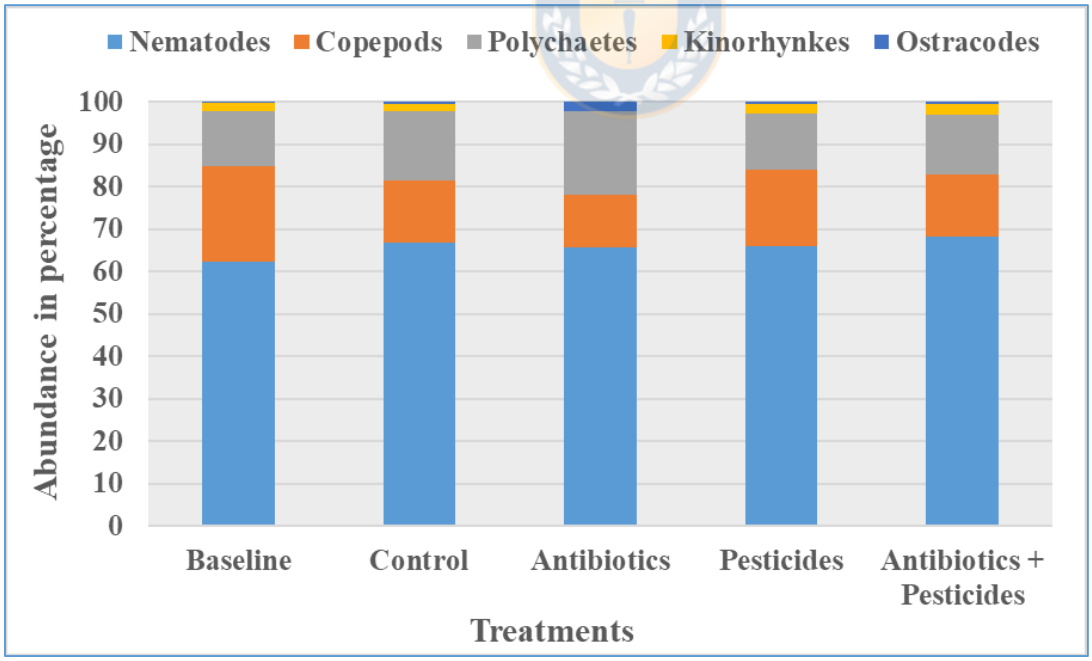

Figure 3. Diversity of meiofauna (average, %) in the sediment at the beginning (baseline) and the final respiration experiment in sediment microcosms.

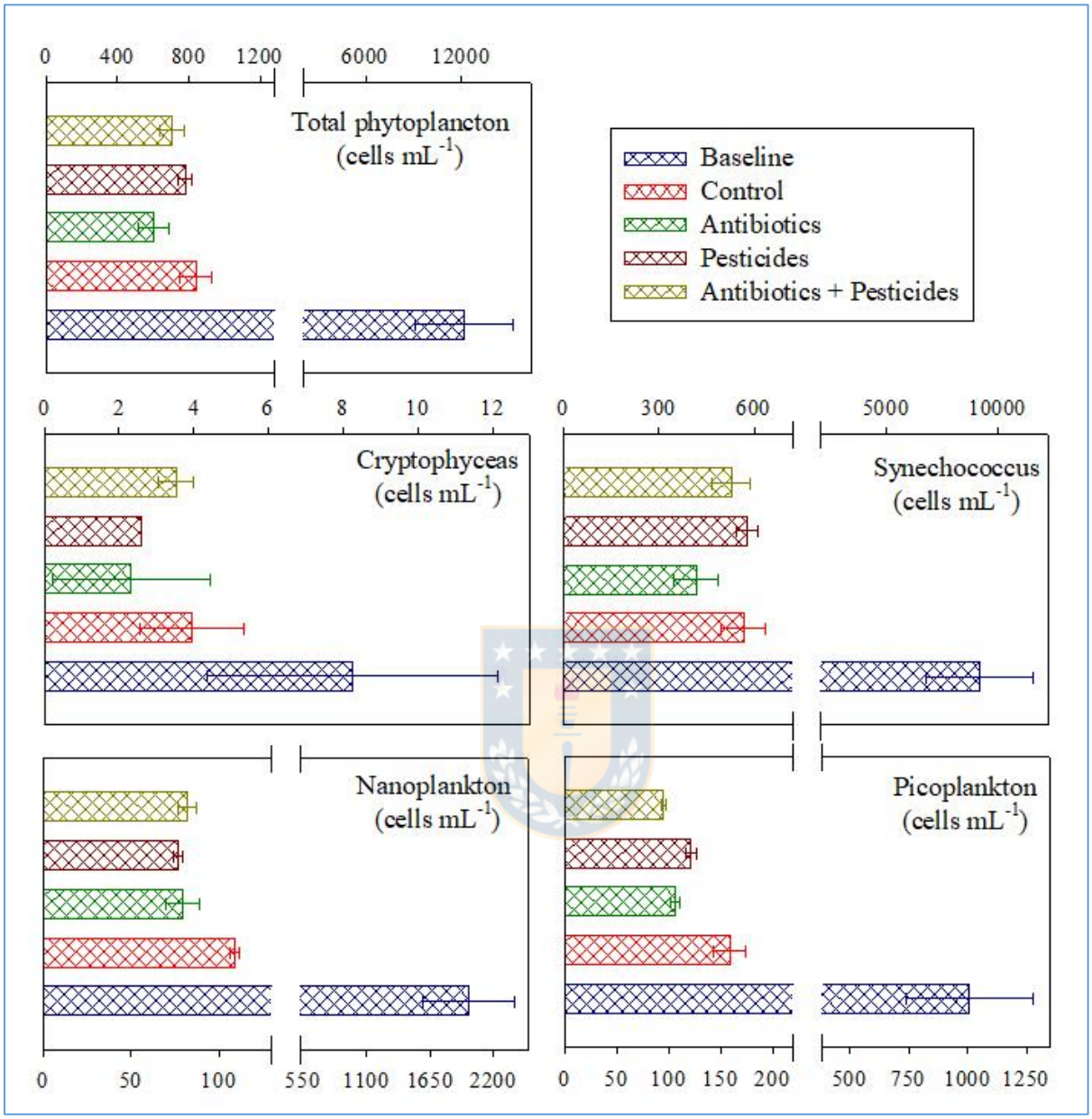

Figure 4. Phytoplankton abundance (average ± standard deviation, cells mL^{-1}) and diversity (by flux cytometry) at the beginning (baseline) and the final respiration experiment in water column microcosms.

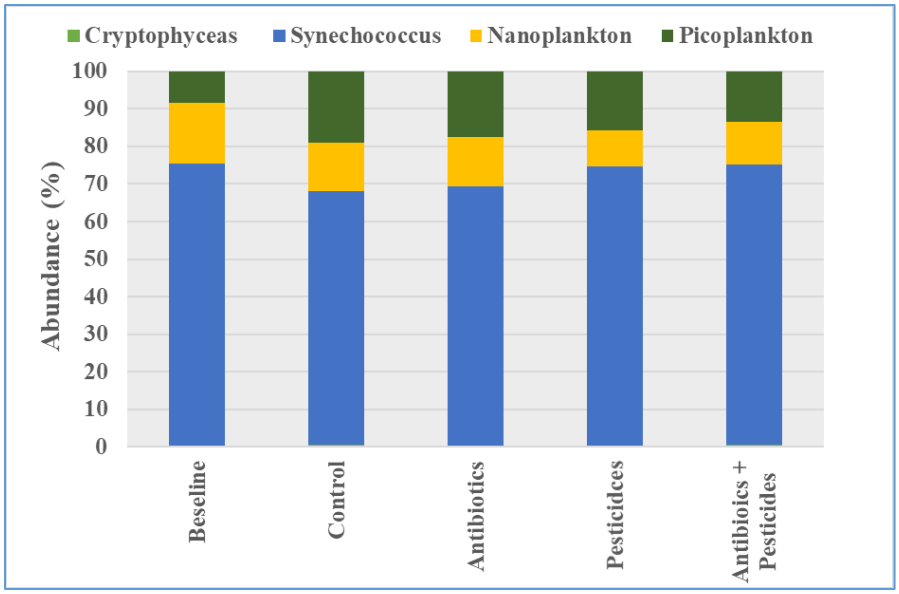

Figure 5. Abundance distribution (%) of phytoplankton group in water column microcosms for the beginning and the final respiration experiment.

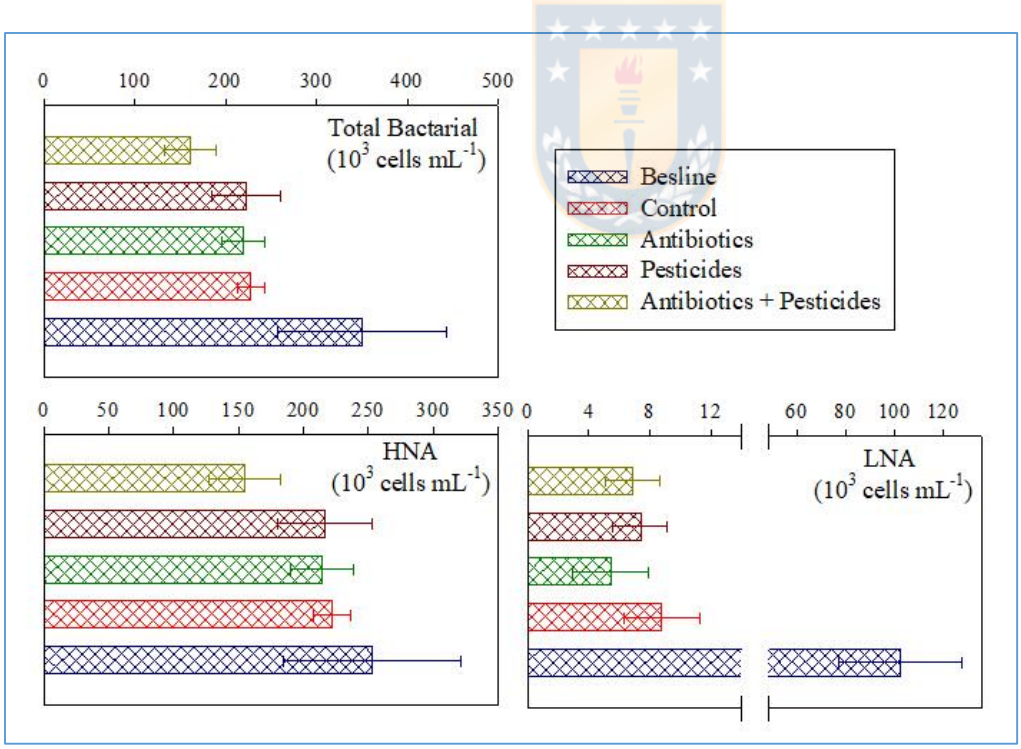

Figure 6. Bacterial abundance (average ± standard deviation, 10^3 cells mL^{-1}) at the beginning (baseline) and the final respiration experiment in water column microcosm. HNA: High acid nucleoid content, and LNA: Low acid nucleoid content.