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Response of stream invertebrates to short-term salinization: A mesocosm approach

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ABSTRACT

Salinization is a major and growing threat to freshwater ecosystems, yet its effects on aquatic invertebrates have been poorly described at a community-level. Here we use a controlled experimental setting to evaluate short-term stream community responses to salinization, under conditions designed to replicate the duration (72 h) and intensity (up to 5 mS cm^{-1}) of salinity pulses common to Mediterranean rivers subjected to mining pollution during runoff events. There was a significant overall effect, but differences between individual treatments and the control were only significant for the highest salinity treatment. The community response to salinization was characterized by a decline in total invertebrate density, taxon richness and diversity, an increase in invertebrate drift and loss of the most sensitive taxa. The findings indicate that short-term salinity increases have a significant impact on the stream invertebrate community, but concentrations of 5 mS cm^{-1} are needed to produce a significant ecological response.

1. Introduction

The artificial increase in salinity caused by human activities (Davis et al., 2003) is a major and growing threat to the world's freshwater ecosystems (Williams, 2001). Among the multiple drivers of freshwater salinization, salt mining is the most widely documented in Europe, where potassium and soda industries are highly developed (Casas et al., 2003; Böhme, 2011; Braukmann and Böhme, 2011). The impacts of salt mining can be particularly severe in Mediterranean streams, where runoff events are precluded and/or followed by prolonged droughts and seasonal low river flows (Prat and Munné, 2000). During runoff events the salt waste from the mines is washed into the rivers producing short-term conductivity peaks, while during the dry-season the reduced dilution capacity of the streams leads to increased conductivities.

It is known that freshwater invertebrates, and especially insects, are highly sensitive to elevated levels of salts (Remane and Schlieper, 1971; Berenzina, 2002; Pinder et al., 2005; Wolf et al., 2009) and their communities can be significantly altered by stream salinization (Hart et al., 1991; Piscart et al., 2006; Böhme, 2011; Braukmann and Böhme, 2011; Kefford et al., 2011). Nonetheless, most studies exploring the effects of salinization on freshwater invertebrates have been based on laboratory assays, in which the toxicity of salinity is assessed by exposing invertebrate species to different salt concentrations (e.g. Hassell et al., 2006; Dunlop et al., 2008). The findings of those studies are valid at a population level, but they do not consider the interactions between taxa, and thus do not provide a realistic representation of the environment that could influence community responses to salinization.

The community-level responses of aquatic macroinvertebrates to salinization are not well known. Long-term studies in the Werra River (Germany) identified a significant increase in species richness after the reduction in the salt mining activity (Bäthe and Coring, 2011) and several studies have reported declines in species richness along conductivity gradients (Pinder et al., 2005; Böhme, 2011; Kefford et al., 2011), but the confounding influence of other environmental factors (e.g. pollution: Barata et al., 2005) make it difficult to quantify the responses of invertebrate communities to elevated salinity levels. Stream mesocosms offer an alternative to field sampling and allow for examination of potential stressors on aquatic communities under controlled experimental conditions (Odum, 1984; Petersen and Englund, 2005). Mesocosms have been extensively used to explore factors and processes that control benthic invertebrate assemblages (see Lamberti and Steinman,

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1993 for a review) and to test the toxicity of potentially hazardous chemicals (e.g. Belanger et al., 2004; Colville et al., 2008).

Quantifying the effects of salinization on aquatic invertebrate assemblages has important implications for freshwater ecosystem management. Aquatic invertebrates have been extensively used in biomonitoring programs (Bonada et al., 2006) and are central to the implementation of the Water Framework Directive (WFD) (EU Directive, 2000/60/EC) (European Commission, 2000). Nevertheless the sensitivity of the invertebrate-based metrics has been usually tested for pollutants (e.g., organic pollution, acidification) rather than salinity (e.g. Buffagni et al., 2006). Thus, there is a need to examine the relationship between stream salinity and aquatic macroinvertebrate responses to determine if community metrics used for biological assessment adequately capture observed responses to salinity impacts.

In the present study, we used a mesocosm approach to examine the response of aquatic invertebrates to short-term salinization typical of salt-mine runoff in Mediterranean streams. We evaluated the effects of conductivity increases of 1.5, 2.5 and 5 mS cm^{-1} (similar to those observed during raining events in the mining-impacted reach of the Llobregat River) over a three-day period. The specific objectives of the study were to i) quantify the response of the stream invertebrate communities to short-term conductivity increases; ii) identify taxon-specific responses to the treatment; and iii) quantify the effects of increased conductivity on different invertebrate-based biotic metrics commonly used for water quality assessment and implementation of the WFD.

2. Methods

2.1. Study site and experimental design

The experiment was designed to mimic the short-term conductivity peaks that are registered in the Llobregat River, a 156 km long river which supplies 35% of the Barcelona's (Spain) drinking water needs. Since the establishment of the mining industry in 1923 a progressive salinization of the Llobregat has been recorded, although since the operation of a brine collector the quality of the surface water was improved for drinking water production (Martín-Alonso, 1994). Mining activities generate large amounts of NaCl waste that are washed into the river during runoff events, causing significant, short-term conductivity increases (Fig. 1). The present experiment intended to replicate the observed dynamics of stream conductivities downstream of salt mines in order to quantify potential effects on aquatic invertebrate communities. Invertebrate communities were subjected to three treatments of salt-concentrated solution at conductivities of 1.5, 2.5 and 5 mS cm^{-1}, in addition to a control (river water only) group (0.4 mS cm^{-1}). These conductivities were representative of the conductivity increases recorded in the Llobregat River (Catalonian Water Agency) and, according to the existing literature (Hart et al., 1991; James et al., 2003; Wolf et al., 2009; Kefford et al., 2011), they were high enough to expect a response from the aquatic macroinvertebrate community. The experimental treatments had a duration of 72 h, which approximates the maximum duration of a rainfall event within the region (Catalonian Meteorological Office).

The experiment was conducted at a flow-through mesocosm of 12 artificial stream channels relying on water pumped from a diversion channel of the Llobregat River (Fig. 2). The pump provided a continuous supply of water to a 4000-L tank. A second 2000-L tank was filled with a salt-saturated solution (250 g l^{-1}), which was a mix of freshwater coming from the river and common table salt (NaCl). Taps on the inlet pipes regulated the proportions of river water and salt-saturated solution entering each of the mixing tanks, which created uniform conductivities in each tank before flowing into three artificial stream channels. Each channel consisted of a 2-m long, 12-cm wide by 8-cm deep polyvinyl chloride (PVC) drain trough. Flows in each of the streams was maintained at a constant rate of 0.33 L s^{-1}, with corresponding hydraulic conditions similar to those present in natural riffles from the river of origin. Further information of the mesocosms facility is provided in Grantham et al. (2012).

The experiment was carried out in November of 2010. On November 2, river cobbles (average surface area of 177 cm^2) were collected from a riffle segment of the Llobregat River. Five cobbles were used as control samples to characterize the river community while 108 were transported in individual mesh baskets to the stream mesocosm site. Nine randomly chosen cobbles were placed in each of the artificial stream channels and submerged by at least 10 mm of flowing water. Baskets with 1-mm vinyl mesh were placed at the outlet of each stream to capture invertebrate drift. The streams were fed by natural river water for six days to allow for stabilization of invertebrate communities. Individual organisms collected in the drift baskets were relocated in the top of each channel every day during the settlement period. All twelve streams were used for the experiment and individual treatments were allocated to each of the four mixing tanks, yielding three replicates of each treatment. The experimental treatments were applied to the streams on November 8, 2010.

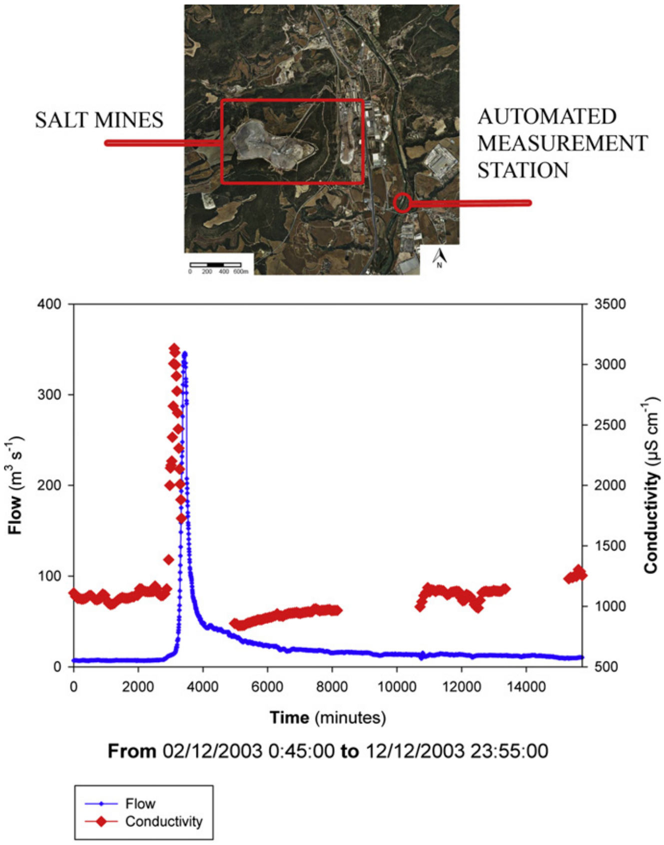

Fig. 1. Conductivity (mS cm^{-1}) versus river flow (m^3 L^{-1}) measured by an automated measurement station placed in the Llobregat River downstream the Sallent salt mines. The data correspond to a runoff event occurred between the 00:45 h of the 02/12/2003 and the 23:55 h of the 12/12/2003. The data were provided by the Catalonian Water Agency (http://aca-web.gencat.cat/aca, last accessed 31.01.12.). The aerial photograph was provided by the Catalonian Cartographic Institute (http://www.icc.cat/, last accessed 31.01.12.).

2.2. Macroinvertebrate and water quality sampling

Benthic macroinvertebrates were sampled by randomly extracting three cobbles in each of the channels and collecting the animals captured in the drift baskets on November 8 (before treatment), November 9 (24 h exposure) and November 11 (72 h exposure). Each cobble was transferred to a 500 µm mesh bag and washed by hand for several minutes to remove the macroinvertebrates (when necessary, forceps were used to capture the attached animals). The contents of the nets were gently emptied into bottles and preserved in formaldehyde. In the laboratory, macroinvertebrates were sorted and identified to the family or genus level. The raw taxon abundance data was scaled by the surface area of each cobble, measured with tin foil, to estimate the density of each taxon present (number of individuals per square meter). The contents of each drift basket were transferred to bottles and preserved in formaldehyde to be later sorted and identified in the laboratory.

Water quality conditions in the stream channels were assessed during each macroinvertebrate sampling event using an YSI multiparameter 63/10 FT to measure *in situ* water temperature, conductivity, pH, and dissolved oxygen (DO).

2.3. Data analysis

Since all the channels were fed with the same water and NaCl addition was the only manipulated variable, differences in the water quality conditions between channels were not expected. However the differences in the water quality (dissolved oxygen, temperature and pH) between treatments and between channels within each treatment (replicates) were assessed using nested ANOSIM (Warwick et al., 1990). Prior to analysis the variables were normalized (subtract means and divide

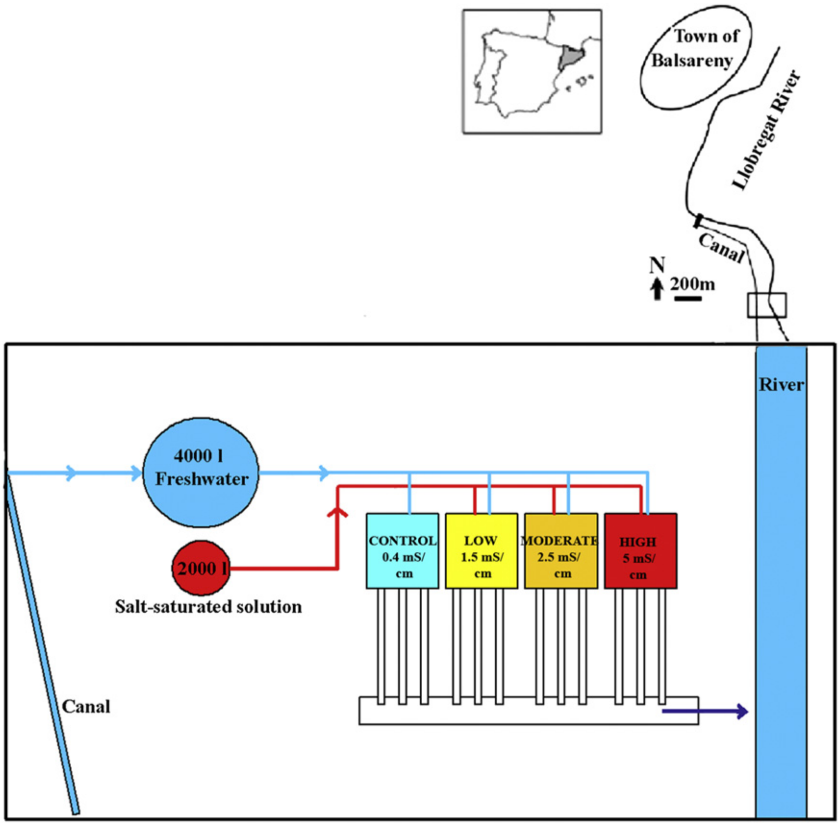

Fig. 2. Stream mesocosm at the Balsereny wastewater treatment plant (WWTP) on the Llobregat River in Catalonia, Spain. River water was diverted from a diversion canal, mixed with salt-saturated effluent in four concentrations [1.5 mS cm^{-1} (Low), 2.5 mS cm^{-1} (Moderate) and 5 mS cm^{-1} (High)] and continuously supplied to 12 artificial channels for 72 h.

by standard deviation). Samples were grouped by treatment (control = 0.4 mS cm^{-1}, low = 1.5 mS cm^{-1}, moderate = 2.5 mS cm^{-1} and high = 5 mS cm^{-1}) and replicate (channels 1, 2 and 3) factors. We used PRIMER 6 to run ANOSIM, which is a non-parametric analysis of variance designed to test if the differences between *a priori* defined groups of samples are statistically significant. The test yields the statistic R (ranging from −1 for no differences between groups to +1 for a clear division of samples between groups) and a significance level (p).

Prior to analysis macroinvertebrate data were $\log(x+1)$-transformed. Average densities from the three cobbles sampled per channel, per sampling date were used. The differences between the macroinvertebrate communities in each treatment were assessed using nested ANOSIM (Warwick et al., 1990). Samples were grouped by treatment (control = 0.4 mS cm^{-1}, low = 1.5 mS cm^{-1}, moderate = 2.5 mS cm^{-1} and high = 5 mS cm^{-1}) and time (0, 24 and 72 h exposure) factors. The indicator taxa of each treatment group were identified using IndVal analysis in PCORD 4.20 software (McCune and Mefford, 1999). The analysis assigned each taxon to a most probable group (control, low, moderate or high) basing on its relative abundances and relative frequencies in each group (Dufrêne and Legendre, 1997), and provided an indicator value (varying between 0 and 100) and a p-value obtained by Monte Carlo permutations (9999 runs). Those taxa registered in only one sample were considered rare and not included into the analysis.

The macroinvertebrate community data from the mesocosm experiment were further analyzed using the Principal Response Curve (PRC) method (van den Brink and Ter Braak, 1999), which is designed to evaluate the temporal responses of communities to controlled, experimental treatments. The PRC method is a form of redundancy analysis and generates series of curves that represent the extent and direction of differences between experimental treatments and controls. It is a useful method for visualizing the trajectories of community responses to treatments over time, and is commonly used in stream mesocosm studies (van den Brink et al., 1996; Colville et al., 2008; Duarte et al., 2008; Grantham et al., 2012). The PRC analysis was performed in CANOCO version 4.5 (ter Braak and Šmilauer, 2002).

The resulting PRC diagram displays the first principal component of each treatment effect over time, and can be interpreted as the deviation in the community response from the controls. In addition, species weights are calculated for each invertebrate taxon that indicates how closely the response of that taxon follows the overall community response defined by the PRC. Taxa with a high weight are inferred to exhibit changes in abundance that follow the pattern of the PRC, whereas taxa with species scores near zero either show no response to the treatment or a response that is unrelated to the PRC (van den Brink and Ter Braak, 1999). Following the inclusion rules, weights of taxa with very poor fit (not characterized well by the explanatory variables used in the analysis) were not calculated (Lepš and Šmilauer, 2003).

To determine the significance of the PRC in describing community trends, Monte Carlo permutations were performed with macroinvertebrate density data from each replicate channel. The significance of treatment effects at each sampling date was also assessed by Monte Carlo permutation tests, restricting the data to each sampling date. Finally, statistical tests were performed to assess which treatments differed significantly from the controls. Because of the limited number of replicates, permutation tests could not be performed and comparisons were made using univariate comparison methods. Therefore, the multivariate data were reduced to a single variable by calculating the first component of the PCA. One-way analysis of variance, with PCA scores for individual stream mesocosm samples, was then used to evaluate differences between treatment and control groups per sampling date. Whenever significant differences were observed, Dunnett's post hoc tests were used to determine which treatments were significantly different from the control.

In addition to the multivariate analysis, several univariate community metrics were calculated from the macroinvertebrate data, including taxon richness (S), Shannon Diversity (H′), number of EPT taxa and log (Sel EPTCD + 1), which is based on pollution sensitive Ephemeroptera, Plecoptera, Trichoptera, Coleoptera and Diptera. The diversity data were then used to calculate two multimetric indices of biological quality: IMMi-T (a combination of the number of families and the EPT, IASPT and, log (Sel EPTCD + 1) metrics) specifically designed to assess the ecological status of rivers in Spain and the Mediterranean (Munné and Prat, 2009), and ICM-Star (a combination of the number of families, the Shannon diversity and the ASPT, 1-GOLD and (Sel EPTCD+1) metrics), developed through the intercalibration exercise for the WFD (Buffagni et al., 2006, 2007). Finally, the biological quality indices for each treatment group were evaluated in relation to an ecological quality classes defined for rivers in Spain (Munné and Prat, 2009).

3. Results

Before starting the treatments conductivity was around 0.39 mS cm^{-1} in all the experimental channels (Table 1). When salt was added the conductivity remained around 0.38 mS cm^{-1} in the control channels and around 1.5, 2.5 and 3 mS cm^{-1} in the low,

Table 1
Physico-chemical characterization of the experimental channels. Mean values (±standard deviation) of the three channels for each treatment (control, low, moderate and high) are presented.

	Temperature (°C)	pH	Oxygen (%)	Conductivity (mS cm^{-1})
Before treatment				
Control	10.8 ± 0	8.22 ± 0.001	101.3 ± 0.05	0.39 ± 0.109
Low	10.8 ± 0	8.22 ± 0	101.1 ± 0.10	0.39 ± 0.001
Moderate	10.9 ± 0	8.23 ± 0.003	100.6 ± 0.05	0.39 ± 0.002
High	11.1 ± 0	8.23 ± 0	100.4 ± 0.03	0.39 ± 0.001
24 h treatment				
Control	10.4 ± 0	8.3 ± 0.005	98.0 ± 0.05	0.38 ± 0.436
Low	10.4 ± 0	8.3 ± 0.003	97.4 ± 0.05	1.40 ± 0.024
Moderate	10.4 ± 0	8.3 ± 0	97.1 ± 0.13	2.47 ± 0.092
High	10.4 ± 0.029	8.3 ± 0.005	99.8 ± 1.00	4.66 ± 0.171
48 h treatment				
Control	10.3 ± 0.028	8.5 ± 0.003	93.3 ± 0.03	0.38 ± 0.436
Low	10.4 ± 0	8.5 ± 0.009	93.4 ± 0.03	1.38 ± 0.048
Moderate	10.4 ± 0	8.5 ± 0.009	93.6 ± 0.03	2.77 ± 0.013
High	10.4 ± 0.029	8.4 ± 0.007	94.1 ± 0.05	4.77 ± 0.072
72 h treatment				
Control	10.5 ± 0	8.4 ± 0.004	95.2 ± 0.10	0.38 ± 0
Low	10.5 ± 0.019	8.3 ± 0.005	95.8 ± 0.04	1.5 ± 0.022
Moderate	10.4 ± 0	8.4 ± 0	96.2 ± 0.03	2.61 ± 0.038
High	10.5 ± 0	8.4 ± 0.006	95.7 ± 0.04	5.06 ± 0.067

moderate and high treatment channels respectively (Table 1). No significant differences in the water quality conditions (dissolved oxygen, temperature and pH) were registered between treatments ($R = -0.41$; $p = 1$) or between channels within the same treatment ($R = -0.04$; $p = 0.7$).

A total of 26 macroinvertebrate taxa were identified. Chironomidae (48% of relative abundance), Oligochaeta (19%) and Psychomyiidae (17%) were the most abundant families, while Diptera (number of taxa (S) = 8) and Trichoptera (S = 7) had the greatest number of family taxa. At the beginning of the experiment the invertebrate density in the river (mean density = 11357 individuals m^{-2}) and experimental channels (mean density = 12760 individuals m^{-2}) were similar, suggesting minimal transport and mesocosm effects. All taxa that had been observed in the river were also present in the experimental channels, with the exception of Dytiscidae (Coleoptera). Prior to treatments, mean invertebrate densities varied within each group (mean densities in individuals m^{-2}: control = 13036; low = 19030; moderate = 6944; high = 6418). After 72 h exposure, invertebrate densities decreased in all groups (mean densities in individuals m^{-2}: control = 9342; low = 8326; moderate = 5060; high = 2378), but the high treatment group had the greatest proportional decline in density. The total number of drifting invertebrates was similar for all experimental channels before the treatment started (Fig. 3). After 24 h and 72 h of exposure the number of drifting individuals was higher in the treatment channels than in the control channels, and was greatest in the high treatment channels. The total number of drifting individuals after treatment was 22 in the control and 88 in the high treatment channels. The invertebrate drift was mainly composed of Chironomidae, Hydropsychidae and Physidae, each of them accounting for a 44%, 13% and 20% of total drift in the control channels and a 24%, 15% and 29% in the treatment channels, respectively.

Based on the ANOSIM analysis there were significant differences between the treatment groups ($R = 0.282$, $p = 0.001$) and between the exposure time groups ($R = 0.379$, $p = 0.001$). The PRC indicated an increase in the scores of the first principal component along time for all the treatments (Fig. 4). The PRC also showed that the low treatment community already deviated from the control community (no treatment effects) at the beginning of the experiment. The response pattern in the first PRC axis was significant (F-ratio = 7.54, $p = 0.004$) and captured 38.5% of the total variance explained by the treatment regime. Differences among treatments accounted for 52.3% of all variance in the invertebrate data, while differences in sampling times accounted for 23.9% of all variance. The second PRC axis was not significant and is not presented. When the data were restricted to each time period (before exposure, 24 h exposure and 72 h exposure), only the longest period of exposure (72 h) registered a significant treatment effect (F-ratio = 4.91, $p = 0.002$). The ANOVA (using PCA scores) also detected significant treatment effects in the 72 h exposure period. Dunnett's post hoc tests indicated that the invertebrate community exposed to high-salinity treatment was significantly different than that control (mean difference = −8.42, $p = 0.001$). There was a large difference in the PC1 score (mean difference = −3.52) between the moderate treatment and control, but it was not significant at 0.05 threshold ($p = 0.073$).

Based on the IndVal analysis (Table 2), 10 taxa were associated to the control group (including the Oligochaeta, the Mollusca, the Ephemeroptera and several Trichoptera and Diptera families), 8 to the low treatment group (including the Coleoptera and several Trichoptera and Diptera families) and only 2 and 1 taxa were associated to the high and moderate treatment groups respectively. In terms of statistical significance 3 taxa were significantly associated to the control group (*Physa acuta*, Baetidae and Simuliidae), 5 to the low treatment group (Elmidae, Hydroptilidae, Psychomyiidae, Chironomidae and Empididae) and none to the moderate and high treatment groups. Based to the PRC analysis (Fig. 4) the families Elmidae, Empididae and Hydroptilidae were the most affected by the treatment effects (negative scores); their densities increased along time in the control channels and decreased in the moderate and high treatment channels (Table 2).

All the unimetric indices declined in response to the salinity treatments (Table 3). For Shannon's diversity and selected Ephemeroptera, Plecoptera, Trichoptera, Coleoptera and Diptera (Sel EPTCD) the decline was evident after 24 h of exposure, while for total taxon richness and EPT (Ephemeroptera, Plecoptera and Trichoptera richness) the decline was only evident after 72 h of exposure (Table 3). The multimetric indices only registered a change in the high treatment channels after 72 h of exposure, indicating that ecological quality (Munné and Prat, 2009) declined from good to moderate conditions (Table 3).

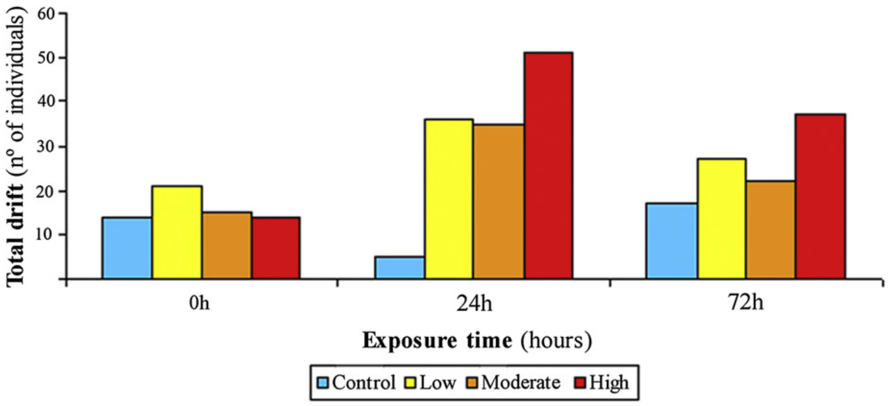

Fig. 3. Total drifting invertebrates (total number of individuals) for each treatment group at 0, 24, and 72 h of exposure to salt-saturated effluent.

4. Discussion

The findings indicate that short-term salinization of streams has a significant impact on the composition and diversity of the aquatic invertebrate community. Treatment effects on the invertebrate community in the artificial stream channels increased with elevated salt concentrations and extended periods of exposure. The stream mesocosm channels receiving the highest salt treatment (5 mS cm^{-1}) showed significant changes in the macroinvertebrate community, characterized by a decline in total invertebrate density, taxon richness and diversity, loss of the most sensitive taxa and a higher number of drifting individuals. Changes in the invertebrate community exposed to low-salinity treatments were not significant. However, the moderate treatment channels (2.5 mS cm^{-1}) registered a higher number of drifting individuals, lower diversity, taxon richness and sensitive taxa abundances than the low treatment and control groups. Therefore, there is some evidence that the lower salinity concentrations had a negative effect on the macroinvertebrate communities, although the responses were not statistically significant. Before the treatments started there were some differences between the experimental channels in terms of invertebrate densities. These differences were mainly related to the Oligochaeta and Chironomidae abundances. These taxa register very high densities in the Llobregat River (Caus and Prat, 2010) and are not evenly distributed in the river (Muñoz et al., 1986; Millet et al., 1987). Therefore, pre-treatment differences registered between the channels could be attributed to the sampling procedure (random collection of naturally colonized stones from the river). The observed community responses to experimental treatments did not appear to be influenced by initial differences in macroinvertebrate densities, which consistently declined among all salinity treatments over time. However, it is possible that

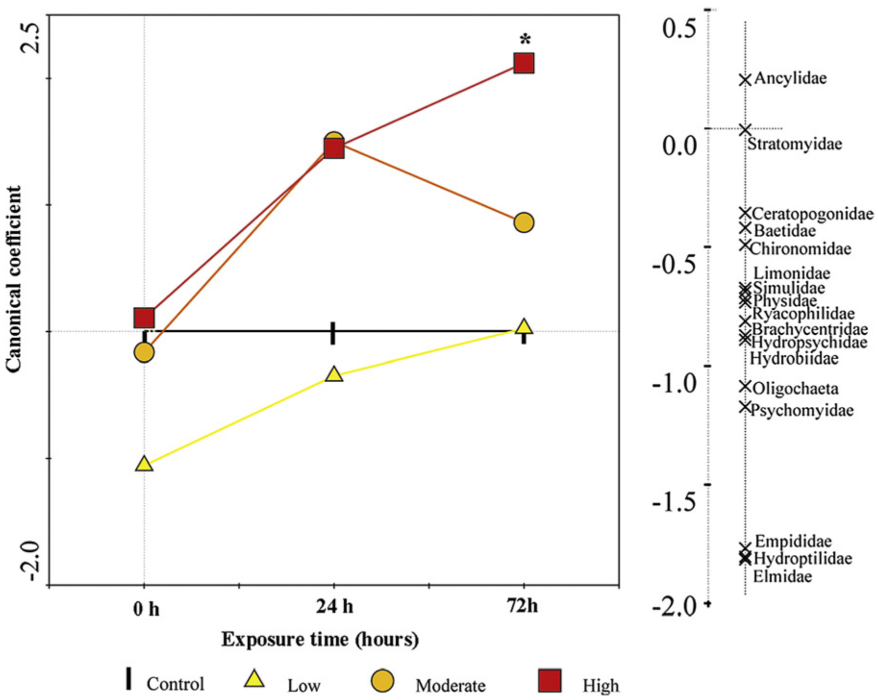

Fig. 4. Principal Response Curve (PRC) with species weights for the aquatic invertebrate community data, indicating the effects of exposure to 1.5 mS cm^{-1} (Low), 2.5 mS cm^{-1} (Moderate) and 5 mS cm^{-1} (High) concentrations of salt. Asterisk (*) indicates significant ($p < 0.05$) differences between treatment and control groups. Following the inclusion rules, weights of taxa with very poor fit (not characterized well by the explanatory variables used in the analysis) were not calculated (Lepš and Šmilauer, 2003).

Table 2
Results from the Indicator Species Analysis (Dufrêne and Legendre, 1997) using the treatments (control, low, moderate and high) as groups. The analysis assigns each taxon to a most probable treatment group (Max. Group) and provides an indicator values (IV) and a *p*-value according to the relative abundances and frequencies of each taxa within each group. * = *p* value ≤ 0.05. Taxa registered in only one sample were considered rare and not included into the analysis.

	IndVal Analysis		
	Max. Group	IV	*p*-value
Oligochaeta			
Oligochaeta spp.	Control	31	0.117
Mollusca			
Ancylidae	Control	12	0.471
Hydrobidae	Control	13	0.184
Physa acuta*	Control	27	0.028
Ephemeroptera			
Baetidae*	Control	20	0.029
Coleoptera			
Elmidae*	Low	47	0.002
Heteroptera			
Corixidae	Moderate	3	0.804
Mesoveliidae	High	6	1.000
Trichoptera			
Brachycentridae	Control	14	0.243
Hydropsychidae	Control	31	0.112
Hydroptilidae*	Low	42	0.003
Polycentropodidae	Control	6	1.000
Psychomyiidae*	Low	54	0.001
Rhyacophilidae	Low	25	0.084
Diptera			
Ceratopogonidae	Control	6	1.000
Chironomidae*	Low	38	0.001
Empididae*	Low	42	0.017
Limoniidae	Low	37	0.076
Psychodidae	High	6	1.000
Simuliidae*	Control	31	0.014
Stratiomyidae	Low	6	0.247

significant treatment effects of moderate salinity concentrations would have been detected had the variability in initial densities prior to treatment been lower.

We found that the threshold beyond which short-term conductivity releases have a significant effect on freshwater macroinvertebrate communities is 5 mS cm^{-1}. This is consistent with previous field studies that reported a significant decline in freshwater taxon richness (Pinder et al., 2005; Böhme, 2011; Kefford et al., 2011) in streams with salinities above 5 mS cm^{-1}, and in the mesocosm study of Marshall and Bailey (2004), in which only the 3.5 g l^{-1} (≈6 mS cm^{-1}) treatment caused significant reductions in invertebrate abundance. The observed threshold of 5 mS cm^{-1} was also consistent with laboratory studies of salt toxicity conducted over similar time periods (e.g. Berenzina, 2002). For example, Dunlop et al. (2008) and Kefford et al. (2006) found that the LC-50 (concentration needed to kill half of the sample population) of aquatic invertebrates exposed to salt over a 72 h period was 6.9–55 mS cm^{-1} and 5.5–76 mS cm^{-1}, respectively. Several field surveys conducted across wide conductivity ranges and including a large number of invertebrate species had reported that salinities in excess of 1.5 mS cm^{-1} were likely to have adverse effects on invertebrate communities (Hart et al., 1991; Dunlop et al., 2005; Horrigan et al., 2005). In contrast, we found no significant response of the macroinvertebrate community to the moderate (2.5 mS cm^{-1}) and low (1.5 mS cm^{-1}) treatments. This could be related to the limited time exposure of the present study (72 h).

The significant effects of short-term salinity exposure have important management implications for Mediterranean rivers affected by mining activities. During rainfall events, the conductivity of rivers is substantially increased (to values close to those used in the experiment) when large amount of salts are washed into surface waters. These events do not usually exceed 72 h; after that period the salts are washed downstream and conductivity drops down again to pre-storm levels. These short and intense rainfall events are characteristic of the Mediterranean climate region. In more temperate-climate regions, where rainfall events are longer and not as intense, salts are generally diluted after the first 24 h and the increase in conductivity is not as pronounced and sudden. Therefore, the effects of salt mining activities are particularly severe on Mediterranean streams. Our findings suggest that management efforts should be focused on controlling sources of salt-mine runoff in order to prevent short-term conductivity increases ≥5 mS cm^{-1}. Currently, runoffs from the salt mines along the Llobregat River are controlled by a 60-km long diversion pipe that runs parallel to the river to the sea. The pipe has a flow capacity of 600 L s^{-1} is designed to collect all of the wastewater produced by the process of separating KCl from other salts. However, leaks in the pipe have occasionally occurred, resulting in the discharge of high-salinity wastewater to the river. As river baseflows are approximately 4000 L s^{-1}, the conductivity of river may increase up to 10 mS cm^{-1}, which based on our findings is likely to adversely affect the biological community. However, sampling for invertebrates and

Table 3
Aquatic invertebrate community metrics and corresponding multimetric indices for ecological status assessment of European rivers.

		Unimetric indices				Multimetric indices	
Treatment	Week	Diversity (H')	Number of taxa (S)	EPT taxa	log (Sel EPTCD + 1)	IMMi - T	ICM-Star
Control	0	1.57	13	5	3.51	0.92	0.88
Low	0	1.60	16	6	3.47	0.91	0.90
Moderate	0	1.59	16	6	2.88	0.84	0.76
High	0	1.59	15	6	2.67	0.83	0.75
Control	1	1.91	14	6	2.82	0.85	0.95
Low	1	1.48	13	5	3.08	0.84	0.86
Moderate	1	1.26	14	5	2.52	0.79	0.73
High	1	1.29	15	6	2.12	0.76	0.82
Control	2	1.58	18	7	2.88	0.87	0.97
Low	2	1.60	14	5	3.13	0.84	0.89
Moderate	2	1.44	14	5	2.41	0.78	0.85
High	2	1.18	14	5	2.05	0.70	0.56

[a]The colors correspond to the ecological status classification based on biotic indices IMMi-T, developed by Munné and Prat (2009), and the ICM-Star, developed by Buffagni et al. (2006). Blue = Very good; Green = Good; Orange = Moderate.

fish has failed to detect a significant impact, probably because the lower Llobregat River is also subject to impacts from sewage discharge that has impaired the aquatic community (Prat and Rieradevall, 2006). Thus, performing the mesocosm experiment upstream of other pollutant sources was critical first step for evaluating the potential ecological effects of salinization. Nevertheless, additional experiments are needed involving longer and/or repeated exposure times to develop effective guidelines for managing and restoring streams affected by persistent exposure to high salt concentrations.

The salt treatments appear to have caused a reduction in all the unimetric community indices, especially in diversity, which has been shown to be a sensitive metric to freshwater salinization in the German river Werra along a 0.46–7.24 mS cm^{-1} conductivity gradient (Braukmann and Böhme, 2011). Although the sampling procedure used in this study did not meet the requirements for calculating the IMMi-T and ICM-Star indices (size of the sample is not large enough to assess the richness of the site according to Buffagni et al., 2006 and Munné and Prat, 2009), these mulimetric indices did indicate a response to the salt treatment. The highest salt treatment appears to have caused a decline in the index values that led to a reduction in the ecological status (from good to moderate) after 72 h. This suggests that although these indices have been designed to respond to pollution they can also be sensitive to detecting the effects of salinization. This confirms findings of previous studies carried out within the study area, (Barata et al., 2005; Prat and Rieradevall, 2006; Damásio et al., 2008), which reported a decline in the biological quality of the Llobregat River along a 0.48–1.94 mS cm^{-1} conductivity range (before and after the salt mines). Nonetheless the response of multimetric indices to salinization warrants additional study, since they only responded to 5 mS cm^{-1} treatments after 72 h. Further research should focus on the incorporation of the biotic salinity preferences of aquatic invertebrates into multimetric indices, which has been successfully used to classify brackish marshes in Germany (Wolf et al., 2009) and is being implemented in some regions of Australia (Schäfer et al., 2011), and could be important for improving the assessment of the environmental conditions of rivers in Spain and other regions threatened by salinization.

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