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Relationship between salt use in fish farms and drift of macroinvertebrates in a freshwater stream

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ABSTRACT: In Chile, salt (NaCl) use per salmon fish farm ranges between 20–30 t yr^{-1} and is used to prevent and control fungal infections. An increase in salinity in freshwater can have adverse effects on freshwater biodiversity and ecosystem functions and services. We studied the effects of fish-farm effluents on benthic macroinvertebrate communities in a northern Patagonian stream (Chile). Benthic samples were collected at 3 sites near a land-based salmon aquaculture facility (one located 100 m upstream from the fish-farm outlet for effluent, 2 sites located 200 and 400 m downstream from the effluent source). We found changes in benthic macroinvertebrate communities downstream from the effluent, with higher abundances of tolerant taxa and lower abundances of sensitive taxa, which was related to nutrient and salt concentration in the water. We also studied the effects of salinity on macroinvertebrate drift in a mesocosm experiment conducted in recirculating channels, measuring the drift of 2 salt-sensitive macroinvertebrates (*Andesiops peruvianus* and *Smicridea annulicornis*), collected from an unpolluted northern Patagonian stream, after exposure to a range of salinity concentration pulses similar to those from fish farms. Our results demonstrate that (1) fish-farm effluent can alter stream macroinvertebrate community composition and dynamics, and (2) such effects are at least partly driven by high salt concentrations in effluent waters.

KEY WORDS: Macroinvertebrate communities · Drift · Sodium chloride · Fish farm · *Andesiops* · *Smicridea*

1. INTRODUCTION

Increased salinity in freshwater is an emerging issue of global concern, with potentially adverse effects on human health, freshwater biodiversity and ecosystem functions and services (Kefford et al. 2004, Cañedo-Argüelles et al. 2016b, Kefford et al. 2016). Anthropogenic salinization has been related to activities such as agriculture, aquaculture, mining, industry, urban effluent treatment and use of road de-icing

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salt, and is known to affect macroinvertebrate drift rates and macrozoobenthos structure in freshwater (Kefford et al. 2002, Webb 2012, Cañedo-Argüelles et al. 2014, Szöcs et al. 2014, Dunlop et al. 2015). Discharges from land-based fish-farms contain dissolved nutrients, disinfectants and other substances that can have potentially harmful effects on freshwater communities. In southern Chile there are around 300 land-based salmon farms which produce up to 2000 t yr^{-1} of fish. Ninety-three of them are located in the La Araucania region (northern Patagonia, southern Chile) and produce in total ca. 207 t yr^{-1}; mainly smolt (2017, www.sernapesca.cl). Each farm utilizes 20–30 t yr^{-1} of salt (NaCl) in order to prevent and control infections (Marking et al. 1994, Zaror et al. 2004, Tello et al. 2010), which have caused an increase in freshwater conductivity from 50 µS cm^{-1} to up to 1000 µS cm−1 downstream from where the effluents are discharged (Nimptsch et al. 2014). In Chile, land-based fish-farm effluents must meet environmental emission standards (MINSEGPRES 2001), which set chloride to 2000 mg l^{-1} (5359 µS cm^{-1}), but there are no specific standards for protecting river biota.

Drift, the downstream transport of organisms, is considered a complex response of macroinvertebrates to abiotic (e.g. current speed, flow, water chemistry, temperature or photoperiod) and biotic factors (e.g. life cycle, density, food resources, predation or competition) (Reisen & Prins 1972, Hildebrand 1974, Brittain & Eikeland 1988, Smock 1996). Increased salt concentration in freshwater is known to cause acute and chronic effects on benthic species, driving changes in communities, e.g. decreasing abundance of salinity-sensitive taxa such as Ephemeroptera, Plecoptera and Trichoptera and increasing abundance salinity-tolerant taxa such as Oligochaeta and Chironomidae (Camargo 1992, Loch et al. 1996, Kefford et al. 2016, Sala et al. 2016). Salt inputs can also increase periphyton abundance, produce harmful algal blooms (Berg et al. 1997), and alter ecosystem processes such as stream metabolism, organic matter decomposition, respiration, nutrient flow or benthic macroinvertebrate drift (Vannote et al. 1980, Loch et al. 1996). No causal relationship between salt addition and changes in benthic communities has been demonstrated to date in studies conducted in larger streams exploring the effects of fish farms on stream communities (Figueroa et al. 2014, 2017, Nimptsch et al. 2015, Kamjunke et al. 2017). The aim of this study was to (1) assess whether the presence of salt from land-based fish farm in a headwater stream in southern Chile has caused changes in environmental factors and benthic macroinvertebrate communities and (2) to test whether there is a causal relationship between conductivity and drift of 2 selected macroinvertebrate taxa using a mesocosm experimental setting. Mesocosms, in the form of artificial stream channels, are a useful tool as these provide controlled conditions while simulating stream flow in which benthic drift can be measured (Warren & Davis 1971, Navarro et al. 2000, Cañedo-Argüelles et al. 2014, 2016a).

2. MATERIALS AND METHODS

2.1. Field survey

Field work was conducted in January (austral summer) 2015 in a rithronic section of the Molco Stream (39° 20′ 0.4″ S, 72° 05′ 44.8″ W), in the surroundings of a fish farm southeast of Villarrica (northern Patagonia, southern Chile). We established 3 sites, one located 100 m upstream from the fish-farm effluent discharge location (E1) and 2 downstream from the discharge location (E2 and E3, located 200 and 400 m, respectively). At each site, we collected 3 benthic samples using a Surber sampler (0.3 × 0.3 m, 250 µm mesh size) which were deposited in plastic bags preserved in 95% ethanol and transported to the laboratory for taxonomic identification to family level and expressed as individuals per m^2 (ind. m^{-2}). At each site, we also collected water samples in 3 l plastic containers which were taken to the laboratory within 4 h for physicochemical analysis at 4°C. Water samples were analyzed for concentrations of total nitrogen (TN), total phosphorous (TP), ammonium (NH_4^+) and nitrite (NO_2^-), biological oxygen demand (BOD) and total suspended solids (TSS), all according the Standard Methods for the Examination of Water and Wastewater (APHA 2005). Conductivity, dissolved oxygen, temperature and pH were measured in the field using a WTW MULTI 340i/SET multiparameter sonde (Wissenschaftlich-Technische-Werkstätten).

2.2. Mesocosm experiment

We used 2 of the most common macroinvertebrate taxa, *Andesiops peruvianus* Lugo-Ortiz & McCafferti, 1999 (Ephemeroptera: Baetidae) and *Smicridea annulicornis* Blanchard in Gay, 1851 (Trichoptera: Hydropsychidae), both found in a stream without any effluent discharge from fish farms, the Catrico

Stream at 39° 08′ 2.1″ S, 72° 20′ 16.4″ W. We used this stream because the Molco Stream does not have any unaffected areas (the fish farm is located at the headwaters). Individuals were collected from the benthos using a Surber sampler (250 µm mesh size) and transported to the laboratory in stream-water filled containers. Mesocosms (based on Warren & Davis 1971 and Navarro et al. 2000) consisted of rectangular glass channels (100 cm long, 14 m wide, 30 cm deep and 1% slope) containing filtered (45 µm) stream water (22.3 l) from the unpolluted Catrico Stream. The bottom was covered with a gravel layer (2–3 cm). Water was recirculated with a continuous flow rate of 19.2 l min^{-1} and a velocity of 0.11 ± 0.06 m s^{-1}, simulating average conditions of the Molco Stream, and a 1 mm mesh size net was placed downstream to capture drifting individuals. Five mesocosms were located within a controlled-temperature room set up at 8–10°C (mean temperature of the Molco Stream) with continuous lighting and left for 2 wk for acclimatization prior to the experiment and to allow for sedimentation of suspended particles and biofilm development.

The experiment consisted of 5 replicates (10 ind. each) for each species and treatment. Treatments consisted of the addition of salt at different concentrations at the beginning of the experiment (3, 6, 9, 21, 30 and 45 mg l^{-1}), resulting in water conductivities of 89.0, 96.8, 104.6, 135.7, 159.0 and 197.9 µS cm^{-1}, respectively, recorded using a multi-parameter instrument YSI Pro30, (Yellow Springs Instruments). The salt concentrations used covered the range of concentrations used at the fish farm. A control with no salt addition (natural conductivity of 49.7 µS cm^{-1}) was also included. Drift rate was estimated as the number of individuals collected at the downstream net 1 h after the start of the experiment (Schulz & Liess 2001, Beermann et al. 2018).

2.3. Data analysis

We first used principal component analysis (PCA) to explore variation in environmental factors across sites both upstream and downstream from the effluent discharge location as well as the PCA function of the 'FactoMineR' package in R (Lê et al. 2008, R Core Team 2019), which scales environmental data to unit variance. Secondly, to evaluate the effect of environmental factors on macroinvertebrate communities, we used redundancy analysis (RDA, Legendre & Legendre 1998), where the taxon dataset is predicted by the environmental dataset. The best RDA model was obtained after a stepwise model selection procedure using the 'ordistep' function from the vegan package (Oksanen et al. 2019), based on the Akaike information criterion (AIC) and adjusted R^2. Collinear predictors in the final RDA model were detected using variance inflation factor (VIF) and removed when VIF > 3 (Zuur et al. 2009). RDA was performed using a Hellinger-transformed macroinvertebrate abundance matrix.

Macroinvertebrate drift response to water conductivity (as an indicator of salt concentration and fit as a smoother term) was evaluated using additive models (separately for *A. peruvianus* and *S. annulicornis*) given the non-linear trends detected visually (in scatterplots) during the initial data exploration (Ieno & Zuur 2015). Additive models were fitted in the 'mgcv' package using the 'gam' function with a normal distribution and identity link function; generalized cross validation (GCV) was the smoothing parameter estimation method (Wood 2004, 2011).

Chronic ecotoxicological endpoints were calculated for both experimental species, including the lowest concentration with significant differences compared to the control (LOEC) and the non-effect conductivity (NOEC), using a Tukey's test multiple comparison analysis (Zar 2010) and the LC_{10} calculated (i.e. concentration at which 10% of individuals die, calculated by interpolation as the distribution of chronic endpoints) (Manly 1997).

3. RESULTS

3.1. Field survey

Environmental data are shown in Table 1. A gradient in water quality was observed between E1 (upstream from the fish-farm effluent discharge outlet) and E2 (located downstream and closest to the effluent outlet), with intermediate values found at the downstream site furthest from the effluent outlet (E3), as obtained through PCA (Fig. 1). Nutrient concentrations (TN and TP), TSS, conductivity and BOD, all indicators of water quality, were strongly correlated to PCA1 (which retained >80% variability), being lower at E1, intermediate at E3 and higher at E2. On the other hand, water temperature and pH did not vary with distance to the effluent, as these were mostly related to PCA2.

The most abundant macroinvertebrate taxa found were Chironomidae (Diptera), Naididae (Oligochaeta), Hyalellidae (Amphipoda), Sericostomatidae (Trichoptera) and Baetidae (Ephemeroptera) (Table 2). Combined conductivity and temperature were found

Table 1. Environmental factors measured at the 3 study sites (E1, E2 and E3). DO: dissolved oxygen ; BOD: biological oxygen demand; TSS: total suspended solids

Environmental factor	E1 Mean	E1 SD	E2 Mean	E2 SD	E3 Mean	E3 SD
Temperature (°C)	9.3	0.15	9.0	0.21	8.8	0.34
pH	6.86	0.07	6.98	0.03	6.98	0.08
Conductivity (µS cm^{-1})	49.7	1.53	165.1	1.16	101.7	0.86
DO (mg l^{-1})	10.1	0.26	11.5	0.15	11.0	0.21
BOD (mg l^{-1})	0.13	0.01	20.73	0.90	9.80	0.98
Total N (mg l^{-1})	0.04	0.01	0.63	0.01	0.24	0.01
Total P (mg l^{-1})	0.05	0.01	0.28	0.01	0.18	0.01
TSS (mg l^{-1})	2.30	0.10	15.31	0.90	9.20	0.26

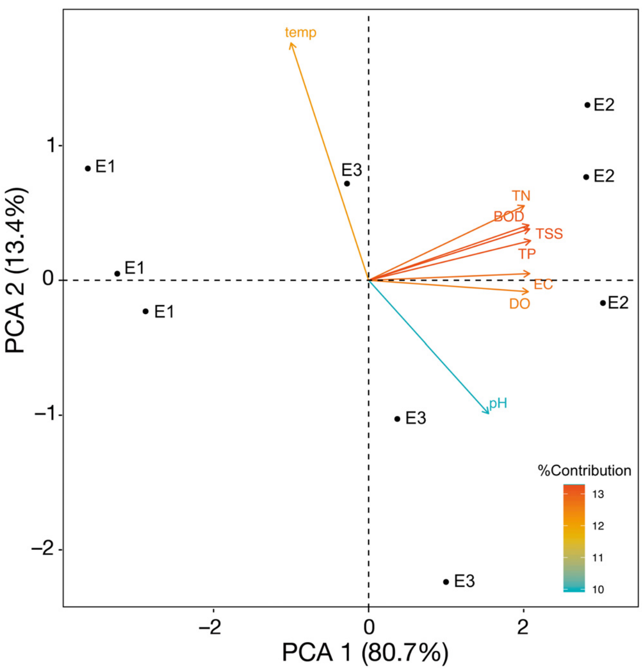

Fig. 1. Principal component analysis (PCA) exploring variation of physical and chemical parameters across sites both upstream (E1) and downstream (E2, E3) from the fish-farm effluent discharge location. TN: total nitrogen; TP: total phosphorous; NH_4^+: ammonium; NO_2^-: nitrite; DO: dissolved oxygen; BOD: biological oxygen demand; TSS: total suspended solids

to explain 92 % of total variance in the macroinvertebrate abundance matrix using RDA (best RDA model: R^2_{adj} = 0.92, AIC = –30.0) The abundance of the most sensitive taxa such as Sericostomatidae and Hyalellidae was higher at E1 and lower at both E2 and E3 and negatively related to TP (Fig. 2). Nonetheless, Sites E2 and E3 showed higher abundances of tolerant taxa such as Naididae, Chironomidae and Baetidae. In addition, Naididae abundance was positively related to TP (Fig. 2).

3.2. Mesocosm experiment

Additive modelling revealed a strong non-linear relationship between water conductivity (a salt concentration indicator) and invertebrate drift (Fig. 3), which was consistent across both invertebrate taxa (*Andesiops peruvianus*: effective df = 2.14, F = 29.5, $p < 0.001$, adj. R^2 = 0.79, deviance explained = 81.6 %; *Smicridea annulicornis*: effective df = 2.41, F = 55.2, $p < 0.001$, adj. R^2 = 0.89, deviance explained = 90.3 %). Invertebrate drift tended to increase exponentially with increasing water conductivity (up to 125 µS cm–1 for S. annulicornis and up to 150 µS cm–1 for A. peruvianus), with an average increase of 1 drifting individual per 23 µS cm–1 and 30 µS cm–1 for *S. annulicornis* and *A. peruvianus*, respectively. For *A. peruvianus*, LOEC compared to that of the control was 166.46 µS cm^{-1} (equivalent to 15.03 mg l^{-1} of NaCl) while NOEC was 151.16 µS cm^{-1} (equivalent to 10.01 mg l^{-1} of NaCl). LC_{10} was estimated to be 146.10 µS cm^{-1} (129.1 to 151.4 µS cm^{-1}) by means of interpolation. In contrast, LOEC, NOEC and estimated LC_{10} for *S. annulicornis* were 186.2, 166.46 and 133.69 µS cm^{-1}, respectively (CI: 121.3–149.1 µS cm^{-1}). *A. peruvianus* LC_{10} was significantly higher than that of *S. annulicornis* (p = 0.006).

4. DISCUSSION

Our results on the effects of salt from fish farm effluents on stream water quality are in agreement with previous studies (Nimptsch et al. 2015, Kamjunke et al. 2017). We found that nutrient concentrations, TSS, conductivity and BOD all increased downstream from the fish-farm effluent discharge location. Conductivity and nutrient concentrations measured upstream from the discharge location (E1) correlated to natural levels in northern Patagonian streams (Rivera et al. 2004, Kristensen et al. 2009), while conductivity downstream from the effluent increased to levels considerably higher than those found upstream from the effluent, mainly due to salt addition and, to a lesser extent, to increased dissolved inorganic nutrients (Nimptsch et al. 2014, 2015). Interestingly, we observed a dilution effect from E2 to E3 (both located downstream from the effluent and 200 m apart). This highlights the self-purification capacity of streams, which is nonetheless

Table 2. Abundance (ind. m^{-2}) per family, representing 90% of the benthos

Family	E1		E2		E3	
	Mean	SD	Mean	SD	Mean	SD
Chironomidae	1591	137	19 521	3157	17 714	2345
Naididae	37	2.5	25 399	1711	29 187	2079
Hyalellidae	3458	580	13	2.5	154	15
Baetidae	4	1.5	62	14.6	315	23.4
Sericostomatidae	3351	130	92	24.1	15	5.6
Total	8441		45 087		47 385	

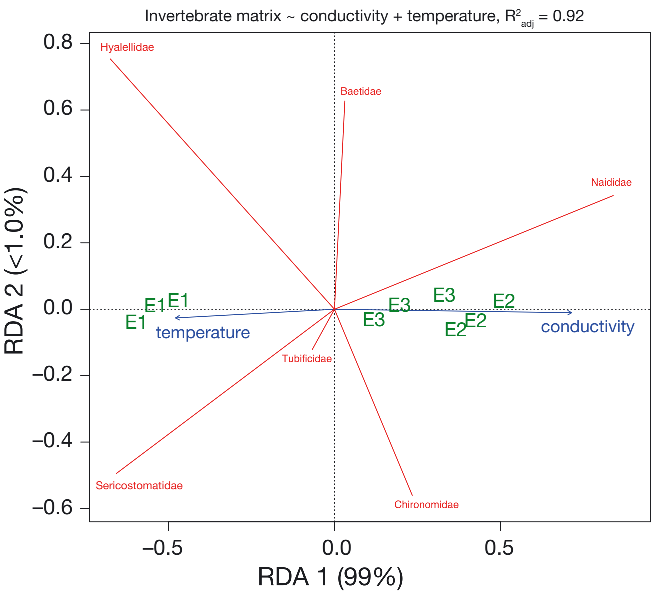

Fig. 2. Redundancy analysis (RDA) exploring the effect of environmental factors on macroinvertebrate communities across sites both upstream (E1) and downstream (E2, E3) from the fish-farm effluent discharge location

often exceeded due to large nutrient inputs and contaminants (Elósegui et al. 1995).

Although the highest conductivity value detected in the field was 165 µS cm^{-1}, higher conductivities are likely to occur because salt is usually added in pulses in fish farms, and previous studies have detected values up to ~1000 µS cm^{-1} in our study stream (Figueroa et al. 2014, Nimptsch et al. 2014). A salinity-monitoring experiment conducted on 10 fish farms in southern Chile found values up to 1070 µS cm^{-1} (Nimptsch et al. 2014).

Benthic macroinvertebrate community composition changes were observed downstream from the fish farm, with a large decrease in abundance of sensitive taxa and a large concomitant increase in abundance of tolerant taxa. Macroinvertebrates are often used as water-quality bioindicators, providing integrating information about alterations in physico-chemical variables (Figueroa et al. 2007). Our results thus reflect long-term effects of fish-farm effluents on stream communities, which most likely result in key ecosystem process changes such as organic matter decomposition or nutrient cycling, where macroinvertebrates play a key role (Boyero et al. 2011).

Shifts in stream macroinvertebrate communities can occur due to local extinction of taxa, resulting in either massive mortality rates (Greathouse et al. 2005) or active migration. Drift is a downstream migration mechanism used by macroinvertebrates which has been linked to natural processes (e.g. diel activity, life cycles or population density regulation; Brittain & Eikeland 1988) as well as to physical or chemical disturbances (e.g. floods, droughts, ice, erosion or effluents; Rieradevall & Prat 1986, Horrigan et al. 2007, Beermann et al. 2018). Here we show that macroinvertebrate drift increased as a result of salt addition, partly explaining the macroinvertebrate community composition changes observed in the field. Both taxa examined drifted as a response to salt concentrations, with strong linear responses at conductivities ranging from 125 to 150 µS cm^{-1}. Although the highest conductivity value detected in the field was 165 µS cm^{-1}, higher conductivities are likely to occur as salt in fish farms is usually applied in pulses. In our study stream, we values of up to ~1000 µS cm^{-1} have been previoulsy detected (Figueroa et al. 2014, Nimptsch et al. 2014). Furthermore, the conductivity values at which *Andesiops peruvianus* and *Smicridea annulicornis* drifts occurred were lower than those reported for other taxa (Blasius & Merritt 2002, Kefford et al. 2004, Zorina & Zinchenko 2009, Elphick et al. 2011, Dunlop et al. 2015). These observations are an indication that these taxa are highly sensitive to increased salinity, which could be explained by the oligotrophic nature of northern Patagonian streams, where conductivity is usually within the range of 20–80 µS cm^{-1} (Rivera et al. 2004).

In our study species, the indicators of chronic toxicity for conductivity, using drift as response, were much lower than those of other taxa reported in the literature (Table 3). possibly reflecting differences in salinity of their natural habitats (Kefford et al. 2004). For example, Blasius & Merritt (2002) found no significant macroinvertebrate drift at conductivities lower than 4424 µS cm^{-1}, and Bidwell & Gorrie (2006) found that survival of Chironomidae measured as acute response was significantly reduced at 1994.6 µS cm^{-1}. Zinchenko & Golovatyuk (2013) showed that the maximum ex-

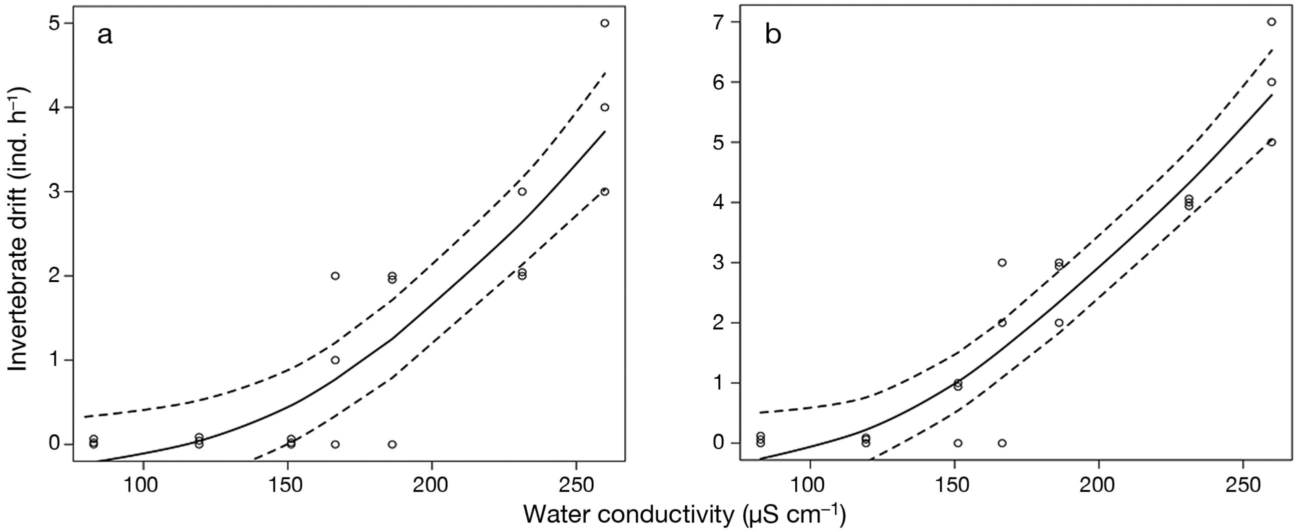

Fig. 3. Adjusted model correlating drift rates (ind. h−1) and conductivity (µS cm−1). Dashed lines are 95% confidence intervals. (a) *Andesiops peruvianus*; (b) *Smicridea annulicornis*

Table 3. Chronic ecotoxilogical endpoint values of freshwater benthic organisms compared to water quality reference values, fish farm effluents and Chilean environmental quality values measured as specific conductivity in water. LC_{10}: salt concentration at which 10% of individuals die; NR: not registered; NOEC: non-effect conductivity; LOEC: lowest-effect conductivity

Conductivity (µS cm^{-1})	Water type/target organism, assessed effect, toxicological end-point	Source
50.0	Freshwater (headwater stream)	Nimptsch et al. (2014)
49.7	E1 (100 m before fish farm)	This study
101.7	E3 (400 m after fish farm)	This study
133.7	*Andesiops*, drift, LC_{10}	This study
146.1	*Smicridea*, drift, LC_{10}	This study
165.1	E2 (200 m after fish farm)	This study
669.2	Annelidae, biomass, NOEC	Elphick et al. (2011)
712.0	Crustacea, NR, LC_{10}	Elphick et al. (2011)
990.1	Oligochaeta, NR, NOEC (96 h)	Elphick et al. (2011)
1054	Cyprinidae, NR, LC_{10}	Elphick et al. (2011)
1070	In the effluent	Nimptsch et al. (2014)
1078	Annelidae, NR, LOEC	Elphick et al. (2011)
1095	Crustacea, NOEC	Birge et al. (1985)
1306	Cyprinidae, NR, NOEC	Elphick et al. (2011)
1399	Oligochaeta, Reproduction, NR	Elphick et al. (2011)
1808	Rotifera, NR, NOEC	Elphick et al. (2011)
2097	Diptera, NR, NOEC	Short et al. (1991)
2151	Amphipoda, Drift, NR	Crowther & Hynes (1977)
2685	Trichoptera, NR, LOEC	Zinchenko & Golovatyuk (2013)
2763	Salmonidae, Reproduction, NOEC	Elphick et al. (2011)
3557	Crustacea, Biomass, NOEC	Elphick et al. (2011)
3749	Rotifera, NR, NOEC	Elphick et al. (2011)
4423	Trichoptera, Reproduction, NR	Blasius & Merritt (2002)
5359	DS 90 (upper limit in Chile)	MINSEGPRES (2001)
6867	Crustacea, NR, LOEC	Elphick et al. (2011)
8835	Ephemeroptera, NR, LOEC	Zinchenko & Golovatyuk (2013)
9637	Crustacea, Reproduction, NOEC	Martínez-Jerónimo & Martínez-Jerónimo (2007)
25 370	Annelidae, NR, LOEC	Zinchenko & Golovatyuk (2013)
29 298	Diptera, NR, LOEC	Zinchenko & Golovatyuk (2013)
32 471	Heteroptera, NR, LOEC	Zinchenko & Golovatyuk (2013)
38 640	Choleoptera, NR, LOEC	Zinchenko & Golovatyuk (2013)
38 755	Diptera, NR, LOEC	Zorina & Zinchenko (2009)

posure was 8835 µS cm^{-1} for Ephemeroptera and 2685 µS cm^{-1} for Trichoptera. Different studies have shown that the Ephemeroptera, Plecoptera and Trichoptera are more sensitive to salinity than other taxa such as Coleoptera, Crustacea and Diptera (Hart et al. 1991, García-Criado et al. 1999, Berezina 2003). Comparing the results of our study with those obtained by Short et al. (1991), Martínez-Jerónimo & Martínez-Jerónimo (2007), Zorina & Zinchenko (2009), Elphick et al. (2011), Zinchenko & Golovatyuk (2013) (Table 3) by means of the NOEC, LOEC or LC_{10} profiles, we conclude that salt has an effect on benthic community reproduction and survival patterns without considering drift as a toxicity indicator. The genera used in this study (*Smicridea* and *Andesiops*) were found to be very sensitive to changes in salinity, showing drift due to conductivity increases between 133.69 and 146.10 µS cm^{-1}, respectively, which were lower than those reported in the literature. Moreover, fish farms effluents significantly increase nutrient loads and salts in streams, posing a risk to benthic communities by changing their compositions and increasing their drift rates.

In our study, macroinvertebrates were exposed to salinity pulses associated with fish farm discharge patterns, producing changes in the drift rates which were corroborated via mesocosm assays. Conductivity values that produce these changes are notoriously lower than those reported in the literature. It is therefore important to take the effects of salinity on benthic communities into consideration when discussing the adjustment of this parameter in Chilean emission regulations, as currently, they allow chloride emissions of up to 5359 µS cm^{-1} (MINSEGPRES 2001).

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