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Effects of ethoxyquin on metabolism and composition of active marine microbial communities

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Highlights

- Ethoxyquin generates changes in the metabolism of the planktonic microbial community.
- Microbial capacity to process different carbon substrates was altered by ethoxyquin.
- Ethoxyquin can alter the composition of the active planktonic microbial community.
- Communities from areas with and without salmon farms respond differently to ethoxyquin.

Abstract

Salmon culture has the potential to generate environmental impacts due to the release of organic matter and chemical products into the water column. Ethoxyquin (EQ) is a food additive widely used as an antioxidant in fish feed, but due to the scarce evidence on its safety for the environment it has been banned by the European Food Safety Authority. To evaluate and compare responses of marine microbial communities to EQ additions (10, 100 and 1000 µg L⁻¹), we carried out 20L seawater microcosm experiments in two distinct areas, Achao and Coliumo Bay, where the first is influenced by salmon farming. We estimated metabolic response (respiration, extracellular enzymatic activity and ECOplates) for two size fractions (< 1 µm and < 63 µm) and changes in the active microbial community diversity. The results revealed different responses associated with study sites and size fractions. For the < 1 µm fraction (bacterioplankton), the addition of EQ increased both oxygen consumption and the degradation of proteinaceous compounds, while for the entire community (< 63 µm), the addition of EQ decrease respiration and the capacity to degrade carbohydrates and lipids. The carbon substrates utilization was variable, decreasing in the 100 and 1000 µg EQ L⁻¹ treatments, suggesting that high EQ concentrations may limit the capacity to process different carbon sources, producing a preference for amino acids and carboxylic acids in the first hours of exposition in Achao. In the area without salmon farming (Coliumo Bay) substrates utilization, mainly carbohydrates and carboxylic acids, was stimulated by the addition of EQ. A shift was observed in the active microbial community between treatments at different taxonomic levels, especially associated with Alphaproteobacteria, which presented lower activity when exposed to EQ. Gammaproteobacteria (K189A), Bacteroidia (NS10, NS3b clade) and MGII showed higher activity associated with EQ additions. Overall, our study indicates that EQ may generate both changes in the metabolism (respiration, enzymatic extracellular activity, carbon sources preferences) and composition of the active microbial community in the water column altering the degradation of organic matter.

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Introduction

Chilean Patagonia, located on the southeast border of the Pacific Ocean, has an intricate geography composed of many islands, peninsulas, fjords and channels (Silva and Palma, 2008), constantly influenced by freshwater from rivers, runoff, precipitation and glaciers (Pantoja et al., 2011b). The area has been of particular interest for the development of tourism, fishing and aquaculture. In fact, aquaculture, particularly salmonid cultivation, has expanded significantly, making Chile the second largest salmonid producer after Norway (FAO, 2018) with annual production of over 700 thousand tons (Avendaño-Herrera, 2018).

Salmon farming can generate strong impacts on the environment, especially due to the organic material load in the water column and sediments around the floating cages (Haya et al., 2001; Soto and Norambuena, 2004; Buschmann et al., 2009; Caruso, 2014; Quiñones et al., 2019; Molina and Fernández, 2020). Salmon farming also liberates chemical products into the environment such as pesticides, antibiotics and food additives, among others (Haya et al., 2001; Burridge et al., 2010; Quiñones et al., 2019), and there is scarce information about their effects on non-target species (Rohr et al., 2006; Rain-Franco et al., 2018; Urbina et al., 2019; Garcés et al., 2020).

The feed additive ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline; hereafter EQ), initially registered as a pesticide, is an aromatic amine derived from quinoline (Bohne et al., 2008). It is used in fishmeal to prevent spontaneous combustion during maritime transport (United Nations, 2015), and also to prevent animal feed rancidity due to its ability to trap free radicals and thus avoid the spontaneous oxidation of lipids and other feed ingredients (Bohne et al., 2008). EQ was also used in Europe until 2022 (EFSA, 2022) to avoid loss of color in apples and pears (Błaszczuk et al., 2013).

Ethoxyquin in feed materials and animal tissues (e.g. fish muscle) leaves detectable amounts of the parent compound and main metabolites (e.g. quinone imine, EQ dimer; Bohne et al., 2008) as well as EQ impurities (e.g. p-phenethidine; EFSA, 2015). EQ and its transformation products have caused controversy due to their possible mutagenicity and carcinogenicity in animal species (EFSA, 2015). To date, it has not been established that ethoxyquin does not have an adverse effect on animal health, human health or the environment when used as a feed additive, which led to its ban as a feed additive in the European Union (EFSA, 2022). In fact, DNA damage in human lymphocytes induced by EQ has been reported (Błaszczuk, 2006), and EQ may also induce apoptosis (Błaszczuk and Skolimowski, 2005). EQ increases the number of aberrations in hamster ovarian cells (Gille et al., 1991) and inhibits the mitochondrial electron transport chain in animals (Reyes et al., 1995). Fish can accumulate this compound in organs and muscles as reported by one study that found an EQ concentration of 1.4 µg in 200g salmon fillets (Lundebye et al., 2010). Moreover, deleterious EQ effects have been reported in zebra fish embryos, *Daphnia magna* and microalgae (Egloff and Pietsch, 2018; Pradhan et al., 2020). The mutagenicity of EQ was evaluated in *Salmonella* sp., but the results were ambiguous (Błaszczuk, 2006; Błaszczuk et al., 2013) with both negative (Joner, 1977; Zeiger, 1993) and positive results (Rannug et al., 1984; Reddy et al., 1983) reported. Shah et al. (2005) also reported the inhibition of methanogenesis in biological treatment systems. Environmental studies related to EQ are scarce. Papadopoulou et al. (2016) showed the EQ produced significant inhibition of potential nitrification of soil microbiota.

Despite the wide use of EQ in the salmon aquaculture industry, knowledge about its effects on microbial metabolism and degradation of organic matter in the marine environment is very scarce. Information about the occurrence of this compound is also scarce. The relatively low concentrations of EQ in open surface waters may be due to its rapid degradation by irradiation (Bintou et al., 2015). However, EQ concentrations between 0.008 and 0.29 µg L⁻¹ have been found in surface waters in Vietnam (Chau et al., 2018). Xu et al. (2018) detected EQ in deposits of drinking water and treatment plants adjacent to the Yangtze Estuary in China, with concentrations of 0.02 to 0.07 µg L⁻¹; they cataloged it as one of the most abundant and recalcitrant “pesticides” in the study area. It must be noted that the European Food Safety Authority (EFSA) decreased the allowed concentration of EQ in fish feed from 300 to 150 mg Kg⁻¹; they determined that there is not sufficient evidence to classify this additive as environmentally safe (EFSA, 2015). In June 2017, the European Commission suspended the authorization for the use of EQ as an additive in the feed of all animal species and categories (EU, 2017) extended until 2022 (EU, 2021). In Chile, the EQ is on the list of authorized additives for the preparation and manufacture of food and supplements for animals (SAG, 2015).

There is a lack of information on the effects of EQ on marine microbial communities in coastal areas. Hence, the objective of this study was to evaluate the metabolic response and composition changes of the active microbial plankton community when exposed

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to ethoxyquin. Microcosm experiments were performed under controlled conditions of temperature and oxygen. Changes under different EQ concentrations over time were assessed measuring respiration rates, extracellular (aminopeptidase, β -glucosidase and lipase) and intracellular (MDH) enzymatic activity, microbial biomass (ATP), metabolic “fingerprints” with Biolog EcoPlates, and molecular techniques (16S rRNA barcode sequencing). These measurements provided information about how the microbial communities that inhabit the water column of Chiloé’s inner sea (Achao), an area highly impacted by salmon farming, and of a contrasting area off central Chile (Coliumo Bay) without salmon farming, respond to the exposure to this antioxidant.

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Study zones

Sampling was conducted in two coastal zones: (i) Achao, located in the inner sea of Chiloé, (Chiloé Island, Los Lagos Region; 42.52°S 73.20°W, Fig. 1), 6km (3.2nm) from the closest salmon farm; and (ii) Coliumo Bay, located in south-central Chile (Biobío Region; 36.5°S 72.944°W, Fig. 1), 2km (1.07 nm) from the coast. No salmon farming takes place in Coliumo Bay or in surrounding areas. Field sampling was done in two oceanographic cruises organized by the Interdisciplinary Center for ...

Environmental conditions of the sampling sites

The hydrographic conditions in Achao show a highly mixed water column with average temperature and salinity of 12.2°C and 33.1 psu, respectively (Fig. 2a). A typical nutrient profile shows lower concentrations at the surface (e.g. NO_3^- 0.5 μM at 2m) in comparison to deeper water strata (8.4 μM and 9.6 μM at 10 and 30m, respectively, Table 1). DOC and Chl-*a* concentrations were higher at 10m in comparison to the surface and 30m. The picoplankton abundance was higher in the surface compared ...

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Discussion

Our study seeks to identify the potential effects of ethoxyquin on the metabolic activity and biodiversity of the active microbial planktonic community in two contrasting areas, Achao and Coliumo Bay where the first is influenced by salmon farming. To the best of our knowledge, this is the first time that the effects of EQ have been evaluated in marine microbial communities of the water column. It is important to consider the physicochemical characteristics of EQ (Table S2), to understand its ...

Conclusions

The use of EQ in the feed of farmed salmon in seawater may generate changes in the metabolism of the microbial community, producing effects on the degradation of organic matter.

Our study indicates that the addition of EQ increases both oxygen consumption and the degradation of nitrogenous compounds by the < 1 μm size fraction (bacterioplankton), while respiration and the capacity to degrade carbohydrates and lipids of the entire community (< 63 μm) decreases, suggesting differential degradation ...

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CRediT authorship contribution statement

María-Lorena González: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Marcelo E. Fuentes:** Methodology, Validation, Formal analysis, Investigation, Writing – review & editing. **Verónica Molina:** Validation, Data curation, Investigation, Writing – review & editing. **Renato A. Quiñones:** Conceptualization, Methodology, Resources, Investigation, Writing – review & editing, Funding ...

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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