

## Environmental issues in Chilean salmon farming: a review

Renato A. Quiñones<sup>1,2</sup>, Marcelo Fuentes<sup>2</sup>, Rodrigo M. Montes<sup>1</sup>, Doris Soto<sup>1</sup> and Jorge León-Muñoz<sup>1,3</sup>

<sup>1</sup> Interdisciplinary Center for Aquaculture Research (INCAR), University of Concepción, O'Higgins 1695, Concepción, Chile

<sup>2</sup> Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile

<sup>3</sup> Departamento de Química Ambiental, Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Concepción, Chile

### Correspondence

Renato A. Quiñones, Interdisciplinary Center for Aquaculture Research (INCAR), University of Concepción, O'Higgins 1695, Concepción, Chile. Email: rquinone@udec.cl

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### Abstract

The growth of Chilean salmon production has not been free of important sanitary and environmental shortcomings. To ensure sustainability, it is necessary to understand the environmental impacts of salmon production on the Patagonian ecosystems. Currently, there is limited regulation or monitoring of impacts in the freshwater phase compared to the marine fattening stage, and there is some evidence of local eutrophication impact and diversity changes downstream the farms. Eutrophication of Patagonian channels and fjords from marine farms has been recognized as crucial environmental risk, although most scientific evidence comes from local effects below and around farms. So far, there are no regulations based on carrying capacity estimates to limit maximum fish biomass per area or water body. There is controversy regarding the potential role of nutrients derived from farming in triggering harmful algal blooms, yet current environmental monitoring and available information does not allow establishing or rejecting a cause–effect relationship. Pesticides used to control sea lice infestation have been shown to be deleterious to some non-target species. There is evidence that the use of high quantities of antibiotics has allowed the development of antibiotic-resistant bacteria in sediments and there is concern that salmon aquaculture has the potential to increase the proportion of antimicrobial-resistant bacteria to antibiotics that are used in human medicine. There is an urgent need for more comprehensive ecosystem (beyond farm) studies on the impacts of antibiotics. Escapes of salmon (exotic species) from farms are a relevant environmental risk, although the most farmed species, *Salmo salar*, has shown little success in establishing wild populations. The review identifies critical knowledge gaps whose fulfilment is essential to advance towards an ecosystem approach to aquaculture and to protect Patagonian ecosystems.

**Key words:** aquaculture pollution, environmental sustainability, Patagonian marine ecosystems, salmon farming.

### Introduction

Marine and inland aquaculture has expanded rapidly in the last three decades, and the relative contribution of marine capture fisheries to the growing total world fish production has shrunk. World aquaculture production of fish accounted for 44.1% of total fish production in 2016 (FAO 2018).

Although the aquaculture sector has shown impressive growth globally, it has also encountered significant environmental issues. The most common issues are those related to unsustainable aquaculture practices, and the

potential negative impacts on ecosystems (Maroni 2000; Tacon *et al.* 2010; Klinger & Naylor 2012) such as habitat loss (Stickney & McVey 2002), pollution (e.g. Tett 2008), escapes, genetic interaction of non-native species with wild fish populations (Svasand *et al.* 2007; Chevassus-au-Louis & Lazard 2009; Lazard & Lévêque 2009), disease transmission and use of fishmeal and fish oil as major feed inputs (Naylor *et al.* 2000; Tacon & Metian 2008), among others.

Recently, considerable focus has turned towards the development of best management practices, codes of conduct and aquaculture certification programmes to promote more environmentally and socially responsible and

sustainable farming practices (Boyd *et al.* 2005, 2008; WWF 2007; Allsopp *et al.* 2008; Belton *et al.* 2009; Umesh *et al.* 2009; FAO 2010). However, globally aquaculture is still confronting many environmental challenges to achieve better levels of sustainability and a better public reputation (Mazur & Curtis 2008; Murray & D'Anna 2015; Froehlich *et al.* 2017).

Since salmon are mostly farmed in well-developed economies such as Norway, United Kingdom and Canada, impacts of salmon farming are among the best studied and known within the aquaculture sector. A recently published environmental risk assessment of salmon farming in Norway revealed the wealth of information, especially in that country (Taranger *et al.* 2017). The environmental impacts of salmon farming elsewhere vary depending on the hosting ecosystem and management practices, but the stressors are similar.

The Chilean aquaculture industry has grown exponentially since the late 1980s, mostly due to the increased production of salmonids and particularly Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*). Total harvest for these species in 2016 was 532, 85 and 111 thousand tons, respectively (SERNAPESCA 2016). Chile is thus the second largest global producer of farmed salmon, with an annual production of over 700 thousand tons (Avendaño-Herrera 2018).

Early life-history phases of salmon farming take place in a wide spatial distribution covering from central to southern Chile, with most freshwater farms and hatcheries located in the Biobío, Araucanía, Los Ríos and Los Lagos Regions (INGELAND 2017) also known as the VIII, IX, XIV and X regions, respectively (Fig. 1). The marine fattening phase takes place further south in the Patagonian fjords and channels of Los Lagos, Aysén and Magallanes (Regions X, XI and XII, respectively; Fig. 1). The salmon industry provides about 30,000 direct and 14,500 indirect jobs (Dresdner *et al.* 2016). However, it is argued that indirect employment has been greatly underestimated (Soto *et al.* 2019).

The growth of Chilean salmon production has not been free of important sanitary and environmental shortcomings, as highlighted by the 2008 crisis produced by the spread of the Infectious Salmon Anemia (ISA) virus. The disease had devastating effects on productivity and caused a social crisis due to job losses (about 20,000), particularly in the Los Lagos and Aysén Regions (Iizuka & Katz 2011; Iizuka & Zanlungo 2016). More recently, at the beginning of 2016, high and persistent harmful algal blooms (HABs) of *Pseudochattonella cf. verruculosa* and *Alexandrium catenella* took place in the estuarine and marine ecosystems of southern Chile. A major mortality event of about 27 million salmon and trout (i.e. 39,000 tonnes) was caused by *P.*

*cf. verruculosa* blooms in the Los Lagos Region, specifically in the northern area of the inner sea of Chiloé and at the Reloncaví Sound (Buschmann *et al.* 2016; León-Muñoz *et al.* 2018; Montes *et al.* 2018). These HABs were followed by others that caused mass mortality of several fish and shellfish near Chiloé Island (León-Muñoz *et al.* 2018). This is the first time that a major *A. catenella* bloom affected coastal areas of Chiloé Island; it produced a major social upheaval. Many local and national stakeholders raised strong concerns about aquaculture-driven eutrophication and its potential association with the widespread harmful algal blooms (HABs) in 2016. Eutrophication of Patagonian channels and fjords from salmon culture in Chile has been recognized as an environmental risk since the early stages of development of the industry (Soto & Norambuena 2004; Niklitschek *et al.* 2013).

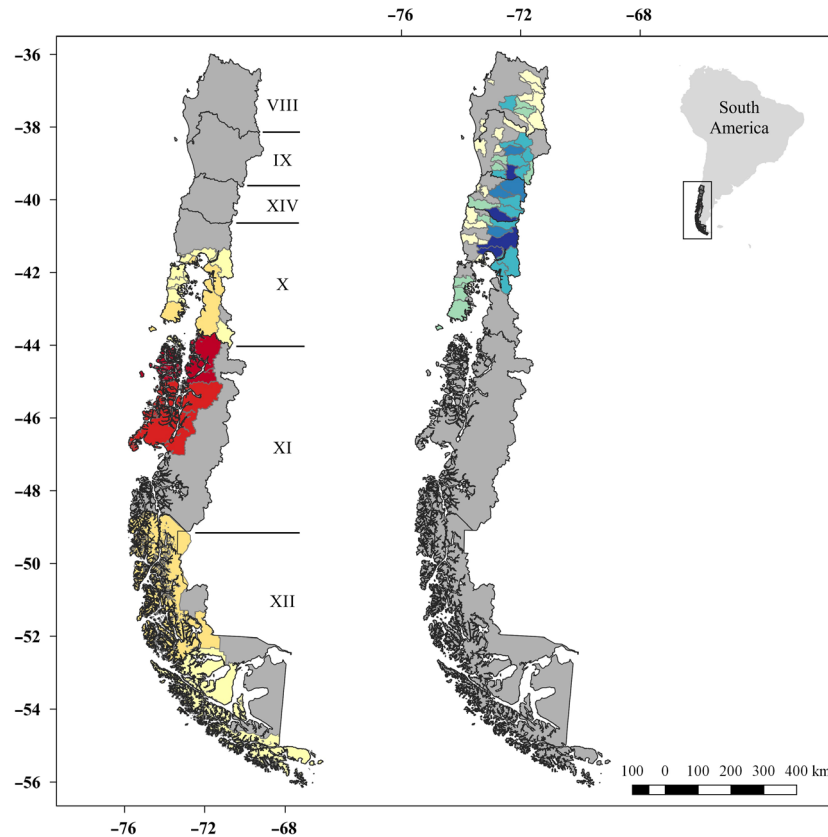
The intensive use of antibiotics to fight the infection caused by the bacterium *Piscirickettsia salmonis* and pesticides to control the parasitic load of the sea lice *Caligus rogercresseyi* have become key environmental concerns in Chilean salmon aquaculture (Millanao *et al.* 2011; Núñez-Acuña *et al.* 2015; Avendaño-Herrera 2018). In fact, the Chilean salmon industry came under the scrutiny and criticism of important international media in 2008, accusing the industry of environmental malpractice and overuse of antibiotics (Barton & Fløysand 2010; Iizuka 2016).

The scientific challenges imposed by the environmental issues confronting Chilean salmon production are significant. Here, we provide an overview of the current state of scientific knowledge of environmental effects of Chilean salmon aquaculture activities. The review also identifies critical gaps and recommends specific research to address these gaps.

## Environmental issues arising from the freshwater phase of salmon production

Eggs, fingerlings and pre-smolts (juveniles up to about 100 g) are produced and reared in land-based farms supplied with stream water in the south-central regions of Chile (Fig. 2). Freshwater farms are mainly located in the pre-Andean slopes where the freshwater quality of streams is suitable for early life cycle stages (Soto *et al.* 2007; Atland & Bjerknes 2009). Andean watersheds are still relatively pristine, with a good proportion of native forest. This also ensures high water quality and suitable flows even in summer (Lara *et al.* 2009).

During the early development of the industry in Chile, freshwater fish farms were mainly located in the Los Lagos Region (X Region, Fig. 1). Given the growing demand for eggs, fingerlings and smolts, the industry then began to build freshwater fish farms in more northern areas, including Los Ríos (Region XIV), La Araucanía (Region IX) and



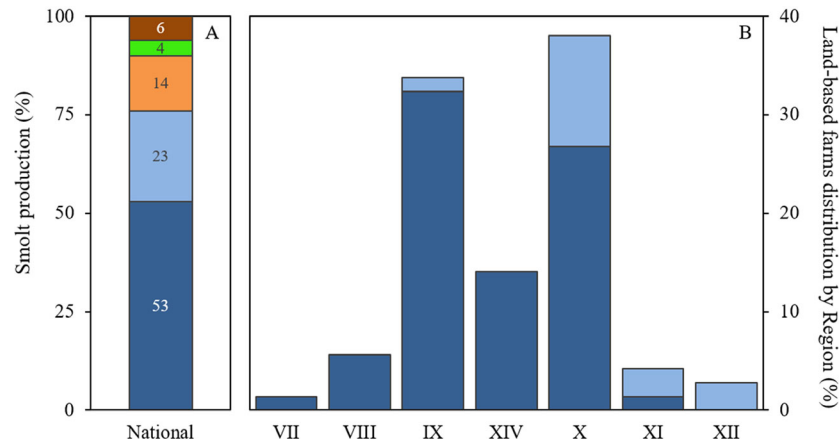
**Figure 1** Geographical location of freshwater and marine salmon aquaculture leases in 2016. Roman numerals correspond to the Chilean administrative regions. Information on land base concessions (hatcheries and smolt production) per county for regions XI and XII was not yet available for 2016. Information for these figures was provided (through the Transparency Law request process) by the Chilean Undersecretariat for Fisheries and Aquaculture (SUBPESCA) and the Chilean National Fisheries and Aquaculture Service (SERNAPESCA). Land-based (freshwater) concessions per county (N°): (□) 1, (□) 1–3, (□) 3–5, (□) 5–8, (□) 8–13; Marine farm concessions per county (N°): (□) 1–50, (□) 50–100, (□) 100–150, (□) 150–200, (□) 200–250, (□) 250–300, (□) 300–350, (□) 350–369.

Biobío (Region VIII) (Figs 1, 2), which are now key suppliers of eggs and smolts. The northern expansion meant that freshwater fish farms had to be located in increasingly less pristine landscapes, where there are strong processes of deforestation and degradation of the temperate native forest, initially associated with agricultural expansion, and in the last 40 years with replacement of native trees by plantations of fast-growing exotic forest species (Heilmayr *et al.* 2016). Loss or alteration of ecosystem services such as soil protection, nutrient recycling and hydrological control has occurred as a result of this transformation (Nahuelhual *et al.* 2007; Lara *et al.* 2009). Thus, many freshwater farms take in and release water in more fragile ecosystems that are subjected to pollution derived from agricultural activities and dumping of industrial and urban waste.

Surprisingly, there are very few comprehensive studies of land-based salmon farming impacts on Chilean streams, especially regarding impacts in the context of other human-related stressors at the ecosystem level (i.e. stream basin,

catchment), including effects of antibiotics and other chemicals. Additionally, there is almost no regulation or monitoring of impact for the freshwater phase in Chile, compared to the marine fattening stage (Avendaño-Herrera 2018).

Egg and fry phases of salmonid production are particularly susceptible to infections (bacterial, viral and fungal) due to their immature immune systems. Infections are treated with antibiotics (florfenicol and oxytetracycline), and prevented using vaccines and a various vaccines, and a variety of technological management solutions (e.g. continuous flow system, water recirculation, (Avendaño-Herrera 2018). NaCl is also used to prevent and control ectoparasites, particularly fungi (Tello *et al.* 2010; Torres & Fajardo 2011). Concerns have been raised mainly regarding the environmental impact of flow through farms (León-Muñoz *et al.* 2007) because they release faeces, unconsumed food and metabolic by-products, which increase the load of nitrogen, phosphorus, carbon and suspended solids in the receiving stream waterbed (Warrer-Hansen 1982; Wang *et al.* 2012; Nimptsch *et al.*



**Figure 2** Smolt production by type of farming system in Chile. (A) Estuary, river and lake correspond to floating cages while recirculation and open flow are inland structures. (B) Bars represent geographical distribution by Chilean administrative regions (represented by Roman numerals) of the inland freshwater farms producing smolts in 2017. The geographical locations of these administrative regions are described in Figure 1. Prepared by the authors based on data supplied by the Salmon Technology Institute (INTESAL). (■) Estuary, (■) River, (■) Lake, (■) Recirculation, (■) Open flow.

2015; Kamjunke *et al.* 2017). Recent studies have focused mainly on the potential impacts of organic and inorganic loads. For example, Kamjunke *et al.* (2017) performed a detailed molecular level characterization of aquaculture dissolved organic matter (DOM) quality and its bacterial degradation in four freshwater streams receiving salmon farms output in Chile. The observed changes in DOM composition led to an increase in bacteria and a decrease in benthic algae downstream of the aquacultures, shifting stream ecosystems to a more heterotrophic state (Kamjunke *et al.* 2017). Figueroa *et al.* (2017) studied the environmental impact of six fresh water salmonid farms located on central-southern Chilean streams (Biobio Region). They found that aquaculture activity modified conductivity, salinity and total dissolved solids, even 100 m downstream from the point of discharge. They also found changes in riparian benthic species composition, with an increase in species able to tolerate significant inputs of organic matter and nutrients. The analyses of total organic carbon and organic matter degradation suggested that the effluent of the fish farms damaged the stream ecosystem for some distance below the farm (Figueroa *et al.* 2017).

While salmon farms have recently started to remove suspended matter from waste water using sediment traps and rotating drum filters, dissolved components are still discharged untreated (Kamjunke *et al.* 2017). For instance, the watershed of Villarrica Lake (IX Region) is one of the most densely used areas for freshwater farms, and recently the Chilean government declared this lake a saturated zone (i.e. a zone in which one or more environmental quality norms are exceeded; Chilean Environmental Act) due to

rapid eutrophication (MMA 2018). Fish farms here have been identified as one of the sources of pollution (N, P); reduction of these nutrient inputs will require improved waste treatment technologies and/or full recirculation.

The relative contribution of freshwater ecosystems to national smolt production declined during 1998–2005, with an increase of smoltification in estuaries and in land-based recirculation systems. According to information provided by SalmonChile ([www.salmonchile.cl](http://www.salmonchile.cl)), by 2017 53% of smolt production was done in land-based flow through fish farms, 23% in recirculation systems, 14% in floating cages in lakes, 6% in estuaries and 4% in rivers (Fig. 2). There has been increasing pressure to remove cages from lakes, especially from those lakes with low flushing rates and from those already affected by agriculture and other human intervention (León-Muñoz *et al.* 2007). Concerns are similar to those described above for riverine ecosystems regarding excess nutrient loads generating eutrophication risks in lakes that are (or used to be) unique in a global context in terms of their oligotrophy and resilience mechanisms (Soto 2002). It is worth mentioning that 20 years ago more than 90% of smolts were produced in floating cages in lakes.

### Salmon farming environmental impacts during the marine fattening phase in southern Chile

#### Main oceanographic characteristics of Patagonian fjords and channels

Chilean Patagonia (41–56°S) is characterized by its highly complex geomorphology and hydrographic conditions, and by strong seasonal and latitudinal patterns in primary

production, light regime, freshwater discharge, precipitation and glacier coverage (Montecino & Pizarro 2008; Pantoja *et al.* 2011; Jacob *et al.* 2014; Viale & Garreaud 2015). This zone extends for 1,600 km and covers an area of roughly 241,000 km<sup>2</sup> (Fig. 1); it has an extensive coastline composed of a large number of islands, fjords, sounds, basins and gulfs. The complex topography is a crucial factor influencing water exchange between coastal regions and the open ocean, creating micro-environments with oceanographic conditions that sustain unique ecosystems (Lange *et al.* 2006) and that could be sensitive to excess nutrient input, chemicals and other stressors. Patagonian fjords and channels have been recognized as having high conservation value due to their biodiversity and uniqueness (Fernández *et al.* 2000; Hücke-Gaete *et al.* 2006; Häussermann & Försterra 2009). The area is important for microbial (Ugalde *et al.* 2013), invertebrate (Hernández *et al.* 2005; Mutschke 2008; Häussermann & Försterra 2009) and vertebrate biodiversity (Schlatter & Simeone 1999; Valenzuela *et al.* 2008; Skewgar *et al.* 2014), and is a hotspot for cetacean diversity (Hücke-Gaete *et al.* 2006; Buchan & Quiñones 2016; Viddi *et al.* 2016).

This region is a transitional marine system, influenced by oceanic deep waters of high salinity/nutrients and surface freshwater of low salinity/nutrients. The estuarine waters are relatively poor in nutrients, with the oceanic Sub-Antarctic Waters (SAAW) being the main source of nutrients (Silva & Neshyba 1979). The Sub-Antarctic Surface Modified Water is rich in macronutrients from the adjacent Pacific Ocean as well as of terrestrial origin (Iriarte *et al.* 2007). High annual rainfall in the fjord region (>3,000 mm year<sup>-1</sup>; Viale & Garreaud 2015; León-Muñoz *et al.* 2018) and high mean annual river discharge (~2,500–3,500 m<sup>3</sup> per s) greatly enhance the supply of sediments (Lange 2012; León-Muñoz *et al.* 2018).

The primary production of Patagonian fjords and channels is co-limited by the annual cycle of solar radiation, wind intensity and direction and nutrient availability (Iriarte *et al.* 2007; Montero *et al.* 2011). Large-scale climate-oceanographic forcing, freshwater input and enhanced solar radiation seem to be crucial in altering phytoplankton composition and in triggering HABs in Patagonia (León-Muñoz *et al.* 2018).

### Salmon farms and their role in eutrophication of coastal marine environments of southern Chile

One of the most important environmental impacts of fish farms is the increase in load of organic matter to the sea-floor (Findlay *et al.* 1995; Kutti *et al.* 2007, 2008; Aranda *et al.* 2010; Hargrave 2010), which may produce a decrease in dissolved oxygen in the water column and in the oxidation-reduction potential of the sediments (Hargrave *et al.*

1993; Sanz-Lázaro & Marín 2008). If the flow of organic matter is sufficiently high, hypoxic or anoxic conditions are formed in surface sediments, and macrofauna requiring oxygen for respiration cannot survive (Haya *et al.* 2001). The decomposition of excessive amounts of organic matter increases microbial oxygen consumption in bottom waters, ultimately forming toxic hydrogen sulphide under anaerobic conditions (Gray *et al.* 2002; Hyland *et al.* 2005).

Eutrophication of Patagonian channels and fjords due to salmon culture in Chile has been recognized as an environmental risk of salmon production (Buschmann & Pizarro 2002; Soto & Norambuena 2004; Buschmann *et al.* 2006). The Chilean aquaculture authority has confronted this risk mainly by regulating site selection and by compulsory monitoring of environmental variables, which are determined by the characteristics of the farm and its environment. According to present Chilean regulations, the productive carrying capacity of a site is mainly reflected by the oxygen condition of the sediments below the site. The Chilean National Fisheries and Aquaculture Service (SER-NAPESCA) administers the preparation of the environmental information report (INFA), which can be delegated by the Service to an environmental consultant, to monitor the sanitary and environmental conditions of cultivation and determine the continuation of productive farm activity at the site. When the site shows anaerobic conditions, the farm is not permitted to cultivate fish until aerobic conditions are re-established (Alvial 2017). A farm is considered to be operating in anaerobic conditions if one of the following thresholds is met: organic material higher than 9%; pH lower than 7.1, Eh (Redox) lower than 50 mV, dissolved oxygen at 1 m from the sea bottom lower than 2.5 mg L<sup>-1</sup>; or visual examination showing visible filamentous bacteria or gas bubbles (SUBPESCA 2009). According to the official Aquaculture Environmental Report (SUBPESCA 2017), 74.5% and 25.5% of the fish farms presented aerobic and anaerobic conditions, respectively, in their sediments in 2016. This diagnosis also suggests that in almost 75% of the cases organic matter does not accumulate significantly under the cages and that the fraction that is not locally degraded (sediments or water column) goes elsewhere.

Excess nutrients with potential eutrophication consequences are mainly due to remains of uneaten food, faeces and excretion of fish confined in cages. Wang *et al.* (2012), analysing Norwegian salmon farms, estimated that of the total feed input, 70% C, 62% N and 70% P were released into the environment. Niklitschek *et al.* (2013) estimated that the annual nutrient discharges from 154 salmon farms operating in the Aysén Region of Chile in 2010 were equivalent to 12,300 t N and 1,600 t P. A crucial factor determining the ecological impacts of these inputs locally is the capacity of the recipient waters to assimilate the nutrients, which is strongly related to water retention time and

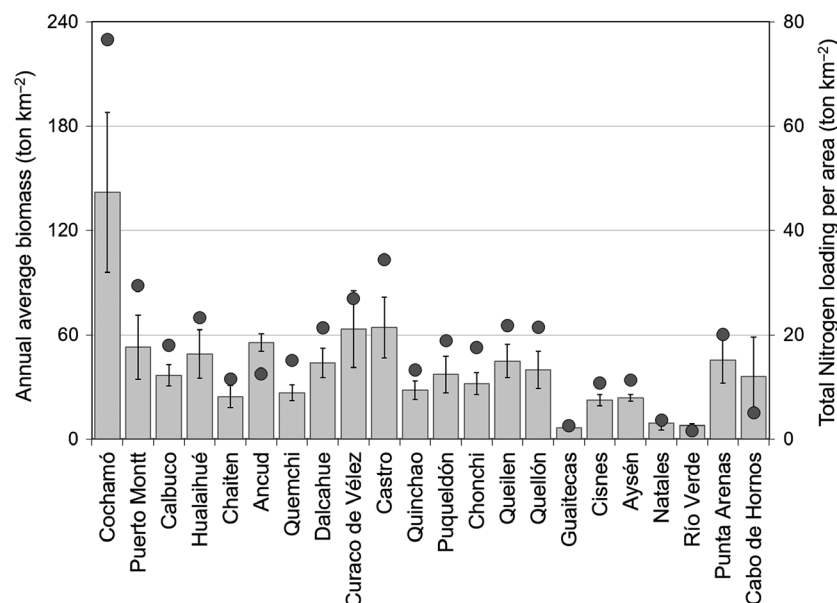


hydrodynamics (Yokoyama *et al.* 2004; Mayor & Solan 2011; Urbina 2016). However, the presence of salmon cages can modify the natural hydrodynamics of the channel/fjord, attenuating the intensity of the local current velocity and generating recirculation and retention zones near the cages (Herrera *et al.* 2018).

The potential impact of inorganic waste generated by fish farms is difficult to elucidate because of dilution and potentially rapid uptake by microorganisms in the water column. Soto and Norambuena (2004) evaluated 43 salmon farm sites, of which 29 were in full operation, and grouped them in nine locations in southern Chile. They reported that no effects of salmon farms were found on water column variables such as nitrate, ammonia, orthophosphate and chlorophyll, while they found an effect of salmon farms on sediment variables such as nitrogen, phosphorus and organic carbon. Lack of association between nutrient conditions in the water column and sediments revealed a rapid dilution process and perhaps rapid biological recycling in the water column. They also found lower species richness in sites below cages compared to control sites. Based on these results, Soto and Norambuena (2004) concluded that salmon farming effects seem to be localized to the areas beneath salmon cages and those immediately adjacent to the salmon cages with some limited broader effects on sediments in nearby reference areas, especially within a system where salmon farming is very intensive such as the Reloncaví fjord. In another study, Soto and Jara (2007) found

increased species richness around farm sites, which may be explained by increased productivity due to nutrient input and/or by enhanced protection from small-scale fisheries that operate in the area. Furthermore, according to Soto *et al.* (2019), considering the accumulated salmon production from 2006 to 2017 in the main communities where they are farmed in southern Chile, the total N load varied between 77 and 2 tons per km<sup>2</sup> of relevant area (area of influence under the “salmon farming concessions grouping” (ACS) or “neighbourhoods” per community). Cochamó in Los Lagos (X) region, one of the oldest and densest salmon farming areas, has received the highest N load, while Río Verde in the southern most Region of Magallanes, the newest salmon area with lower aggregation of farms, has received the lowest load (Fig. 3).

Mayr *et al.* (2014) applied carbon isotopes and C/N ratios to a sediment record from Comau Fjord in southern Chile (42°S) with the aim of reconstructing carbon and nitrogen mass accumulation rates and to determine their allochthonous and autochthonous sources for the last century. They found an environmental shift in nitrogen and carbon accumulation rates in the last 2–3 decades, reflecting an increase in primary productivity within the fjord. They concluded that anthropogenic eutrophication caused by aquaculture is the most likely explanation for this observed increment in carbon and nitrogen accumulation rates. At the landscape level, the area where marine fattening is concentrated, particularly the Aysén and Magallanes



**Figure 3** Bars indicate annual average salmon biomass (harvested) from 2007 to 2017 ( $\pm$ SE) per commune from the northern part of the X region (Cochamó) to the southernmost locations in the XII Region (Cabo de Hornos). Circles represent estimated total nitrogen loading per relevant area of impact during the 10-year period. Figure redrawn from Soto *et al.* (2019), figures on production per commune were estimated from public information provided by the Chilean National Fisheries and Aquaculture Service (SERNAPESCA). (■) Annual average biomass, (●) Nitrogen loading.

Regions, is still quite pristine. Thus, the watersheds of the main tributary rivers are dominated by native forests and their exports of nutrients are significantly lower than those in temperate forest ecosystems of the Northern Hemisphere, which experience greater anthropogenic perturbation (Perakis & Hedin 2002).

Based on benthic samples collected from two salmon farms located in Southern Chile, Aranda *et al.* (2010, 2015) reported *Beggiatoa* spp. mats directly beneath the fish cages but not outside, which indicates localized significant benthic organic enrichment. The coexistence of different sulfur cycling functional groups associated with mat forming bacteria *Beggiatoa* spp. strongly suggests intense sulfur cycling in these aquaculture-impacted sediments. These results suggest that at the sampling sites there was decomposition of excessive amounts of organic matter, ultimately generating toxic hydrogen sulfide under anaerobic conditions (Gray *et al.* 2002; Hyland *et al.* 2005). Recently Hornick and Buschmann (2018) compared bacterial communities and functional bacterial diversity in surface sediments in three salmon aquaculture locations and reference sites in Chiloé Island. Their results indicate that sediment bacterial communities influenced by salmon aquaculture presented changes in taxonomic diversity, composition, and function. Hornick and Buschmann (2018) hypothesized that these changes are due to organic loading and pollution resulting from salmon aquaculture and recommended the use of bacterial shifts as indicators of aquaculture perturbations.

A comparison of the impacts on sediments and benthic communities of two salmon farms located in zones with differing hydrodynamic characteristics in the Inner Sea of Chiloé was conducted during one year by Urbina (2016). Environmental variables and diversity indexes were quantified and calculated for the exposed (high speed currents) and protected salmon farms (low speed currents). The results indicated that exposed zones are more resilient to the impacts of salmon farming than protected zones. Oxygen availability in the sediments seems to be a major driver of the impacts. In both protected and exposed zones, the impact of salmon farming was first seen as a decrease in overall biodiversity and an increase in the dominance of opportunistic species. The environmental variables that were determined to be most appropriate to assess the impacts in the marine sediments of both zones were redox potential, sulphurs and phosphorus. Total organic carbon was not a good explanatory variable for evaluating salmon farming impact.

In summary, most studies in Chile have found localized eutrophication impacts under salmon cages. However, there are no broader analyses that could allow better understanding of the fate of nutrients from salmon farming, especially in deep steep-sided fjords where

sediments and particulates do not accumulate under the cages and which under current Chilean regulation are considered excellent sites for salmon culture. However, all the organic and inorganic material goes somewhere, and although greater circulation could facilitate recycling, organic matter may also be accumulating in the deeper bottom of the fjords beyond the farms. Such impacts are not being examined or monitored. Also, inorganic material (e.g. dissolved inorganic N) may be producing new biomass and entering food webs in the ecosystem, including microalgae.

### Carrying capacity

Carrying capacity is a complex concept and requires many biological, physical and socioeconomic components for its estimation, especially when integrated into the Ecosystem Approach to Aquaculture framework (Costa-Pierce 2008, 2010; Ross *et al.* 2013; Aguilar-Manjarrez *et al.* 2017). Many definitions of carrying capacity in aquaculture are available, however the concept has expanded over the last decade into a four-pillar approach based on physical, production, ecological and social carrying capacity, encompassing all elements of sustainability (McKindsey *et al.* 2006; Gibbs 2009; McKindsey 2012; Ferreira *et al.* 2013).

In Chile, there is an increasing need for a sound productive carrying capacity estimation tool to establish the maximum number and/or volume of fish that can be supported by an area and or relevant water body without causing “unacceptable change” to ecosystems (Soto *et al.* 2008; Byron & Costa-Pierce 2013; Ferreira *et al.* 2013). Current Chilean regulations focus on a number of variables contained in the environmental impact assessment norm (RAMA; Environmental Regulation for Aquaculture; Supreme Decree N° 320/2001) especially focusing on the aerobic status of the site. However, there have been very few studies attempting to understand the cumulative environmental effects of a number of farms in a common recipient water body such as a fjord or a channel. Some water bodies such as the Reloncaví estuary have a very large number of farms which generate risks for salmon production and for the ecosystem (Soto *et al.* 2019). As water bodies capacities are estimated individually (site by site) and not in broader spatial scales, no sound estimates of carrying capacity at fjords/channels scale are available. Current organization of farms in neighbourhoods (i.e. administrative spatial units where the farms should carry out coordinated actions regarding fish inputs, prophylaxis and therapeutic treatments, sanitary issues, harvest and resting periods; Ibieta *et al.* 2011) could provide a first step towards the implementation in Chile of the carrying capacity concept at a broader spatial scale, although the current grouping of farms in

neighbourhoods is only related to biosecurity concerns (Alvial 2017).

As emphasized by researchers, there is an urgent need to estimate the carrying capacity of water bodies in Chilean Patagonia before allowing an increase in current farmed salmon production levels in new areas further south (Iriarte *et al.* 2010; Niklitschek *et al.* 2013; Soto *et al.* 2019).

### Research on carrying capacity in Chilean fjords/channels

Historically, few assessments of carrying capacity at cage, farm and/or fjord scales have been conducted in Chile. For example, the MOM (Modelling - Ongrowing fish farm - Monitoring) model (Ervik *et al.* 1997; Hansen *et al.* 2001; Stigebrandt *et al.* 2004; Stigebrandt 2011) was used to estimate carrying capacity for four sub-basins (Boca, Mari-melli, Puelo, Cochamó) of the Reloncaví fjord (ca. 41°S) (Daneri *et al.* 2007). For the estimation of carrying capacity, the effect that the spatial arrangement of salmon farm cages and their locations (relative to the main current) have on carrying capacity estimates was also analysed (Daneri *et al.* 2007). Dissolved oxygen (in the water column and benthos) was selected by Daneri *et al.* (2007) as the critical parameter that drives carrying capacity estimations within the fjord. MOM simulations for the Reloncaví fjord showed that estimated values of carrying capacity significantly increase if the main axis of the net pens was located perpendicular to the main direction of the current flow. According to our knowledge, the model was not subsequently validated with field data, and hence the usefulness of this model to regulate salmon production in the Reloncaví fjord has not been proved.

A numerical model, at a much finer scale, to predict the environmental capacity of a farm and its effects on production was developed for salmon farms in Chiloé channels by Salamanca *et al.* (2009, 2010). Oxygen availability within and outside salmon cages was modelled in relation to its seasonal changes and according to different levels of salmon biomass produced. Validation of the model was conducted with field observations; its accuracy varied between 78% and 83%. The results showed that based on actual biomass production levels, dissolved oxygen levels decreased from 10 mg O<sub>2</sub>L<sup>-1</sup> outside the cages to 3.7 mg O<sub>2</sub>L<sup>-1</sup> inside the cages. If salmon biomass production increased by 20%, a decrease of oxygen to critical levels (2.5 mg L<sup>-1</sup>) inside the cages was detected, which strongly affected salmon growth. In contrast, a 20% reduction of salmon biomass was associated with an oxygen decrease inside the cages to 5.5 mg L<sup>-1</sup>, which can be considered a well-oxygenated water column (Salamanca *et al.* 2009, 2010). It was proposed that this information be used to implement a biomass management programme to maintain fish growth at optimal levels (Salamanca *et al.* 2009, 2010).

A conceptual model, which should help in the integrated management of physical–ecological–social systems, was designed for the Aysén fjord using STELLA (Visual Modeling Environment), identifying components and processes related to nutrient cycling and carrying capacity of salmon farms (Marín *et al.* 2008). No empirical application of this model has been reported to date in the scientific literature.

A recent attempt to evaluate the carrying capacity of Patagonian coastal sea waters was conducted focusing on a highly concentrated salmon farm area, the inner sea of Chiloé (Rojas 2017). For that purpose, a biogeochemical model (NPZD) coupled to a bio-optical model was implemented using dynamic systems modelling (STELLA). Outputs from the coupled model were used, with the help of a hydrodynamic 3D model (MOHID, Water Modelling System), to evaluate the dynamics of dissolved nutrients (nitrates, phosphates, silicates) and oxygen in the water column. Preliminary simulations showed different primary productivity dynamics between the northern and southern areas of the inner sea of Chiloé, and also between seasons within an area. For example, the northern area during the fall showed oxygen levels that fluctuated around 7–8 mL L<sup>-1</sup> and after that, during the spring season, they decrease to ca. 5–6 mL L<sup>-1</sup> (Rojas 2017).

A detailed review of carrying capacity models used worldwide was conducted by Tapia and Giglio (2010) in an effort to promote the use of these models in Chilean salmon aquaculture by the government and private sector. In this study, the requirements and outputs of each model applicable to Chilean fjords and/or channels were clearly identified. In addition, a monitoring program was suggested for critical variables that are necessary to implement carrying capacity models in Chilean fjords. According to Tapia and Giglio (2010), this program should include the monitoring of: (i) the spatial and temporal variability of currents and/or circulation patterns inside a fjord, (ii) the freshwater input into a fjord including the entrance of organic matter and nutrients, (iii) a high resolution (<100 m) bathymetry of fjords and channels, (iv) main inorganic nutrients and dissolved oxygen in the water column, primary production rates and carbon fluxes during an annual cycle, (v) up-to-date salmon farm statistics, which include monthly farmed biomass, mortality and harvest levels, type of feed, pellet dimension, feeding frequency and conversion factors.

So far none of the scientific efforts to estimate the carrying capacity of water bodies described above have been used by the government or private sector to limit maximum fish biomass per area or water body. It is relevant to emphasize that there has been no attempt to address the carrying capacity of Patagonian ecosystems to receive antibiotics and pesticides used in salmon production.



### On the interactions between harmful algal blooms (HABs) and salmon farming

Harmful algal blooms are a crucial issue for salmon farming in Chile. On one hand, HABs have strongly affected the Chilean salmon industry by producing massive mortality, and on the other hand there is a deep controversy regarding the potential role of nutrients derived from salmon farming in triggering HABs. In what follows, we briefly treat both environmental issues.

HABs have been reported in Chile for more than 40 years, affecting wild and farmed marine resources and human health (Lembeye 1994; Uribe & Ruiz 2001; Guzmán *et al.* 2002; Suárez *et al.* 2003; Iriarte *et al.* 2005; Sandoval *et al.* 2018). Blooms of the dinoflagellate *Alexandrium catenella* in Chile have been reported in the scientific literature since 1972 (Guzmán *et al.* 1975). Most blooms of this species took place in remote southern areas long before salmon farming began. An intense bloom was detected in the Aysén Region in 2002, which expanded northward to the Los Lagos Region (42°10'S). Since then, there have been recurring outbreaks of *A. catenella* (Molinet *et al.* 2003). Exceptionally intense blooms affected the coastal zone of southern Chile in the austral summers of 2009 and 2016. The first *Pseudochattonella cf. verruculosa* bloom was detected in 2004, and blooms recurred in 2005, 2009 and 2011 (Mardones *et al.* 2012; Eckford-Soper & Daugbjerg 2016). The *P. cf. verruculosa* blooms of early 2016 were economically devastating for the Chilean salmon farming industry, killing ca. 27 million farmed salmon and trout (39,000 tonnes) (Montes *et al.* 2018). In February 2017, a massive mortality event (ca. 150,000 smolts) was reported in a shipment of smolts transported to the southern Magallanes Region for seeding at sea, as well as a cargo of salmon travelling from Magallanes to the more northern Los Lagos Region for harvest and processing. The mortalities occurred when the wellboats took seawater from the Golfo de Penas (Aysén Region; Southern Patagonia) and adjacent areas. Several harmful algae were found, including the dinoflagellates *Gymnodinium* spp., *Azadinium* spp., and *Karenia mikimotoi* and the diatoms *Skeletonema* spp. and *Pseudonitzschia* spp. (Anabalón *et al.* 2017).

León-Muñoz *et al.* (2018) analysed the *P. cf. verruculosa* harmful bloom that took place during 2016, and suggested that this was especially triggered by a strong El Niño event and the positive phase of the Southern Annular Mode that altered the atmospheric circulation in southern South America and the adjacent Pacific Ocean. This led to very dry conditions, with one of the lowest inputs of freshwater in the last seven decades and higher than normal solar radiation reaching the surface (approximately 30% higher on average) (Garreaud 2018; León-Muñoz *et al.* 2018). This allowed the advection of more saline and nutrient-rich

waters generating optimum conditions for harmful phytoplankton species to bloom in the coastal waters of western Patagonia.

Recently, Montes *et al.* (2018) estimated the threshold concentrations of algal cell abundance for *A. catenella* and *Pseudochattonella cf. verruculosa* that could be harmful to the health of farmed salmon in southern Chile. Critical concentration levels, that is, thresholds at which the behaviour of farmed *Salmo salar* is affected by harmful algae, were quantified using generalized linear mixed models (GLMM) and an extensive database from southern Chile covering the period from 1989 to 2016. The hypothesis underlying this study is that changes in salmon behaviour (e.g. loss of appetite, erratic swimming) and consequent changes in general wellbeing are related to the abundance of harmful microalgae. For both species analysed, the higher the cell abundance, the greater the probability of detecting anomalous behaviour. A threshold of 397 cells mL<sup>-1</sup> was estimated for *A. catenella*, although it can increase up to 620 cells mL<sup>-1</sup> at a Secchi depth >6 m and up to 874 cells mL<sup>-1</sup> during flood tide. A threshold value ≤1 cell mL<sup>-1</sup> for *Pseudochattonella cf. verruculosa* was found to be associated with anomalous salmon behaviour, which increased significantly at a water temperature of 11°C.

In spite of the general consensus that changes in the quantity and composition of nutrient supply (chronic and episodic) derived from anthropogenic activities affect phytoplankton growth and abundance, frequency and toxicity of HABs (Officer & Ryther 1980; Anderson *et al.* 2008; Heisler *et al.* 2008), the scientific evidence sustaining this causal link is scarce and strongly depends on local nutrient conditions (Davidson *et al.* 2012). Evidence that alteration in nitrogen:phosphorus ratios may promote HABs has been detected only for specific locations, but an unequivocal link between changes in nutrient ratios and HABs has not been demonstrated (Davidson *et al.* 2012). Nutrient ratios have been associated with the amount and rate of toxin production only under laboratory conditions (Fehling *et al.* 2004; Granéli & Flynn 2006), but the direct extrapolation of this relationship to field conditions is difficult (Marchetti *et al.* 2004; Davidson *et al.* 2012). Furthermore, several species have the ability to acquire P in organic or particulate form via a range of enzymes that convert organic to inorganic forms, or by mixotrophy, the ability to ingest particulate nutrients (Cochlan *et al.* 2008; Glibert & Burford 2017).

Therefore, considering the limited knowledge of the factors promoting the development and decay of harmful algal blooms in Chile (Sandoval *et al.* 2018), it is crucial to allocate effort to elucidate the relationship between nutrient availability and ratios and HABs in Chilean Patagonia. So far, there is no scientific evidence that salmon farming is or is not affecting the frequency and extent of HABs in Chile, because of major knowledge gaps and limited monitoring

of environmental conditions. For example, there is no regular monitoring of areas without salmon farms that could serve as reference sites during HABs.

#### Effects of pharmaceutical (antibiotics) and chemical (pesticides, antifoulants, disinfectants) discharges from salmon farms to the marine environment

Infections by bacteria, parasites, fungi and viruses are frequent causes of morbidity and mortality in salmon aquaculture in Chile, and they have important negative effects on production and profit (Asche *et al.* 2010; Cabello *et al.* 2013; Dresdner *et al.* 2019). The best approach to control pathogens in Chilean aquaculture is by improving sanitary conditions, husbandry and environmental practices, as well as discovering effective and environmentally friendly treatments (e.g. vaccines, immune modulators, in-feed masking compounds and non-pharmacological therapies). Nevertheless, in the short term antimicrobials and pesticides remain as the most used methods to prevent and provide treatment for bacterial infections and caligidosis (sea lice), respectively.

The potential effect of pharmaceuticals used in salmon farming has been explored in non-target species (mainly metazoan) in recent years (DeLorenzo *et al.* 2001; Burrige *et al.* 2004; Canty *et al.* 2007; Bhanu *et al.* 2011), but our understanding of potential impacts on the structure and functioning of aquatic ecosystems is still poor (Buschmann *et al.* 2006; Burrige *et al.* 2010).

#### Antibiotics

Chile has one of the highest rates of antibiotic use in salmon production of the world (Buschmann *et al.* 2009a; Millanao *et al.* 2011). One of the reasons it is so high compared to other countries is that the main bacterial threat to salmon farming in Chile is *Piscirickettsia salmonis*, a facultative intracellular bacterium which has proven very difficult to address through vaccination (Miranda *et al.* 2018; Figueroa *et al.* 2019). In fact, in the fattening phase in seawater, 94.7% of the antibiotics used were for piscirickettsiosis, 4.5% for Bacterial Kidney Disease (BKD) and 0.73% for other diseases (SERNAPESCA 2017). In 2017, the antibiotics used by the salmon industry in Chile were equivalent to 393.9 tons of active principle and the consumption index (percentage relation between the antibiotic used quantity and the tons of harvested biomass) was 0.05% (SERNAPESCA 2017). For the freshwater phase, the antibiotics used in 2017 were oxytetracycline (62.1%), florfenicol (19.8%) and erythromycin (18.1%). In the marine fattening phase 92.2%, 6.7% and 1.0% corresponded to florfenicol, oxytetracycline and flumequine, respectively (SERNAPESCA 2017). The use of antibiotics in salmon production in Chile has changed since 2005, showing an increase in the use of florfenicol and oxytetracycline compared to a decrease in

the use of the quinolones, oxolinic acid and flumequine (Miranda *et al.* 2018).

The main method to prevent bacterial infections is providing antibiotics through medicated feed and seldom by immersion baths (Sørum 2006; Smith *et al.* 2009). A significant percentage of the antibiotics used is released into the environment through unconsumed food, urine and faeces (Cabello *et al.* 2013; Miranda *et al.* 2018). The accumulation of antibiotic molecules in sediments may induce resistance among benthic bacteria (Shah *et al.* 2014), as well as changes in species composition and biogeochemical function (Costanzo *et al.* 2005; Tamminen *et al.* 2011). Unfortunately, very few studies have focused on benthic bacteria under marine salmon cages in Chile (Miranda & Zemelman 2002; Miranda *et al.* 2003; Buschmann *et al.* 2012; Aedo *et al.* 2014; Cabello *et al.* 2016), let alone on pelagic food webs around treated farms. From an environmental standpoint, the study of Buschmann *et al.* (2012) has the largest temporal coverage. They measured the numbers of cultured bacteria and antimicrobial-resistant bacteria in marine sediments of the Calbuco Archipelago over a 12-month period at a site approximately 20 m from a salmon farm and at a control site 8 km distant without observable aquaculture activities. Three antimicrobials extensively used in Chilean salmon aquaculture (oxytetracycline, oxolinic acid and florfenicol) were studied. Buschmann *et al.* (2012) did not detect any of these antimicrobials in sediments from either site, but they found traces of flumequine, a fluoroquinolone antimicrobial also widely used in Chile, in sediments from both sites during this period. They also found significant increases in bacterial numbers and antimicrobial-resistant fractions to oxytetracycline, oxolinic acid and florfenicol in sediments from the aquaculture site compared to those from the control site. Buschmann *et al.* (2012) concluded that these preliminary findings in one location may suggest that the current use of large amounts of antimicrobials in Chilean aquaculture has the potential to select for antimicrobial-resistant bacteria in marine sediments. This is consistent with the results from other studies showing that the use of high quantities of antibiotics has allowed the development of a high frequency of antibiotic-resistant bacteria in sediments, with values for tetracycline, sulphonamides, trimethoprim and amoxicillin of 32%, 50%, 47% and 45%, respectively (Fernández-Alarcón *et al.* 2010; Contreras & Miranda 2011; Ibieta *et al.* 2011; Millanao *et al.* 2011; Cabello *et al.* 2013).

Miranda and Rojas (2007) studied the occurrence of florfenicol-resistant bacteria in two Chilean freshwater salmon farms (Rupanco Lake and Llanquihue Lake) with different history of florfenicol use. Samples from surface water, pellet, *Salmo salar* fingerlings and control and under-cage sediments were collected from each salmon farm. They concluded that the important occurrence of florfenicol-

resistant and antibacterial-multiresistant microorganisms in unpolluted and farm-impacted sites suggests that use of florfenicol is not a necessary causal condition for the development of elevated frequencies of florfenicol resistance. They recommend that further research should be conducted to understand how florfenicol resistance spreads among environmental microbiota and the ecological significance of the occurrence of multidrug-resistant bacteria in Chilean fish farm environments.

Furthermore, there is concern that salmon aquaculture in Chile has the potential to increase the proportion of antimicrobial-resistant bacteria to antibiotics that are used in human medicine, such as oxytetracycline, oxolinic acid and florfenicol (Samuelsen *et al.* 1992; Coyne *et al.* 2001; Navarro *et al.* 2008; Buschmann *et al.* 2012; Cabello *et al.* 2013, 2016; Rozas & Enríquez 2014; Lozano *et al.* 2018). Globally, there is evidence that bacteria from sites used by aquaculture are more resistant to antibiotics and they have been able to transfer the resistance to bacteria in non-contaminated areas (Kerry *et al.* 1996; Winsby *et al.* 1996; Miranda & Zemelman 2002; Chelossi *et al.* 2003; Shakouri 2003). Several studies in Chile have also reported that *P. salmonis* has developed antimicrobial resistance to antibiotics (Shah *et al.* 2014; Tomova *et al.* 2015, 2018; Cartes *et al.* 2017; Lozano *et al.* 2018).

Tomova *et al.* (2015) studied acquired antimicrobial resistance genes (ARG) in the presence of tetracycline, florfenicol and quinolones in antimicrobial-resistant bacteria (ARB) from the water column and sediments of aquaculture-influenced sites. To investigate genetic links between quinolone-resistant marine bacteria and human pathogens, plasmid-mediated quinolone resistance genes in quinolone-resistant marine bacteria in Chile and in clinical isolates of quinolone-resistant human urinary tract *Escherichia coli* from coastal areas near aquaculture sites were compared. They found that levels of ARG were significantly higher in antimicrobial-selected marine bacteria than in unselected bacteria from these sites. While ARG in tetracycline- and florfenicol-selected bacteria from aquaculture and non-aquaculture sites were equally frequent, there were significantly more plasmid-mediated quinolone resistance genes per bacterium and significantly higher numbers of qnrB genes in quinolone-selected bacteria from the aquaculture site. Quinolone-resistant urinary *Escherichia coli* from patients in the Chilean aquaculture region were significantly enriched for qnrB, qnrS, qnrA and aac(6')-1b compared to isolates from New York City. Their results suggest horizontal gene transfer between antimicrobial-resistant marine bacteria and human pathogens (Tomova *et al.* 2015).

Henríquez *et al.* (2016) conducted a large-scale field study in Chile addressing the antimicrobial susceptibility profiles of *P. salmonis* for quinolones, florfenicol and

oxytetracycline. They analysed 292 field isolates collected from fish tissue samples (pool of kidney, liver and brain) from different farm sites over 5 years. Their results showed the existence of resistant types with a high incidence of resistance to quinolones and that resistance to florfenicol and oxytetracycline, despite its high therapeutic use, is still in the onset. Oxytetracycline and quinolone residues have been found in muscle samples from wild native species captured around salmon cages in Chiloé (Fortt *et al.* 2007).

Unfortunately, there have not been more comprehensive ecosystem (beyond farm) studies on the impacts of antibiotics. This is especially relevant in areas where there is salmon farming but also where urban sewage water goes directly into the ecosystem, as is the case of the city of Puerto Montt where sewage from 250 thousand people goes to the sea untreated (there is only retention of large solids). Here, it may be important to track the fate of antibiotics from both sources.

Finally, in 2016 the Chilean National Fisheries and Aquaculture Service (SERNAPESCA) started a certification programme for marine salmon farms free of the use of antibiotics. Presently, SERNAPESCA has certified 63 salmon farms as free of antibiotics (Lara *et al.* 2018).

#### Pesticides

The sea lice *Caligus rogercresseyi*, is one of the main threats to the Chilean salmon industry because it can generate severe skin damage leading to chronic stress, reduced growth and reduced feed conversion efficiency (González & Carvajal 2003; Rozas & Asencio 2007; Revie *et al.* 2009; González *et al.* 2015). In addition, the disease commonly known as “caligidosis” increases the vulnerability of fish to other diseases and increases production costs (Bravo 2003; Johnson *et al.* 2004; Lhorente *et al.* 2014; Dresdner *et al.* 2019). Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) are the main species farmed in Chile which are affected by *C. rogercresseyi* (Bravo 2003; González & Carvajal 2003). In contrast, Pacific salmon (*Oncorhynchus kisutch*) is less susceptible to *C. rogercresseyi* infection (Pino-Marambio *et al.* 2007).

*C. rogercresseyi* is better adapted to live in Patagonian waters with higher temperature and salinity (González & Carvajal 2003; Bravo *et al.* 2008a, 2015). Between 2011 and 2016, approximately 32% of marine fattening farms were located in sites strongly influenced by freshwater, brackish waters and estuaries (Soto *et al.* 2019). For the near future, General Circulation Models (GCM) forecast a reduction in precipitation in northern Patagonia (Garreaud 2018), which could favour sea louse proliferation.

Since the very early reported infections, sea lice in Chile have been controlled using chemicals (Reyes & Bravo 1983). Organophosphates (metrifonate (Neguvon<sup>TM</sup>) and

dichlorvos (Nuvan<sup>TM</sup>) applied by immersion treatments were used between 1981 and 2001, followed by oral treatment (Bravo *et al.* 2014). Avermectins administered as in-feed additives were introduced to Chile at the end of the 1980s (Ivermectin), and emamectin benzoate has been used since the end of the 1990s (Bravo *et al.* 2008b, 2010). Due to the detected resistance of *C. rogercresseyi* to emamectin benzoate (Bravo *et al.* 2008b, 2010), the industry started using the pyrethroids deltamethrin in 2007 (immersion bath treatment), cypermethrin in 2009 (immersion bath treatment) and the chitin synthesis inhibitor diflubenzuron in 2010 (in-feed additive). Subsequently, the organophosphate azamethiphos (immersion bath treatment) entered the Chilean market in 2013 (Helgesen *et al.* 2014). These pesticides have low solubility in water, a high octanol-water partitioning coefficient and high capacity for absorption by suspended matter, thereby reaching bottom sediments (Tucca *et al.* 2017). Recently, based on the prevalence of anti-parasite treatment results, the resistance of *C. rogercresseyi* to pyrethroids is suspected in neighbourhoods located in the central Aysén Region and northern zone of the Los Lagos Region (Arriagada *et al.* 2018). A previous study detected a severe reduction of sensitivity to pyrethroids in areas with high parasite abundance located in the Los Lagos Region (Marín *et al.* 2015).

Tucca *et al.* (2017) measured the pesticides emamectin benzoate (EB), diflubenzuron (DI), teflubenzuron (TE) and cypermethrin (CP) in sediments near salmon cages in southern Chile. Concentrations of EB were between 2.2 and 14.6 ng g<sup>-1</sup>, while the benzoylphenyl ureas DI and TE were detected in the ranges of 0.1 to 1.2 ng g<sup>-1</sup> and 0.8 to 123.3 ng g<sup>-1</sup>, respectively. These results were similar to data reported for the Northern Hemisphere (Scotland, Norway, Canada). However, the pyrethroid CP was detected in higher concentrations, ranging from 18.0 to 1323.7 ng g<sup>-1</sup>. According to reported toxicity data, this range represents a potential risk for benthic invertebrates.

Recently, Gebauer *et al.* (2017) assessed the effects of cypermethrin, deltamethrin, azamethiphos and hydrogen peroxide on the performance of the larvae of *Metacarcinus edwardsii*, an important resource for artisanal fishers. They concluded that the pesticides used against *Caligus* affect these non-target crustacean larvae negatively. The lethal effects of the pyrethroids probably are restricted to immediately around the time and area of application, while the action of azamethiphos may extend to a wider area (Gebauer *et al.* 2017). Nevertheless, these authors concluded that current data are insufficient to assess the effects of these compounds in the ecosystem accurately. The early stages of development of the giant mussel *Choromytilus chorus*, another resource for artisanal fishers, did not show a clear effect to pesticide exposure (Deltamethrin, Emamectin benzoate, azamethiphos) in bioassays (Sanhueza-

Guevara *et al.* 2018). However, trochophore development seemed accelerated in the presence of pesticides compared to that of control larvae.

Rain-Franco *et al.* (2018) recently investigated the response of natural microbial communities to the addition of azamethiphos, deltamethrin and emamectin benzoate and their potential impact on photoautotrophic and chemoautotrophic carbon fixation in central-southern Chile and in Cauahue Channel (Chiloé Island). They concluded that the use of these pesticides in marine waters can produce changes in microbial photo- and chemoautotrophic carbon uptake. Nevertheless, these effects are variable and show significant alterations of carbon fixation flux if a single pesticide is applied as opposed to a combination of two or more compounds. Emamectin benzoate can potentially act as a depressor of carbon fixation while azamethiphos can stimulate primary production in conditions of nutrient limitation or deficiency. They also concluded that the effect of pesticides may be related to the magnitude of primary production and environmental conditions for phytoplankton activity, including nutrient deficiency.

Current scientific information on the potential impact of pesticides on the ecology of Patagonian ecosystems is still very scarce. Further research is urgently needed to address this issue.

#### Antifoulants

Most contemporary antifouling formulations employ an active pigment and a variety of organic or organo-metallic booster biocides which are embedded in or linked to an organic matrix. Cuprous oxide (Cu<sub>2</sub>O) is the usual choice of pigment, although Cu(I) thiocyanate and Zn(II) oxide are also commonly employed (Singh & Turner 2009). The highest risk is the accumulation of copper and zinc in the sediments surrounding the salmon cages. There is extensive scientific evidence that Cu and Zn can produce harmful effects (e.g. growth, reproduction, mortality, behaviour, etc.) in bivalves and macroalgae and that the damage is dependent on the metal concentration, duration of exposure, environmental factors (e.g. pH, salinity) and intrinsic characteristics of the species (Contreras *et al.* 2007; Ali & Taylor 2010; Lawes *et al.* 2016). Almost all antifouling paints in Chile currently contain Cu<sub>2</sub>O (Bravo *et al.* 2005; Vera *et al.* 2015). Oyarzún *et al.* (2017) reported that most salmon companies (61%) in Chile prefer to use water-based antifoulant paint instead of solvent-based paints. They estimated that a conservative figure for the introduction of copper into the marine ecosystem by salmon farms in southern Chile would be 64 tons per production cycle (i.e. 18 months). For zinc, they estimated an input of 42 kg per production cycle (Oyarzún *et al.* 2017).

Evidence of reduced microbial biodiversity has been reported in copper-enriched sediments from Southern



Chile salmon farms (Buschmann & Fortt 2005). Vera *et al.* (2015) analysed sediment samples from three sites off Chiloé Island where salmon farms were using antifouling paints in order to study possible changes in the abundance and diversity of benthic macrofauna due to the accumulation of metals such as Cu and Zn in sediments. They found Cu concentrations in the sediments ranging from 8.73 to 1034.37  $\mu\text{g g}^{-1}$  and Zn between 20.25 and 119.28  $\mu\text{g g}^{-1}$  (Vera *et al.* 2015). Despite the Cu accumulation found in sediments adjacent to the fish cages, no significant changes in the composition or abundance of benthic macrofauna were found even when concentrations of Cu exceeded 800  $\mu\text{g g}^{-1}$  in sediments (Vera *et al.* 2015). However, the concentrations of Cu found in the sediments were much higher than those considered as non-damaging to the biota (Debourg *et al.* 1993; Stark *et al.* 2003; Dean *et al.* 2007; Vera *et al.* 2015). Recently, Oyarzún *et al.* (2017), working in a different zone of Chiloé Island, found copper concentrations in sediments <13.36  $\mu\text{g g}^{-1}$  in winter and <25.78  $\mu\text{g g}^{-1}$  in summer. The concentration of Zn was <19.5  $\mu\text{g g}^{-1}$  in winter and <51.31  $\mu\text{g g}^{-1}$  in summer. Local environmental and oceanographic conditions are important in determining metal accumulation in sediments near salmon farms and beyond.

#### Disinfectants

Prevention and reduction of the spread of animal pathogens are largely dependent on the principles of good biosecurity, decontamination, disinfection and sanitation (Ford 1995). In several salmon culture production procedures, disinfectants are important tools to ensure farm biosecurity. They are used, for instance, to reduce or eliminate pathogenic microorganisms on surfaces and to disinfect equipment (Burka *et al.* 1997). Bravo *et al.* (2005) reported that the following products are used as disinfectants in Chilean aquaculture: Virkon<sup>®</sup>, iodine+detergents, Chloramine-T, hypochlorite ( $\text{HClO}_2$ ), chlorine dioxide ( $\text{ClO}_2$ ), benzalkonium chloride, Superquats<sup>®</sup>, glutaraldehyde, formalin 40%, calcium oxide (CaO or quicklime), calcium hydroxide ( $\text{Ca(OH)}_2$  or slake lime), sodium carbonate ( $\text{Na}_2\text{CO}_3$  or soda ash), Creolin, synthetic phenols, halophenols and ethanol (95% and 70%). Most of these chemicals have been used to inactivate pathogenic bacteria and viruses on rearing equipment, seawater pipes, air hoses, tanks and nets, as well as on staff's hands and footwear. During the ISA virus crisis, disinfectants were an important tool to combat the dispersion of the disease. It is known that ISA is spread by waste waters from processing plants containing ISAV-infected blood in an untreated state (Smail *et al.* 2004). Therefore, effective disinfectants were needed for wastewater disinfection. In addition, mitigation measures included the avoidance of the entry of staff and visitors to the farms with inadequately disinfected implements and equipment

(García *et al.* 2013). Regulations were implemented for each of these identified risk factors; cleanliness and disinfection were considered fundamental in controlling the disease (García *et al.* 2013).

Mardones *et al.* (2016) found that the use of peracetic acid, peroxides, glutaraldehyde and active chlorine dioxide are the most effective for minimizing the load of *Piscirickettsia salmonis* contaminated on salmon fillets. Recently, Muniesa *et al.* (2018) also showed that disinfection using peracetic acid, peroxides and both active and inactive chlorine dioxides is effective against *P. salmonis*.

In Chile, there are several regulations regarding the type of disinfectants and their proper use by the salmon farms and processing plants (e.g. Cleanliness and disinfection applicable to fish production, R.E. N° 2011/2011; Techniques and methods of disinfection of tributaries and effluents, control and treatment of organic solid waste, R.E. N° 4866/2014; Manual of Food Safety and Certification, Res. Ex. N°5125 – 2016; Disinfection of salmonid fish eggs, R.E. N° 65/2003). There is no information on the total amount of disinfectants used by the Chilean salmon aquaculture industry including processing plants (Bravo *et al.* 2005) or on their environmental impact. Burrige *et al.* (2010) stated that some disinfectant formulations may contain surfactants which are endocrine disruptors for marine biota. So far, there is no scientific research published assessing the possible effects of disinfectants used in salmon production on the Patagonian environment.

#### Escapes from fish farms and potential effects on wild populations

Escapes of salmon from farms can have significant ecological consequences on native biota and ecosystems, and accordingly is considered one of the key environmental risks associated with salmon aquaculture (Morris *et al.* 2008; Baskett *et al.* 2013). There are several factors that can produce the escape of fish from salmon farms, including damage to net pens from storms, tides, currents, predators, vandalism, boats and farming equipment, accidental spills of fish during transport from hatcheries and handling during harvest or grading (EVS 2000). Farmed salmon escapees can impact native species through predation, competition and spreading of disease and parasites, among others (Soto *et al.* 2001; Naylor *et al.* 2005; Young *et al.* 2009, 2010; García de Leaniz *et al.* 2010; Jensen *et al.* 2010; Buschmann *et al.* 2012; Sepúlveda *et al.* 2013).

The escape from the net pens of salmon farms occurs by small but frequent operational leakages from local farms (1–5% of the total culture individuals), whereas millions of salmon individuals may escape when extreme meteorological events take place and destroy part of the culture facilities (Soto *et al.* 2001; Niklitschek *et al.* 2011a; Outeiro &

Villasante 2013; Beveridge *et al.* 2018). Estimates suggest that more than 900,000 salmon individuals escape annually from the salmon farms in Chile (Thorstad *et al.* 2008; Sepúlveda *et al.* 2013). While a decrease in the magnitude of escaped Atlantic and Coho salmon has occurred during the last several years, escaped rainbow trout have not followed the same pattern. Rainbow trout have become a greater threat to native ecosystems due to their greater potential to establish self-sustaining naturalized populations (Sepúlveda *et al.* 2013). However, trout were actively introduced in Chile for recreational fishing in the early 1900s and they are widely spread along the country (Soto *et al.* 2006), thus it is difficult to estimate additional impacts from farmed escapees.

Escaped salmonids can impose a strong predatory pressure upon wild fish in Patagonia, including species of commercial importance to artisanal fishers (Soto *et al.* 2001; Arismendi *et al.* 2009; Niklitschek *et al.* 2011b). Should escapees and/or purposely introduced salmon develop ocean migrating populations, they could make a contribution to the freshwater trophic food web by providing marine-derived nutrients to microbial communities and epilithion and food for invertebrates and fish through carcass material and eggs (Chaloner *et al.* 2002). This proved to be the case after the successful establishment of Chinook salmon (*Oncorhynchus tshawytscha*) in Chile, which originated mainly from active introduction through ranching (Soto *et al.* 2007; Arismendi *et al.* 2009).

Up to now, there is no evidence of naturalized populations of Atlantic salmon (*Salmo salar*), the most widely cultured species in Chile, in the southern part of the country (Soto *et al.* 2001, 2006; Schröder & García de Leaniz 2011; Niklitschek *et al.* 2013). Published information on previous large escapes (Soto *et al.* 2001) supplemented by additional indirect information suggests that Atlantic salmon escapees in Chile are not very successful from a trophic standpoint and they do not thrive well in the wild (Arismendi *et al.* 2014). Also, as shown by Soto *et al.* (2001), escaped salmon in southern Chile can be efficiently caught by artisanal fishers. This scenario contrasts with the situation generated by escapes of *S. salar* in their native environment, such as Norway (Thorstad *et al.* 2008; Beveridge *et al.* 2018).

Fortunately, a significant reduction in escape events has occurred during the past 5 to 10 years, mostly due to improved cages and better management. However, a very recent event shows that farming systems are almost never completely secure against escapes. On July 5, 2018 there was a large escape (approximately 800,000 adult fish weighting 3.4 kg each on average) from a farm in Huar Island in front of Puerto Montt, in the middle of the Reloncaví sound (X Region; Fig. 1). Approximately, 250,000 live fish were recovered by the farming owner and many more were removed by fishing by the artisanal fisheries in the

area. Unfortunately, a small fraction of the escaped fish had been recently treated with antibiotics and thus their consumption may have represented a food safety risk, aside from potential risks to the environment.

Risk to indigenous species is a relevant potential issue to consider when discussing the ecological impacts of salmonid escapes, because they can introduce new pathogens, alter disease patterns, and even act synergistically to increase the impact of other stressors (García de Leaniz *et al.* 2010; Habit *et al.* 2010; Sepúlveda *et al.* 2013). Escaped salmonids can also become potential vectors for parasites and diseases at a broad scale (Thorstad *et al.* 2008; Sepúlveda *et al.* 2013) due to their dispersal capabilities (Melo *et al.* 2005; Whoriskey *et al.* 2006; Skilbrei *et al.* 2009). Indeed, there is evidence that infectious pancreatic necrosis (IPN) can be transmitted from salmon escapees to native fish species (Hnath 2002; Guy *et al.* 2006). There are also reports of decrease in the abundance of *C. rogercresseyi* infecting native hosts after the closing of a salmon farm (Costello 2009).

Thus, the monitoring of fish escapes from farming systems and the understanding of ecological and sanitary effects of these on native fish and communities should remain a priority for Chilean salmon aquaculture.

### Interaction of salmon farms with marine mammals, birds and sharks

Mobile fauna, including crustaceans, fish, birds and marine mammals, may have complex interactions with aquaculture operations, including attraction or repulsion to farm operations (for a global review see Callier *et al.* 2017). The most common effects on wild fauna are those related to the provision of physical structure (farm infrastructure acting as fish aggregating devices), the provision of food (e.g. farmed animals, waste feed and faeces, fouling organisms associated with farm structures) and some farm activities (e.g. boating, cleaning) (Callier *et al.* 2017). The interaction can also have negative effects for salmon producers, as is the case of attacks by predators, which are an important factor in the escapes of salmonids in Chile (Sepúlveda & Oliva 2005; Vilata *et al.* 2010).

In Southern Chile, there is a well-known negative interaction between salmon farms and the South American sea lion *Otaria flavescens*, because the high density of fish stocked inside the salmon pens constitutes a strong attraction for these pinnipeds (Sepúlveda & Oliva 2005; Vilata *et al.* 2010). Sea lion control is presently ensured by predator nets with mesh size less than 10 inches, to avoid catching or drowning predators (Oliva *et al.* 2004; Schrader 2005). Oliva *et al.* (2008) reported that this mitigation strategy has been effective and that entangling or enmeshing of sea lions at salmon farms is not a significant

conservation issue. The attacks by *O. flavescens* on salmon farms follow seasonal patterns, with salmon predated more in autumn and winter, and daily patterns, with more interactions at night (Vilata *et al.* 2010). In addition, Vilata *et al.* (2010) found that attacks were more frequent on larger salmon, suggesting the existence of a prey-size preference; they suggested that currents linked to tidal flux might facilitate the access of the sea lions to the farmed salmon.

Sepúlveda *et al.* (2015) examined the degree of spatial overlap between the South American sea lions and salmon farms using satellite telemetry and stable isotope analysis, and quantified the amount of native prey *versus* farmed salmonids in their diets. The most important prey were farmed salmonids, with an estimated median of 19.7% and 15.3% for hair and skin, respectively. They also observed a switch in diet composition in two South American sea lion from farmed salmonids to pelagic fish, which coincided with the decrease in salmon production due to the ISA virus that affected salmon farms in Chile at the end of 2008. They suggested that *O. flavescens* are able to adapt to spatiotemporal shifts in the abundance of potential prey, including feral and farmed salmonids (Sepúlveda *et al.* 2017).

The presence of farm structures and their associated activities can potentially exclude or modify how particular species of marine mammals use critical or sensitive habitats and/or modify historical migratory routes (Heinrich 2006). Huckle-Gaete *et al.* (2013) reported that the level of ship traffic has increased considerably during the last decade as a result of more cargo and supply shipping for the salmon farming industry, as well as public transportation, tour boats and fishing. High shipping traffic can be negative to cetaceans due to increasing the risks of collisions and noise pollution. In fact, Huckle-Gaete *et al.* (2006) reported records of boat collisions with blue whales in the Corcovado Gulf. Furthermore, Huckle-Gaete *et al.* (2013) reported that a humpback whale calf became entangled in a salmon farm's 'anti sea lion' nets during the summer of 2007.

Ribeiro *et al.* (2005) have shown that Chilean dolphins (*Cephalorhynchus eutropia*) in southern Chiloé Island react negatively to boat presence, with behavioural responses such as changes in swimming reorientation rate and speed. However, Ribeiro *et al.* (2007) reported that the presence of salmon farms did not seem to influence or alter movement patterns and habitat use of Chilean dolphins directly. Animals were neither attracted to nor tried to avoid salmon farm structures, since there was no significant association between areas close to the farms and the intensity of habitat use (absence, little, intermediate or high). The lack of direct interference on habitat use patterns and movements of dolphins could also be explained by the fact that salmon farm cages in the study area were located outside the dolphin's preferred habitat (i.e. distant from the coast and with

depths >15 m) Ribeiro *et al.* (2007). In contrast, they stated that in the fjords of southern Chile salmon farm structures are commonly close to the shore, and there Chilean dolphins have been observed avoiding farm cages (Ribeiro *et al.* 2007).

It is interesting to note that research on marine mammals in Patagonia should become more important for aquaculture activities in Chile because a new US trade rule requires countries to demonstrate that their fishery and aquaculture activities are equivalent in effectiveness to the US Marine Mammal Protection Act or risk losing the permit to export seafood products to the US market (Williams *et al.* 2016; Bedriñana-Romano *et al.* 2018).

Gaitán-Espitia *et al.* (2017) studied the interaction between the piked dogfish *Squalus acanthias* and salmon farms in Patagonian fjords. They found that the spatial overlap of shark nursery areas and the salmon industry influences the trophic niche of *S. acanthias* in this region by adding new food items (i.e. uneaten pellets) and by changing prey availability around the cages. Despite differences in the trophic patterns of *S. acanthias* due to the spatial association with intensive salmon farming, there appeared to be no difference in fecundity or size at maturity compared to other populations (Gaitán-Espitia *et al.* 2017).

Birds may also be attracted to farmed and associated fish. For instance, Buschmann *et al.* (2009a,b) found that the abundance of omnivorous diving and carrion-feeding marine birds were two and five times, respectively, as abundant in areas with salmon farms compared to nearby control areas. Many salmon farms have installed bird nets on top of the containment nets to protect from bird predation.

While the potential for more negative indirect ecosystem effects on marine mammals due to salmon farming such as antibiotics (Cabello 2006; Sanino *et al.* 2014) and pesticides (Buschmann *et al.* 2009a,b) has been considered in the literature, no direct scientific evidence on such indirect effects has yet been documented in Patagonian ecosystems.

## Final remarks

The lessons from the Infectious Salmon Anemia (ISA) crisis in 2007–2008 changed the Chilean production model of salmon farming (Iizuka & Jorge Katz 2015; Hosono *et al.* 2016; Chávez *et al.* 2019), and especially the regulatory framework (for a deep analysis see Fuentes & Engler 2016). The ISA crisis showed the need to strengthen the legal framework for environmental protection, including marine spatial planning, environmental protection and sanitary measures. For instance, the current framework includes environmental impact assessment of new farms, regular monitoring of the environmental conditions of the water column and

**Table 1** Summary of the main knowledge gaps on the environmental effects of Chilean salmon aquaculture and research needs to address these gaps

Issue	Research needs
1. Impacts of feed and organic waste	<ul style="list-style-type: none"> <li>To study the far-field effects of salmon farming on nutrient flow and nutrient mass balance in the benthic and pelagic food webs (from microorganisms to wild predators) and ecosystem functioning (e.g. biogeochemical cycles), considering natural and anthropogenic sources.</li> <li>There is a need for studies that examine the impact of salmon production on benthos over a longer timescale and to examine the cumulative impacts of multiple farms in conjunction with other human activities.</li> </ul>
2. Absence of adequate carrying capacity models	<ul style="list-style-type: none"> <li>To develop and/or refine models for estimating productive carrying capacity in key Patagonian ecosystems in order to promote sustainable aquaculture. These models require crucial information from the research gaps described under issue (1) above.</li> <li>To address the ecological carrying capacity of Patagonian ecosystems to receive chemicals and pharmaceuticals (e.g. antibiotics, pesticides) used in aquaculture production.</li> </ul>
3. Potential salmon farming effects on harmful algal blooms	<ul style="list-style-type: none"> <li>To improve the understanding of the drivers of HABs in Patagonian waters and the role that salmon aquaculture may (or may not) play in their emergence.</li> <li>To improve the understanding of physical and chemical variability of the Patagonian marine ecosystems at different temporal and spatial scales by creating a state-of-the-art ocean observation system.</li> </ul>
4. Impacts of antibiotics	<ul style="list-style-type: none"> <li>To identify and quantify environmental and oceanographic factors that can influence the spread and persistence of <i>Piscirickettsiosis</i>.</li> <li>To understand the processes and mechanisms that modulate persistence of antibiotics in the environment and the effects on non-target organisms, ecological processes and biogeochemistry.</li> <li>Further research should be conducted to understand how antibiotic resistance spreads among environmental microbiota and the ecological significance of the occurrence of multidrug-resistant bacteria in Chilean fish farm environments.</li> <li>To conduct comprehensive studies at the ecosystem level (beyond farms) on impacts of antibiotics and to track the fate of antibiotics from different anthropogenic sources (e.g. sewage).</li> <li>To study horizontal gene transfer between antimicrobial-resistant marine bacteria and human pathogens.</li> </ul>
5. Impacts of sea lice pesticides	<ul style="list-style-type: none"> <li>To identify and quantify major environmental and oceanographic factors that can influence the outbreak, spread and persistence of <i>Caligidosis</i>.</li> <li>Current scientific information on the potential impact of pesticides to the ecology of Patagonian ecosystems is still very scarce. Further research is urgently needed to address this issue, including the effects of pesticides on food webs from microorganisms to top predators with emphasis on relevant fisheries and aquaculture target species (e.g. <i>Mytilus chilensis</i>).</li> </ul>
6. Impacts of antifoulants	<ul style="list-style-type: none"> <li>To understand the mechanisms of metal level fluctuations in sediments such as spatial variability, partition of metals between solid and pore water, dispersal, trophic transfers and accumulation processes.</li> <li>To evaluate the potential effects on benthic organisms of chronic exposure to elevated copper and zinc in sediments near salmon aquaculture sites.</li> </ul>
7. Impacts of disinfectants	<ul style="list-style-type: none"> <li>Information is needed on the amounts of disinfectants used by the salmon aquaculture industry, including processing plants.</li> <li>To assess the presence and potential impacts of disinfectants near salmon farms in the Patagonian marine environment.</li> </ul>
8. Impacts of escaped farmed salmon	<ul style="list-style-type: none"> <li>To develop an efficient and permanent monitoring system of salmon escapes from salmon farms.</li> <li>To assess the ecological impacts of salmon escapees, including disease and parasite spread, predation, competition, and other types of effects on native species, communities and ecosystems (e.g. food webs, biogeochemical cycling).</li> </ul>
9. Impacts of salmon farms on marine mammals, birds and sharks	<ul style="list-style-type: none"> <li>To study salmon farming effects on home ranges, migration routes, location and quality of habitats for relevant marine mammal species in Patagonia</li> <li>Research is needed on hearing capabilities and the effects of sound on marine mammals, birds and sharks.</li> <li>Research is needed on long-term health implications of noise exposure in marine mammals, seabirds and sharks.</li> <li>To improve data on the number and nature of entanglements of marine mammals and other animals at aquaculture sites and assess factors (e.g. net design, husbandry practices) affecting entanglement rates.</li> </ul>
10. Impacts of the freshwater phase of salmon production	<ul style="list-style-type: none"> <li>There is a need for a systematic survey of noise sources in the salmon aquaculture industry.</li> <li>To study land-based salmon farming impacts in Chilean streams, specially analysing impacts in the context of other human-related stressors at the ecosystem level (i.e. stream basin, catchment).</li> <li>To study the amount and fate of particulate organic matter derived from salmon farms in freshwater ecosystems and its impact on benthic habitats, communities and food webs (from bacteria to top predators in streams and lakes), including effects of antibiotics and other chemicals.</li> <li>To estimate loads, fate and impacts of dissolved phosphorus, nitrogen and carbon from aquaculture facilities on freshwater communities, food webs and the relevant ecosystems at large.</li> <li>To develop an efficient monitoring system of salmon escapes from freshwater salmon farms.</li> <li>To evaluate the ecological effects of escaped salmon in Chilean freshwater ecosystems.</li> </ul>



benthos, adoption of sediment aerobic conditions as an indicator of an operation compatible with the carrying capacity of the water body, the prohibition to introduce fish to the farm until aerobic conditions are re-established and protocols to prevent escapes and to recover escaped fish. The amendments introduced to the legal framework also stated that renewal of aquaculture leases is subject to strict compliance with environmental regulations as reflected in the environmental monitoring reports. The Aquaculture Reform Act strengthened the available sanctions for the most important sanitary and environmental infractions, including fines, suspension of operations for five years and termination of the lease.

In general terms, the Chilean the regulatory framework has evolved to include many of the international recommended best practices (Fuentes & Engler 2016; Alvial 2017). However, the present review of the most relevant environmental issues affecting salmon production in Chile clearly shows that there are crucial knowledge gaps to ensure sustainability. The large spatial scales and heterogeneity of oceanographic and geomorphologic characteristics of Patagonia impose a major challenge in terms of the generation of the scientific knowledge to fulfil the gaps. In the last 10 years, there has been a clear increment in research efforts focused on Chilean Patagonian ecosystems, with new centres of research funded by the State (Center for Research in Ecosystems of Patagonia, CIEP; Center of Oceanographic Research COPAS Sur-Austral; Interdisciplinary Center for Aquaculture Research, INCAR; Center for the Study of Multiple Drivers on Marine Sociological Systems, MUSSELS; Millennium Nucleus of Invasive Salmonids, INVASAL; Research Center Dynamics of High Latitude Marine Ecosystems, IDEAL) located in universities or linked to Regional Governments (e.g. CIEP). Furthermore, the government has allocated funding for specific programmes to strengthen the institutional capacity of the Fisheries Development Institute (IFOP) in monitoring and research in areas such as HABS and oceanographic modelling. The government has also funded research on environmental modeling and pathogens through the Fund for Strategic Investment administered by SERNAPESCA.

Despite these efforts, there are still major gaps in baseline knowledge about the structure and functioning of the mosaic of marine ecosystems conforming the Chilean Patagonia, including biodiversity, trophic interactions, populations and community connectivity, biogeochemical cycling and circulation patterns, among others. The majority of publications addressing salmon farming sustainability and policies in Chile are focused on fish health issues. Contrastingly, there are very few evaluations of the impacts of nutrients, chemicals and pharmaceuticals

(e.g. antibiotics, pesticides) used in salmon production beyond areas beneath salmon cages and those immediately adjacent to the farms. Few studies provide a more ecosystemic perspective of salmon farming impacts considering larger spatial scales (see Soto & Norambuena 2004; Buschmann *et al.* 2009a), while the study of Mayr *et al.* (2014) reports the first indirect evidence of eutrophication due to aquaculture in a whole Patagonian fjord.

There are two major structural weaknesses to address salmon farming environmental issues properly from the research perspective in Chile: (i) although, as indicated above, there are research centres and initiatives to address relevant environmental issues, these are not well coordinated, and (ii) there is no integrated environmental monitoring or other ocean observation system (Sandoval *et al.* 2018) of the Patagonian marine ecosystems. These observation systems are much needed to facilitate understanding and forecasting of ocean conditions in order to confront environmental risks such as HAB events and pathogen dispersal, as well as to improve modelling (e.g. physical, biogeochemical), aquaculture zoning, carrying capacity estimates and to detect long-term trends (e.g. regime shifts, climate change), among other uses. Based on the information reviewed here, we provide in Table 1, a summary of the main knowledge gaps regarding environmental sustainability of salmon culture in Chile. Fully addressing these gaps is essential to advance towards an ecosystem approach and to protect marine ecosystems, which are embedded as obligations in the Chilean General Fisheries and Aquaculture Act. Possibly, the most important of all the knowledge gaps (Table 1) is to define an approach to address carrying capacity (both ecological and productive considering biosecurity) for relevant water bodies (fjords, channels etc.), since this could lead to establish maximum biomass to be produced per unit area, even if only proxies are used initially. This information would lead to policy making that ensures a more sustainable salmon farming and could guarantee minimizing risks.

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