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Tolerance of glacial-melt stoneflies (Plecoptera) and morphological responses of chloride cells to stream salinity

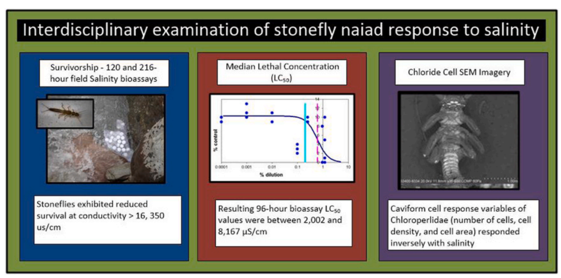
Heather Fair [a,*], Roman Lanno [a,b], Peter C. Smiley Jr. [c]

[a] Environmental Science Graduate Program, Ohio State University, Columbus, OH, USA
[b] Department of Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus, OH, USA
[c] USDA Agricultural Research Service, Soil Drainage Research Unit, Columbus, OH, USA

HIGHLIGHTS

- Climate warming is predicted to increase salinity in glacial-melt streams.
- Glacial-melt stonefly responses to elevated salinity were examined.
- Stoneflies exhibited decreased survival at high salinity levels.
- Stoneflies having greater survival exhibited changes in caviform cell morphology.
- Results suggest stoneflies may be tolerant of moderate salinity increases.

GRAPHICAL ABSTRACT



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ABSTRACT

Aquatic insects within glacial-melt streams are adapted to low dissolved inorganic ion concentrations. Increases in ion concentrations in glacial-melt streams are predicted with increasing air temperatures, which may impact future aquatic insect survival in these streams. We hypothesized that stonefly (Plecoptera) naiads from glacial-melt streams acclimated to different conductivity would differ in survival, median lethal concentrations, and chloride cell responses to elevated conductivity above that expected in our study streams. We conducted field bioassays in remote glacial-melt streams in southwestern China in 2015 and exposed representative stonefly naiads (Chloroperlidae, Nemouridae, Taeniopterygidae) from stream sites differing in conductivity to experimental conductivity ranging from 11 to 20,486 µS/cm for up to 216 h. We examined survivorship, calculated 96-h median lethal concentrations, and measured chloride cell responses with scanning electron microscopy. Chloroperlidae survival after 120 and 216 h did not differ ($P > 0.05$) among conductivity treatments. The combined Nemouridae/Taeniopterygidae survival after 120 and 216 h was the least ($P < 0.05$) in conductivity treatments >16,349 µS/cm. Taeniopterygidae survival after 120 h was also the least ($P < 0.05$) in conductivity treatments >16,349 µS/cm. The 96-h median lethal concentrations did not differ ($P > 0.05$) between the combined Nemouridae/Taeniopterygidae group (2306 µS/cm) and Taeniopterigydae (2002 µS/cm) and were lower ($P < 0.05$) than the 96-h median lethal concentration for Chloroperlidae (8167 µS/cm). Chloroperlidae caviform cell number, density, and area decreased ($P < 0.05$) with increasing conductivity. Taeniopterygidae caviform cell count decreased ($P < 0.05$) with increasing conductivity, but cell density and area did not. Chloroperlidae and Taeniopterygidae coniform cell characteristics and Nemouridae bulbiform cell characteristics

* Corresponding author. National Science Foundation Postdoctoral Research Fellow in Biology, University of Minnesota/Chinese Academy of Sciences Institute of Mountain Hazards and Environment/USDA Agricultural Research Service, 590 Woody Hayes Drive, Columbus, OH, 43210, USA.
E-mail address: hfair@umn.edu (H. Fair).

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were not affected by conductivity. Our results suggest that Chloroperlidae, Nemouridae, and Taeniopterygidae from glacial-melt streams in China may be able to tolerate moderate increases in conductivity (i.e., 100 to 200 μS/cm).

1. Introduction

Within glacial-melt streams, a small number of aquatic insects are physiologically adapted to low water temperatures, hydrological extremes, variable turbidity, and low dissolved ion concentrations (Brundin, 1966; Danks, 1971; Brodsky, 1980; Denlinger and Lee, 2010). As glaciers melt and retreat, cold-adapted, stenothermic aquatic insects are progressively tracking cold water temperatures by migrating to higher altitude sites near the glaciers and the increased isolation of these newly colonized sites may reduce population viability (Jacobsen and Dangles, 2012; Finn et al., 2013). With the reduction of glaciers due to climate-induced warming, the water source of these streams will shift to a greater proportion of groundwater inputs with higher total ion concentrations (Ward et al., 1999; Brown et al., 2010). Warming temperatures may also thaw the bottom of perennially frozen glaciers and result in movement of the glacier against bedrock, which may enhance dissolution of glacier sediments and increase total ion concentrations in the streams (Stallard, 1995; Anderson et al., 1997; Liu and Liu, 2010). Thus, understanding the physiological responses of aquatic insects adapted to low ion concentrations to increased ion concentrations predicted to occur with warming temperatures will help with determining the ability of these insects to persist under altered conditions and to colonize non-glacial streams after glacial-melt streams dissipate (Wooton, 1988; Kefford et al., 2007; Clements and Kotalik, 2016; Griffith, 2017; Schiebener et al., 2017).

Total dissolved ions or total dissolved solids at certain thresholds have been found to filter out certain taxa of invertebrates and result in spatial partitioning of invertebrates based on their sensitivity to dissolved solids (Olson and Hawkins, 2017). Increasing concentrations of dissolved inorganic ions (salinity) is a physiological stressor that can cause lethal and sublethal effects in aquatic insects (Kefford et al., 2012; Bray et al., 2019). The physiological stressor of increasing ion concentrations is especially notable in aquatic insects that are adapted to regional water sources of low ion concentration (Carter et al., 2020). Glacial-melt streams are characterized by having a conductivity of <50 μS/cm (Milner and Petts, 1994) with lowest conductivity at the glacier terminus, and higher conductivity further downstream where well-mixed conditions of glacial-melt, groundwater, and riverbed sediments occur (Gurnell and Fenn, 1985). In glacial-melt streams around the globe, conductivity has been found to range from 7.9 to 113.1 μS/cm in central Iceland streams (Gislason et al., 2001), 3 to 47 μS/cm in Ecuador glacial-melt streams (Kuhn et al., 2011), averages 47.93 to 71.74 μS/cm in Svalbard (Blaen et al., 2014), and averages approximately 65 μS/cm in New Zealand (Milner et al., 2001). In general, previous bioassay results indicate that elevated dissolved inorganic ion concentrations above the hemolymph ion concentration in aquatic insects causes greater mortality (Kapoor, 1978; Castillo et al., 2018; Tiwari and Rachlin, 2018). It is uncertain how glacial-melt insects adapted to low conductivity waters will respond to increased conductivity and inorganic ion concentrations.

Aquatic insects living in waters of low ion concentration hyper-osmoregulate to maintain hemolymph ion concentrations much greater than the external environment by absorbing the dominant Na^+ and Cl^- ions through chloride cells (coniform, caviform, filiform, and bulbiform cells) on the integument, epithelium, anal papillae, and by using the Malpighian tubules and ileum to excrete excess ions (Wichard et al., 1972; Komnick, 1977; Kapoor, 1979; Choe and Strange, 2009; Griffith, 2017). Negative correlations with conductivity have been observed in physiological characteristics of chloride cells of aquatic insects and fish. For example, the number of coniform cells on abdominal gills of mayflies (Ephemeroptera) decreased as external ion concentrations increased (Wichard et al., 1973) and the apical surface area of branchial chloride cells in rainbow trout (*Oncorhynchus mykiss*) increased within 24 h in response to decreases in external ion concentration (Perry and Laurent, 1989). Little is understood of the ability of aquatic insects that hyper-regulate their ion balance, such as mayflies and stoneflies (Plecoptera), to colonize streams having different ion concentrations and/or different natural mineral content from the streams that they originated from (Clements et al., 2012; Griffith et al., 2012; Clements and Kotalik, 2016). Additionally, there is limited information on the ability of the chloride cells on the integument of aquatic insects to respond to changing dissolved ion concentrations (Wichard et al., 1973; Nowghani et al., 2017; Carter et al., 2020).

Our hypotheses were: 1) stoneflies acclimated to waters of low conductivity found close to the glaciers in southwestern China will exhibit lower survival and lower median lethal concentrations when exposed to elevated conductivity above that expected in our study streams than stoneflies originating from higher conductivity downstream glacial-melt or groundwater sites and 2) the mean number, surface area, and density of different types of chloride cells (coniform, caviform, and bulbiform) will decrease in response to increased conductivity and stonefly naiads acclimated to low conductivity will exhibit greater decreases in mean chloride cell responses to conductivity increases than those originating from downstream sites. Our goals were: 1) to investigate the survival of stonefly naiads from different glacial-melt stream sites in southwestern China when exposed to differing conductivity; 2) to compare 96-h median lethal concentration (LC_{50}) values of stoneflies adapted to differing conductivity; and 3) to document the effects of the conductivity ranging from 11 to 20,486 μS/cm on stonefly chloride cells. Stoneflies were selected as the experimental animals for our study because they possess extensive chloride cell distribution on their osmobranchiae, thorax, and abdomen and they are one of the most common insect taxa captured in glacial-melt streams in our study region of southwestern China (Fair et al., 2021). This is the first time field bioassays with aquatic insects have been conducted in glacial-melt streams of high altitude remote locations in the circum-Himalayan region.

2. Materials and methods

2.1. Study sites

Stonefly bioassays were conducted in the Mingyong glacial-melt stream located 2500 m from the Mingyong Glacier in the Meilixueshan mountain range, Yunnan Province, China (28°26′14″N, 98°41′04″E) and in a snowmelt stream that is 1200 m from the Hailuogou Glacier in the Daxueshan mountain range, Sichuan Province, China (29°35′48″N, 101°52′43″E). The bioassays in Meilixueshan were conducted from August 5 to 14, 2015 for nine days (216 h) and bioassays in Daxueshan were conducted from August 23 to 28, 2015 for five days (120 h).

Representative families of stonefly naiads were collected for bioassays based upon their abundance at the glacial-melt stream sites in Yunnan and Sichuan provinces (Fair et al., 2021). The most abundant family allowing the collection of sufficient numbers to conduct bioassays in Yunnan Province were Chloroperlidae in the Niuba Glacier stream site (N1) approximately 8760 m from the Niuba glacier (28°32″84′N, 98°07″29′E) and Nemouridae from the Mingyong Glacier stream site 800 m from the glacier (28°27″44′N, 98°46″29′E). In Sichuan Province, Taeniopterygidae were the most abundant stonefly captured 800 m from the Glacier #3 site (GL3) in Daxueshan (29°33″00′N, 101°58″30′E). The three stonefly families belong to the monophyletic group Arctoperlaria based on their common male structural and behavioral patterns of drumming or tremulation and are placed into the superfamilies of Nemouroidea (Nemouridae and Taeniopterygidae) and Systellognatha (Chloroperlidae) (Zwick, 2000).

2.2. Water quality characteristics

At each of the three stonefly collection sites, water temperature, dissolved oxygen, pH, and conductivity were measured with a multi-parameter meter (YSI, 2009). Conductivity was used as a proxy for the salinity and total dissolved ion concentration in water. We used conductivity as an integrated measure of exposure to dissolved ion concentrations in experimental treatments (NaCl solutions), a distilled water control, and in field water samples, knowing that the composition of dissolved ions contributing to conductivity will differ depending upon the water source or dilution water used in preparing experimental treatments. Nutrient and trace metal concentrations were measured in water samples taken from the stonefly collection sites and filtered through 0.45 μm Whatman filters (GE Healthcare Sciences, Pittsburg, Pennsylvania) into acid-washed vials. All nutrient and trace metal concentrations except for chloride were measured using an optical emission spectrophotometer (Agilent 720 ICP-AES) following U.S. Environmental Protection Agency method 6010C (USEPA, 2007). Chloride concentrations were determined by ion chromatography using a Shimadzu LC-20AD pump, CDD-10A conductivity detector (Shimadzu, Columbia, Maryland), and IonPac As 14 4 × 250mm column (Franson et al., 1992). Quality control practices for all nutrient and trace metal concentration measurements included: 1) method detection limit determination at the lowest calibration standard; 2) initial calibration verification and blanks; and 3) continuing calibration verification and blanks every 10 samples.

2.3. Acute toxicity bioassay

Stonefly naiads were collected for two to three hours at each stream site by disturbing substrate and capturing larvae in a Surber net or by hand washing larvae from stones into a pan filled with stream water. Three stonefly taxa from three stream sites had sufficient numbers to conduct the experiment. After collection, four stoneflies were placed into each 30-mL plastic vial containing stream water from their site of capture to acclimate to the vial conditions. The vials with stoneflies and their originating stream water were placed into the bioassay stream site to provide the naiads with an acclimation period of 24 h before being gently removed and then placed into the same vials with the experimental treatments, stream reference, or distilled water. Additionally, prior to conducting the field bioassays we determined the amount of experimental grade NaCl (Merck Limited, Hong Kong S.A.R.) needed to produce experimental solutions corresponding to six of our conductivity treatments (11 μS/cm, 243 μS/cm, 1500 μS/cm, 4690 μS/cm, 16350 μS/cm and 20486 μS/cm). We confirmed that the identified amounts of NaCl resulted in the desired conductivity in test solutions using a multiparameter meter in the laboratory (YSI, 2009). We then prepared packets containing the identified amounts of NaCl needed for all experimental conductivity solutions. In the field, we prepared the solutions by mixing premeasured NaCl packets with 1L distilled water. This ensured accurate experimental treatment solutions to conduct the experiments in remote field locations. We placed the 1L vials of solution in the experimental stream sites to enable the temperatures of the solutions to equilibrate with the temperatures of the stream water before exposing the naiads to the treatments.

The combined Nemouridae/Taeniopterygidae and Taeniopterygidae stonefly bioassays consisted of six replicates of each of five NaCl treatments, the control, and a stream reference: 1) 0.01% NaCl (243 μS/cm); 2) 0.10% NaCl (1500 μS/cm); 3) 0.25% NaCl (4690 μS/cm); 4) 0.95% NaCl (16,350 μS/cm); and 5) 1.2% NaCl (20,486 μS/cm); 6) control - distilled water (11 μS/cm), and 7) stream reference (M1 - 60 μS/cm or GL3 – 71 μS/cm) (Supplemental Table 1). Chloroperlidae bioassays consisted of two replicates of each of the five NaCl treatments, the control, and the stream reference (N1 – 106 μS/cm) (Supplemental Table 1). The replicates were 30-mL plastic vials which were filled with the experimental treatment, distilled water, or water from the three stream sites and contained four naiads in each vial (42 vials with 4 stoneflies per vial) for the combined Nemouridae/Taeniopterygidae group and Taeniopterygidae bioassays or two to four naiads in each vial (14 vials with 2–4 stoneflies per vial) for the Chloroperlidae bioassays for a total of 213 stoneflies used in the bioassays.

Chloroperlidae naiads from N1 and Taeniopterygidae naiads from GL3 were placed in vials containing only one taxa as it was possible to identify them visually in the field (Supplemental Table 1). However, it was not possible to visually identify stonefly naiads collected at M1 to the family level due to their small size. We originally thought that stonefly naiads collected from M1 were exclusively Nemouridae, but at the conclusion of the field bioassays when we were able to use microscopy to inspect the naiads we discovered that 75% of 84 stonefly naiad specimens used in the M1 bioassays were Nemouridae and 25% were Taeniopterygidae. All treatments in the M1 bioassays contained more Nemouridae than Taeniopterygidae specimens. Specifically, the ratio of Nemouridae and Taeniopterygidae (N:T) in each treatment was: 1) 9 N:2T in 0.01% NaCl (243 μS/cm); 2) 9 N:3T in 0.10% NaCl (1500 μS/cm); 3) 7 N:5T in 0.25% NaCl (4690 μS/cm); 4) 8 N:4T in 0.95% NaCl (16,350 μS/cm); 5) 10 N:2T in 1.2% NaCl (20,486 μS/cm); 6) 10 N:2T in the control; and 7) 9 N:3T in the stream reference (Supplemental Table 1). Thus, we refer to the field bioassay with naiads collected at M1 as combined Nemouridae/Taeniopterygidae (CNT).

Vials with stoneflies were grouped by stream site in mesh bags, placed into the bioassay stream sites at Meilixueshan and Daxueshan and attached securely to a boulder on the river bank. By placing the vials at the same location, we were able to logistically perform daily monitoring, and water temperature regime and sunlight were controlled. Stonefly naiads were monitored every 24 h for mortality by using a small mesh tool to scoop them and place them gently into a white container with experimental treatment, remove and record dead naiads, and place the live naiads back in vials with a fresh solution of the respective treatment. The field bioassays were conducted for five days (120 h) for all three stonefly taxa (Chloroperlidae, Taeniopterygidae, and CNT) and the field bioassays for only two taxa (Chloroperlidae and CNT) were conducted for 216 h.

2.4. Measurement of chloride cell responses

The presence of coniform and caviform chloride cells were verified on Chloroperlidae, Nemouridae, and Taeniopterygidae naiads collected from our study streams prior to conducting the bioassays. The specimens were stained in the field with a histochemical silver nitrate ($AgNO_3$) solution (Koch, 1938) (99.8% $AgNO_3$, Tianjin Damao Chemical Reagant Factory, Tianjin, China). The stained specimens were preserved in 1.2% glutaraldehyde (Ted Pella Inc., Redding, California), transported to the Hong Kong University Electron Microscopy unit, dehydrated using a graded ethanol series (30%–100%), and critical point dried using a Pelco CPD2 dryer (Ted Pella Inc., Redding, California) with liquid carbon dioxide as a transitional fluid. The dried and uncoated specimens were mounted on scanning electron microscopy (SEM) stubs using carbon tape and then imaged with variable pressure scanning electron microscopy at Hong Kong University (Hitachi S3400 N Variable Pressure SEM). Both coniform and caviform cells were found on the ventral side of the anterior thoracic area, prothorax, mesothorax, metathorax, abdominal segments, mandible, and mentum of the Taeniopterygidae (Fig. 1). Bulbiform and caviform cells were verified on images of Nemouridae from M1 (Supplemental Fig. 1). Both coniform and caviform cells were noted on Chloroperlidae from N1.

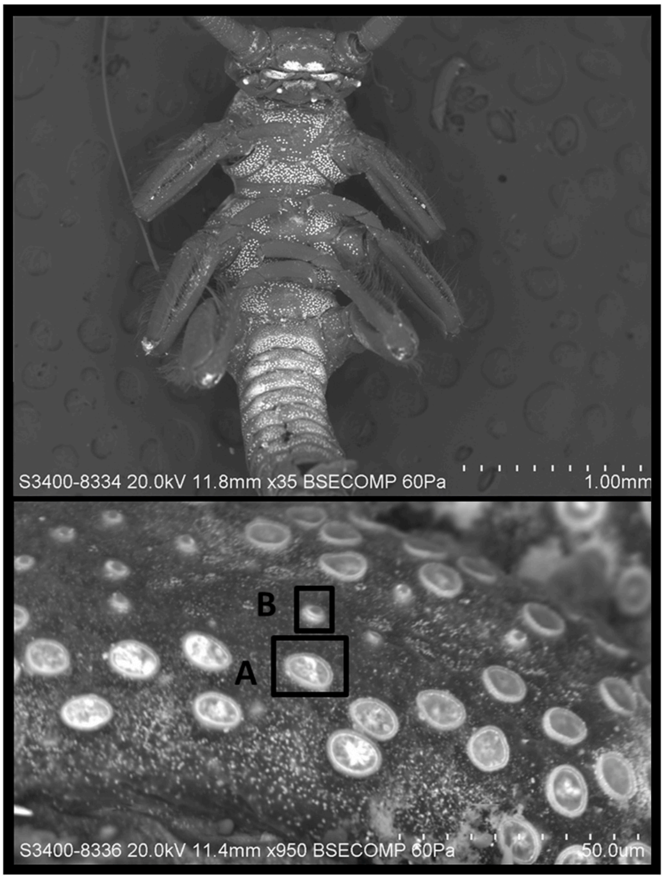

Fig. 1. Taeniopterygidae from Mt. Gongga Hailuogou Glacier stream in the Daxueshan mountain range stained with silver nitrate and imaged with variable-pressure scanning electron microscopy. Both the coniform (A) and smaller forming/caviform chloride cells (B) contain precipitated silver nitrate, which indicates that the cells were absorbing chloride and sodium ions.

To examine morphological responses of the chloride cells to the different NaCl treatments, twelve naiads from each experiment were selected from three treatments (n = 4 naiads per treatment) for examination using SEM: 1) the highest concentration treatment in which stoneflies survived the longest [e.g., 1.2% (20,486 μS/cm) or 0.95% NaCl (16,350 μS/cm)]; 2) the control; and 3) stream reference. If naiads survived less than 48 h in 1.2% NaCl (20,486 μS/cm), then we selected for chloride cell imaging those naiads that survived for longer than 48 h from the next lowest concentration of 0.95% NaCl (16,350 μS/cm). This was done to ensure the examined specimens experienced the maximum exposure to a high concentration of NaCl to increase the probability of detecting a chloride cell response. Nemouridae specimens exposed to the 20,486 μS/cm treatments were desiccated, mounted, and sputter coated in preparation for imaging. However, an accident occurred in the shared vacuum chamber that destroyed the prepared specimens and instead we imaged Nemouridae exposed to 0.95% NaCl (16,350 μS/cm).

Similar-sized naiads were chosen for SEM imaging to control for developmental differences (Supplemental Table 2). Specimens selected for SEM imaging were dehydrated using a graded ethanol series (30%–100%) and then critical point dried using a Pelco CPD2 dryer. Dried specimens were mounted on SEM stubs using carbon tape and sputter coated with a thin layer (5–10 nm) of gold-palladium 60:40. Specimens were stored in a vacuum sealed desiccator until SEM image analysis took place at either Hong Kong University (Hitachi S-4800 field emission SEM, Hitachi High Technologies, Corp., Tokyo) or at Ohio State University (FEI Nova NanoSEM 400 SEM, Thermo Fisher Scientific Inc., Waltham, Massachusetts). SEM images were standardized at 1000× magnification.

SEM images of chloride cells were processed using Fiji ImageJ software (Schindelin et al., 2012). Region-of-interest (ROI) images were generated from ventral sections of the stonefly naiads to quantify chloride cell measurements (Supplemental Table 3; Supplemental Fig. 1). ROI images were analyzed to calculate the following dependent variables for caviform, coniform, and bulbiform cells: 1) cell count; 2) cell density; and 3) cell area. The porous plates of chloride cells were hand-traced due to the similarity in grayscale color intensity between the cell and surrounding tissues and inability of the software to detect the circumference of the cells.

Chloride cells that were partially visible in the image were excluded from our calculation of response variables. Body parts that were smaller than the image area (e.g., osmobranchiae) were traced and cell density measurements were based on the area of the traced body part. Taeniopterygidae and Chloroperlidae ROIs were analyzed for calculation of coniform and caviform cell response variables. Although caviform cells occurred on Nemouridae, many of the smaller caviform cells were not visible due to the presence of debris on the integument. Therefore, only bulbiform chloride cell response variables were measured from Nemouridae ROIs. Additionally, for each experimental replicate (30 mL vial) we calculated the mean values of each chloride cell response variable from our SEM measurements on two to four specimens in each vial for use in our statistical analyses to determine if the mean response variables differed among our conductivity treatments.

2.5. Statistical analysis

All response variables were first examined for normal distribution with the Shapiro-Wilks test using the shapiro.test function from the stats package (R Core Team, 2020) and homogeneity of variance with the Levene test using the leveneTest function from the car package (Fox and Weisberg, 2019). Water temperature, dissolved oxygen, pH, and conductivity met the assumptions of normality and homogeneity of variances and the differences in conductivity among the three collection sites were analyzed with analysis of variance using the aov function from the stats package (R Core Team, 2020). Follow-up Tukey post hoc tests to identify differences among means were conducted with the TukeyHSD function from the stats package (R Core Team, 2020). Percent survivorship and chloride cell responses to conductivity treatments did not meet the assumptions of normality and homogeneity of variances so these response variables were evaluated using the Kruskal-Wallis test with the kruskal.test function in the stats package (R Core Team, 2020). Dunn's post-hoc tests were conducted with the dunn.test function from the dunn.test package (Dinno, 2017) as needed following the Kruskal-Wallis tests to determine differences among experimental treatments.

The median lethal concentration (LC_{50}) representing the conductivity at which 50 percent of the population died after 96 h of exposure was calculated for Chloroperlidae, Taeniopterygidae, and CNT. We reported 96-h LC_{50} values here to enable our calculated LC_{50} values to be comparable with those of others that usually report 72-h or 96-h LC_{50} values rather than 120- or 216-h LC_{50} values. Acute toxicity dose-response curves for the NaCl treatments and the distilled water control were fitted to a log-logistic model using the LC_logit function in the ecotox package (Hlina et al., 2021) and log10 transformed conductivity values. The LC_logit function uses generalized linear model analysis with the binomial family and the logit link transformation to calculate LC_{50} and its associated confidence intervals (Hlina et al. 2021). We also conducted ratio tests using the ratio_test function from the ecotox package (Hlina et al. 2021) to determine if the calculated LC_{50} differed among the three stonefly taxa (Chloroperlidae, Taeniopterygidae, and CNT). For all statistical analyses we used R (Wheeler et al., 2006; R Core Team, 2020) and a level of significance α = 0.05.

3. Results

3.1. Water quality characteristics

Water temperature was the greatest ($P < 0.05$) at the site furthest from the glacier (N1) and the lowest ($P < 0.05$) in the sites closest to the glacier (M1 and GL3) (Table 1). Dissolved oxygen was similar among the three sites, which reflects the low water temperatures and turbulent flow conditions in these glacial-melt stream sites. All three sites exhibited slightly alkaline conditions and pH was the least ($P < 0.05$) in the GL3 site and the greatest ($P < 0.05$) in sites M1 and N1 (Table 1). Conductivity was low at all sites (<133 μS/cm), but the N1 site furthest from the glacier had greater ($P < 0.05$) conductivity than the M1 and GL3 sites closest to the glaciers (Table 1). Calcium concentrations indicated soft water conditions in all three sites (Table 1). Other parameters such as chloride, sodium, arsenic, cadmium, copper, lead, strontium, and vanadium were similar across all stream sites (Table 1).

3.2. Survivorship and median lethal concentrations

Chloroperlidae survival after 120 and 216 h of exposure did not differ ($P > 0.05$) among conductivity treatments (Table 2). CNT survival after 120 and 216 h of exposure was the least ($P < 0.05$) in the 0.95% NaCl (16,350 μS/cm) and 1.2% NaCl (20,486 μS/cm) treatments and the greatest in the distilled water (11 μS/cm), stream reference (60 μS/cm), and 0.01% NaCl (243 μS/cm) treatments (Table 2). Taeniopterygidae survival after 120 h was the least in the 0.95% NaCl (16,350 μS/cm) and 1.2% NaCl (20,486 μS/cm) treatments and the greatest ($P < 0.05$) in the distilled water (11 μS/cm), stream reference (71 μS/cm), and 0.01% NaCl (243 μS/cm) treatments (Table 2). However, from a broader perspective all three stonefly families exhibited lower survivorship with increasing conductivity, with the most precipitous decline in survival occurring between 0.25% NaCl (4,690) and 0.95% NaCl (16,350 μS/cm) (Table 2). More subtle declines in percent survival were also observed for each family at conductivity treatments less than 0.25% NaCl (4690 μS/cm). Median lethal concentrations for 96-h exposures were 2306 μS/cm for CNT, 2002 μS/cm for Taeniopterygidae, and 8167 μS/cm for Chloroperlidae (Table 3). The Chloroperlidae LC50 was significantly greater ($P < 0.05$) than the Taeniopterygidae and CNT LC50 values and Taeniopterygidae and CNT LC50 values did not differ from each other ($P > 0.50$) (Table 4).

Table 1
Mean (SD) of water quality variables (physico-chemical, nutrients, and metals) from three glacial-melt stream sites located at different distances from the glaciers in Yunnan and Sichuan Provinces, China). Physico-chemical measurements were collected in the summer and autumn of 2011 and 2015. Water samples for nutrients and metals were collected during the summer 2015. Different letters within a row for physico-chemical variables indicated a significant difference ($P < 0.05$) among means.

	M1	GL3	N1
Size of Glacier (km^2)	12.6	2.0	10.0
Distance from glacier (m)	800	800	8760
Water temperature (°C)	1.8 (0.60) a	3.75 (2.00) a	8.58 (1.07) b
Dissolved oxygen (%)	89.93 (16.21) a	78.80 (22.37) a	80.70 (5.65) a
pH	8.59 (0.14) a	8.18 (0.17) b	8.52 (0.14) a
Conductivity (μS/cm)	60.4 (2.6) a	70.8 (12.6) a	105.8 (19.2) b
NaCl (mg/L)	–	–	–
Calcium (mg/L)	9.4 (2.0)	10.7 (4.2)	17.5 (14.8)
Chloride (mg/L)	<5	<5	<5
Potassium (mg/L)	0.6 (0.3)	5.1 (2.1)	0.5 (0.6)
Magnesium (mg/L)	2.0 (0.9)	4.0 (3.1)	2.7 (2.6)
Sodium (mg/L)	0.7 (0.8)	0.6 (0.3)	0.6 (0.6)
Arsenic (mg/L)	<0.002	<0.002	<0.002
Cadmium (mg/L)	0	0.001	0
Copper (mg/L)	<0.001	0.005	0.004
Iron (mg/L)	<0.000	7.0 ± 3.8	0.6 ± 0.8
Lead (mg/L)	<0.002	0.003	<0.002
Strontium (mg/L)	0.05	0.1	0.08
Vanadium (mg/L)	0.001	0.02 ± 0.02	0.001

3.3. Chloride cell responses

Chloroperlidae caviform cell count and density were the greatest ($P < 0.05$) in distilled water control (11 μS/cm) and the least ($P < 0.05$) in the stream reference (106 μS/cm) and the 1.2% NaCl (20,486 μS/cm) treatments (Fig. 2). Chloroperlidae caviform cell area was the greatest ($P < 0.05$) in distilled water control (11 μS/cm), the least ($P < 0.05$) in the 1.2% NaCl (20,486 μS/cm) treatment, and intermediate but not different from the other treatments ($P > 0.05$) in the stream reference treatment (106 μS/cm) (Fig. 2). Taeniopterygidae caviform cell count was the greatest in the stream reference treatment (71 μS/cm), the least ($P < 0.05$) in the 0.95% NaCl (16,350 μS/cm) treatment, and intermediate but not different from the other treatments ($P > 0.05$) in the distilled water control (11 μS/cm) (Fig. 2). Taeniopterygidae caviform cell density was the greatest ($P < 0.05$) in the stream reference treatment (71 μS/cm) and the least ($P < 0.05$) in the distilled water control and 0.95% NaCl (16,350 μS/cm) treatments (Fig. 2). Taeniopterygidae caviform cell area did not differ ($P > 0.05$) among conductivity treatments (Fig. 2).

Chloroperlidae coniform cell count did not differ ($P > 0.05$) among conductivity treatments (Fig. 2). Chloroperlidae coniform cell density was the greatest ($P < 0.05$) in the stream reference treatment (106 μS/cm), the least ($P < 0.05$) in the distilled water control (11 μS/cm), and intermediate but not different from the other treatments ($P > 0.05$) in the 1.2% NaCl (20,486 μS/cm) treatment (Fig. 2). Chloroperlidae coniform cell area was the greatest ($P < 0.05$) in distilled water control (11 μS/cm), the least ($P < 0.05$) in the stream reference treatment (106 μS/cm), and intermediate but not different from the other treatments ($P > 0.05$) in the 1.2% NaCl (20,486 μS/cm) treatment (Fig. 2). Taeniopterygidae coniform cell characteristics did not differ ($P > 0.05$) among conductivity treatments (Fig. 2).

Nemouridae bulbiform cell count, cell density, and cell area did not differ ($P > 0.05$) among conductivity treatments (Fig. 2).

4. Discussion

4.1. Survivorship and median lethal concentrations

The range of 96-h median lethal concentrations for stoneflies in this study (2002 to 8167 μS/cm; Table 3) and the observation that the most precipitous decline in survival in all species tested occurred between 4690 and 16,350 μS/cm (Table 2) suggests that stoneflies in Himalayan glacial-melt streams would be tolerant of moderate increases in conductivity (i.e., between 100 and 200 μS/cm) in glacier streams comparable to the increases in conductivity that we have observed between glacial melt and groundwater streams in our study. Bioassay results reported in this study represent the first median lethal concentration values for these Plecoptera families from China (Table 5). Our median lethal concentrations for Chloroperlidae are lower and the associated confidence intervals (Table 3) do not overlap with those reported 72- and 96-h median lethal concentrations reported for Eusthenidae, Gripoterygidae, Leuctridae, Perlidae from Australia, France, Canada, and the United States (Table 5). Our median lethal concentrations for CNT were also lower and their associated confidence intervals do not overlap with those reported for Notonemouridae, Eusthenidae, Gripoterygidae, Leuctridae, and Perlidae from other parts of the world (Table 5). In contrast, we consider our median lethal concentrations for Taeniopterygidae similar to the median lethal concentrations reported by others (Table 5) because the associated confidence intervals for our median lethal concentrations overlapped with all other reported median lethal concentrations. Our results comparing median lethal concentrations among the three Plecoptera taxa groups supports our hypothesis that glacial-melt stonefly naiads colonizing sites close to glaciers would be

Table 2
Means (SD) of percent survival of Chloroperlidae, combined Nemouridae and Taeniopterygidae group (CNT), and Taeniopterygidae, subjected to seven conductivity treatments for 120 h and 216 h. Means with the same letters within a column are not significantly different ($P > 0.05$).

Treatment	Conductivity (μS/cm)	120-h Survivorship			216-h Survivorship	
		Chloroperlidae	CNT	Taeniopterygidae	Chloroperlidae	CNT
Distilled Water	11	100.0 (0.0) a	72.3 (4.62) a	75.0 (25.0) a	100.0 (0.0) a	64.0 (12.8) a
Stream Reference	60–106	62.5 (53.0) a	66.7 (28.8) a	91.7 (14.4) a	62.5 (53.0) a	55.6 (9.60) ab
0.01% NaCl	243	87.5 (17.7) a	67.7 (38.1) a	76.7 (25.2) a	87.5 (17.7) a	47.3 (21.1) ab
0.10% NaCl	1500	83.5 (23.3) a	58.3 (14.4) a	58.3 (38.2) ab	83.5 (23.3) a	41.7 (14.4) abc
0.25% NaCl	4690	75.0 (35.4) a	25.0 (25.0) ab	50.0 (25.0) ab	75.0 (35.4) a	16.7 (14.4) bc
0.95% NaCl	16350	33.5 (47.4) a	0.0 (0.0) b	8.3 (14.4) b	0.0 (0.0) a	0.0 (0.0) c
1.2% NaCl	20486	25.0 (35.4) a	0.0 (0.0) b	0.0 (0.0) b	0.0 (0.0) a	0.0 (0.0) c

Table 3
Median lethal concentrations (LC_{50}) (μS/cm) and their associated 95% confidence intervals in parentheses for the combined Nemouridae and Taeniopterygidae group (CNT), Taeniopterygidae, and Chloroperlidae from three glacial-melt streams in southwestern China exposed to seven conductivity treatments for 96 h.

Family	Stream Collection Site (Average Conductivity)	LC_{50}
CNT	M1 (60.4 μS/cm)	2306 (321–10,018)
Taeniopterygidae	GL3 (70.8 μS/cm)	2002 (25–58,647)
Chloroperlidae	N1 (105.8 μS/cm)	8167 (3696–14,710)

Table 4
Standard errors, test statistics, and associated P values from the ratio tests conducted to compare 96-h median lethal concentrations (LC_{50}) (μS/cm) values for all pairwise comparisons among three Plecoptera taxa groups (Chloroperlidae – Chloro; Taeni – Taeniopterygidae; and CNT - combined Nemouridae and Taeniopterygidae group) from three glacial-melt streams in southwestern China exposed to seven conductivity treatments in August 2015.

Ratio Test Comparison	LC_{50}	LC_{50}	SE	Test statistic	P value
Chloro-Taeni	8167	2002	0.08	7.72	<0.001
Chloro-CNT	8167	2306	0.06	8.46	<0.001
CNT-Taeni	2306	2002	0.09	0.65	0.52

more sensitive to future conductivity changes due to climate change and anthropogenic alterations to water quality. Carter et al. (2020) found that caddisflies from historically low salinity streams in Chile had lower median survival times and more marked behavioral responses than caddisflies originating from historically greater salinity streams when exposed to salinity treatments. Our results and those of Carter et al. (2020) suggest that aquatic insects from stream sites with low conductivities may be more sensitive to increases in conductivity both at the lethal and sublethal response levels.

The greatest observed percent survivorship at 120 and 216 h of the three stonefly families was observed in the distilled water control with the lowest conductivity (11 μS/cm), which is supportive of Tiwari and Rachlin's (2018) observation that Plecoptera appear to tolerate hypotonic conditions better than hypertonic conditions. Kapoor (1978) also observed the greatest Nemouridae survival after 72 h of exposure to hypotonic and isotonic conductivity conditions. Our percent survivorship results indicated that Chloroperlidae, Nemouridae, and Taeniopterygidae from glacial-melt streams in China can tolerate elevated conductivity above that typically observed in our study streams for short periods of time, which is consistent with the Zinchenko and Golovatyuk (2013) report of Plecoptera being able to survive in conductivity up to 13,235 μS/cm. Additionally, the 96-h bioassays of Williams et al. (1999) found no significant changes in survivorship of Nemouridae when exposed to elevated conductivity ranging from 1471 to 8824 μS/cm and Blasius and Merritt (2002) found no significant changes in survivorship of Perlidae when exposed to elevated conductivity ranging from 1471 to 14,706 μS/cm.

Our percent survivorship and median lethal concentration results suggest that salinity tolerance among the representative stonefly families increased with increasing conductivity of their respective collection sites, which is supportive of our survival and median lethal concentration hypotheses. Chloroperlidae from the downstream glacial-melt stream site having the greatest mean conductivity (106 μS/cm) exhibited greater salinity tolerance than the other two families from glacial-melt sites with lower conductivity. Our Chloroperlidae tolerance results are consistent with Olson and Hawkins (2017) conclusion that Chloroperlidae were tolerant to increases in conductivity based on their 83-day streamside experiment results in Nevada (USA) that documented that Chloroperlidae exhibited <10% difference (1–2 days) in survival between moderate conductivity (>120 μS/cm) and low conductivity (<25 μS/cm) water. Notably, Chloroperlidae possess a greater degree of sclerotization on their body than Nemouridae and Taeniopterygidae, with notable dorsoventral sclerotization of the abdominal segments with chloride cells present on the soft integument between sclerotized abdominal segments. The increased amount of sclerotization on Chloroperlidae may reduce the porous surface area exposed to external ion concentrations, which in turn may reduce the stress of increasing conductivity (Buchwalter et al., 2003).

4.2. Chloride cell responses

Chloroperlidae caviform cell count, density, and area responded to increases in external conductivity within 216 h and declined with increasing conductivity as predicted. However, Chloroperlidae coniform cell characteristics, Taeniopterygidae caviform and coniform cell characteristics, and Nemouridae bulbiform cell characteristics did not decline with increasing conductivity. Thus, our hypotheses related to chloride cell responses were only partially supported. The clear responses of the Chloroperlidae caviform cells within 216 h may be due to greater Chloroperlidae survival in the 1.2% NaCl (20,486 μS/cm) treatment that resulted in greater exposure to this elevated conductivity treatment. As well, caviform cells are single-celled whereas coniform and bulbiform are multi-cellular complexes (Wichard et al. 1972), which may enable caviform cells to exhibit a faster response time to changing conductivity. In contrast, the lesser survival of Taeniopterygidae and Nemouridae in 0.95% NaCl (16,350 μS/cm) treatments resulted in less exposure to this elevated conductivity treatment and as such the chloride cells did not have time to respond. The Chloroperlidae naiads selected for imaging were from the greatest 1.2% NaCl (20,486 μS/cm) treatment rather than 0.95% NaCl (16,350 μS/cm) treatment selected for Nemouridae and Taeniopterygidae chloride cell image analyses, which may have also resulted in a clearer Chloroperlidae caviform cell response. Additionally, Wichard et al. (1972) suggested that bulbiform cell morphological responses may occur at a slower rate than other cell types, which combined with the reduced Nemouridae survivorship in elevated conductivity treatments of 0.95% NaCl (16,350 μS/cm) and 1.2% NaCl (20,486 μS/cm) may have hindered us from detecting an effect.

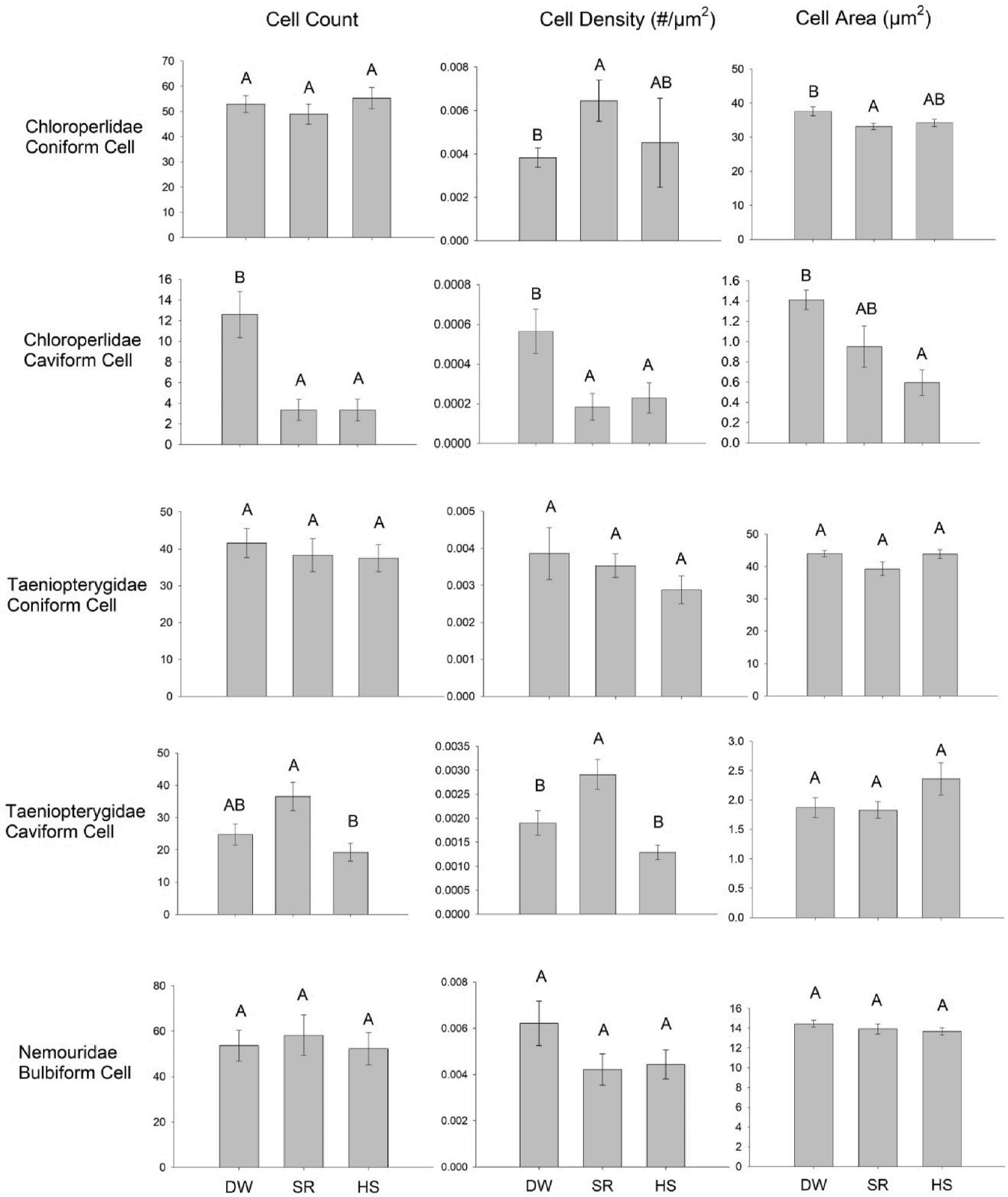

Fig. 2. The response of Chloroperlidae and Taeniopterygidae coniform and caviform cells to distilled water control (DW), stream reference (SR), and highest salinity (HS) (1.2% NaCl or 0.95% NaCl) treatment, and Nemouridae bulbiform responses to DW, SR, and HS (0.95% NaCl) experimental treatment. Bar graphs display chloride cell count, cell density, and mean cell area. Significant differences ($P < 0.05$) are denoted by different letters.

Table 5
Summary of reported median lethal concentrations (LC_{50}) of specific conductance (μS/cm) for Plecoptera families from Australia, France, South Africa, Canada, and the United States.

Family	Country	Duration	LC50	Reference
Eusthenidae	Australia	72	18300	Castillo et al. (2018)
Gripoterygidae	Australia	72	18300	Castillo et al. (2018)
Gripopterygidae	Australia	72	18000	Castillo et al. (2018)
Leuctridae	France	72	10800	Castillo et al. (2018)
Leuctridae	France	72	23400	Castillo et al. (2018)
Notonemouridae	South Africa	72	12600	Castillo et al. (2018)
Nemouridae	Canada	96	>8800	Williams et al. (1999)
Perlidae	Canada	72	15930	Kapoor (1978)
Perlidae	United States	96	>14706	Blasius and Merritt (2002)

Wichard et al. (1972) indicated that coniform chloride cells may be the most efficient at absorbing ions because it was the only type of chloride cell found by itself on several mayflies. All other chloride cell types in aquatic insects (e.g., bulbiform, filiform) co-occur with caviform cells (Wichard et al., 1972; Komnick, 1977). We found that coniform cells co-occur with caviform cells in Chloroperlidae and Taeniopterygidae and our chloride cell image analyses suggest that it is the caviform cells and not the coniform cells that respond to external ion concentrations. Previous studies found that caviform and coniform cells have the same abilities to absorb salt, but the uncertainty remains as to why both chloride cell types are found in many species (Wichard et al., 1972). Our results suggest that single-cell caviform cells may be able to exhibit phenotypic plasticity like the chloride cells on mosquito larvae anal papillae change their shape, mitochondria density, and infolding of plasma membranes in response to conductivity changes in aquatic environments (Hildebrandt et al., 2018). Subsequently, we recommend that future research examine caviform cell growth and response patterns between ecdysis events and at the molecular mechanistic level to increase our understanding of cellular responses underlying stonefly phenotypic plasticity to changing environmental conditions.

4.3. Study limitations

One study limitation that we acknowledge is the use of a different stonefly family from each stream site for our field bioassays. Ideally, we would have used the same stonefly family from each site in the bioassays to account for potential taxonomic differences in survival, median lethal concentrations, and chloride cell responses. The reason we selected representative stonefly families was due to the difficulty we had in collecting sufficient numbers of the same family from each stream site during the glacial ablation and monsoon season, which is when the streams exhibit elevated discharge. We originally planned to compare the responses of Nemouridae with horn-shaped osmobranchiae from a low conductivity site with Nemouridae with rounded osmobranchiae from a downstream site with medium conductivity, and Nemouridae with tracheated filiform gills found in a higher conductivity mixed glacial-melt and groundwater site (Fig. 3a). Tracheated gills with chloride cells covering the gill integument (Komnick, 1977; Shepard and Stewart, 1983) are hypothesized to counter metabolic and osmoregulation stressors due to the proximity of the trachea and chloride cells. Unfortunately, we were not able to collect enough Nemouridae from the other two sites for the bioassays and instead accounted for this issue while onsite at our remote field locations by using Taeniopterygidae and Chloroperlidae instead of Nemouridae. Even if our results are partially influenced by taxonomic differences, our results with representative stonefly families provide novel information about Plecoptera responses to elevated salinity and conductivity above that expected in glacial-melt streams.

5. Conclusions

Our results represent the first documentation of the survivorship, median lethal concentrations, and chloride cell responses of stoneflies from glacial-melt streams in Asia to varying conductivity in field bioassays. These results suggest that Chloroperlidae, Nemouridae, and Taeniopterygidae from glacial-melt streams in Asia may be able to tolerate short term (up to 9 days) and moderate increases in conductivity (i.e., 100 to 200 μS/cm) with stream temperatures ranging between 0.6 °C and 8.1 °C. Our median lethal concentration results and those of others from different regions of the world (Table 5) suggest that glacial-melt stoneflies from China may have similar tolerances to predicted climate-induced increases in conductivity like those of stoneflies from other regions. However, we note these conclusions are based on results that reflect stonefly responses to elevated conductivity above that expected in glacial-melt streams in the absence of biotic interactions and changes in water temperature and dissolved oxygen concentration that may result in interaction effects that heighten effects of climate-induced increases in conductivity of glacial-melt streams (Bray et al., 2019; Birrell et al., 2020). Future research needs to evaluate the long-term effects of the combined changes in water temperature, dissolved oxygen, and conductivity to fully understand the effects of climate change scenarios on glacial-melt stonefly survival and their ability to colonize groundwater streams.

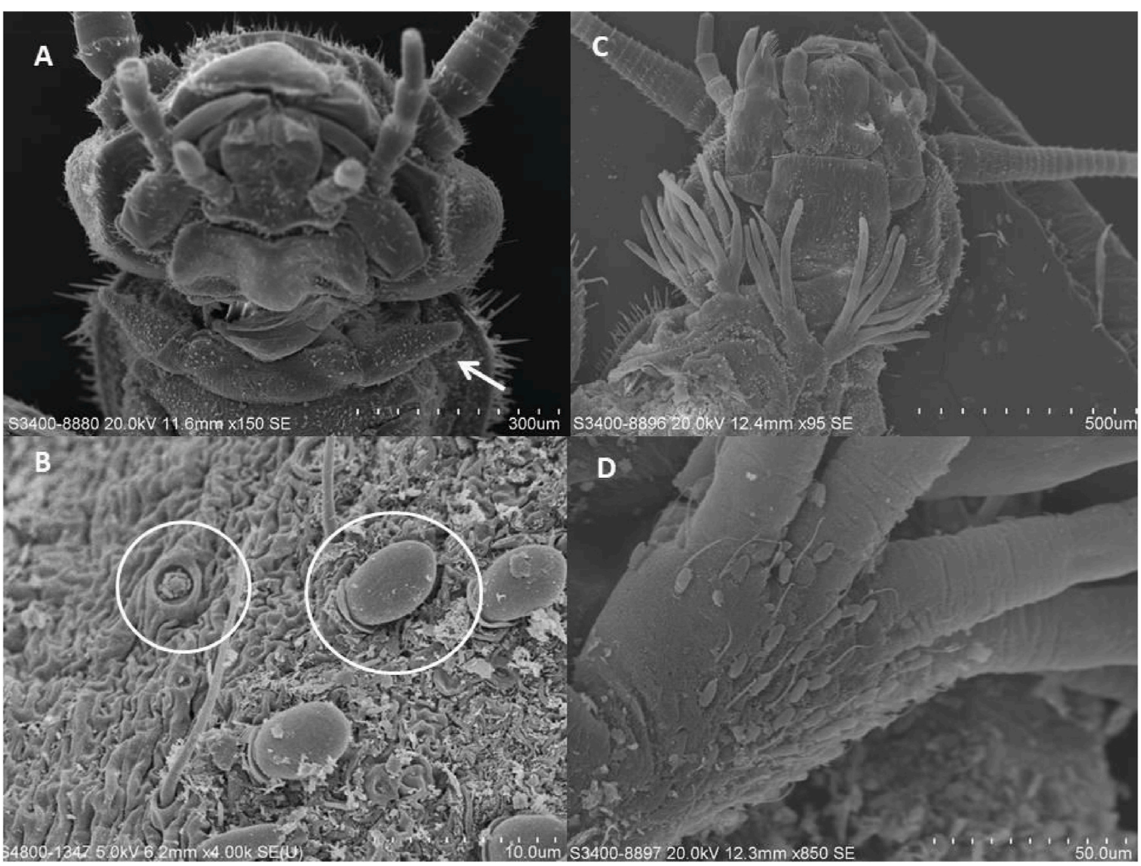

Fig. 3. Scanning electron microscopy images of Nemouridae from glacial-melt stream (A and B) and from a mixed glacial-melt spring stream (C and D). Horn-shaped osmobranchiae of glacial-melt Nemouridae naiad (A). Caviform chloride cell (B, left circle) and bulbiform chloride cell (B, right circle) from the cervical region of the Nemouridae naiad. Nemouridae tracheated cervical gills (C) and bulbiform chloride cells at the tracheated gill bases (D).

Author statement

Heather Fair: Conceptualization, Methodology, Investigation, Fieldwork, Writing – original draft Preparation. **Roman Lanno:** Supervision, Conceptualization Supervision, Writing – original draft preparation. **Peter C. Smiley:** Data reanalysis; Writing – Reviewing and Editing.

Declaration of competing interest

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

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Appendix A. Supplementary data

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References

Anderson, S.P., Drever, J.I., Humphrey, N.F., 1997. Chemical weathering in glacial environments. Geology 25, 399–402.

Birrell, J.H., Shah, A.A., Hotaling, S., Giersch, J.J., Williamson, C.E., Jacobsen, D., Woods, H.A., 2020. Insects in high-elevation streams: life in extreme environments imperiled by climate change. Global Change Biol. 26, 6667–6684.

Blaen, P.J., Brown, L.E., Hannah, D.M., Milner, A.M., 2014. Environmental drivers of macroinvertebrate communities in high Artic rivers (Svalbard). Freshw. Biol. 59, 378–391.

Blasius, B.J., Merritt, R.W., 2002. Field and laboratory investigations on the effects of road salt (NaCl) on stream macroinvertebrate communities. Environ. Pollut. 120, 291-231.

Bray, J.P., Reich, J., Nichols, S.J., King, G.K.K., Mac Nally, R., Thompson, R., O'Reilly-Nugent, A., Kefford, B.J., 2019. Biological interactions mediate context and species-specific sensitivities to salinity. Philos. Trans. Royal Soc. B 374, 20180020. https://doi.org/10.1098/rstb.2018.0020.

Brodsky, K.A., 1980. Mountain Torrent of the Tien Shan. A Faunistic-Ecology Essay, ume 39. Monographiae Biologicae. Springer, Berlin.

Brown, L.E., Hannah, D.M., Milner, A.M., 2010. Predicting river ecosystem response to glacial meltwater dynamics: a case study of quantitative water sourcing and glaciality index approaches. Aquat. Sci. 72, 325–334.

Brundin, L., 1966. Transantarctic relationships and their significance, as evidenced by chironomid midges with a monograph of the subfamilies Podonominae and Aphroteniinae and the Austral Heptagyiae. K. - Sven. Vetenskapsakademiens Handl. 11, 1–472.

Buchwalter, D.B., Jenkins, J.J., Curtis, L.R., 2003. Temperature influences on water permeability and Chlorpyrifos uptake in aquatic insects with differing respiratory strategies. Environ. Toxicol. Chem. 22, 2806–2812.

Carter, M.J., Flores, M., Ramos-Jiliberto, R., 2020. Geographical origin determines responses to salinity of Mediterranean caddisflies. PLoS One 15 (1). https://doi.org/10.1371/journal.pone.0220275.

Castillo, A.M., Sharpe, D.M., Ghalambor, C.K., De Leon, L.F., 2018. Exploring the effects of salinization on trophic diversity in freshwater ecosystems: a quantitative review. Hydrobiologia 807, 1–17.

Choe, K., Strange, K., 2009. In: Evans, D.H. (Ed.), Volume Regulation and Osmosensing in Animal Cells, *Osmotic and Ionic Regulation: Cells and Animals*. CRC Press, Baton Rouge, pp. 37–67.

Clements, W., Kotalik, C., 2016. Effects of major ions on natural benthic communities: an experimental assessment of the US Environmental Protection Agency aquatic life benchmark for conductivity. Freshw. Sci. 35, 126–138.

Clements, W.H., Hickey, C.W., Kidd, K.A., 2012. How do aquatic communities respond to contaminants? It depends on the ecological context. Environ. Toxicol. Chem. 31, 1932–1940.

Danks, H., 1971. Overwintering of some north temperate and arctic Chironomidae (Diptera). II. Chironomid biology. Can. Entomol. 103, 1875–1910.

Denlinger, D.L., Lee Jr., R.E., 2010. Low Temperature Biology of Insects. Cambridge University Press, Cambridge.

Dinno, A., 2017. Dunn's Test of Multiple Comparisons Using Rank Sums. Portland State University, Portland, Oregon.

Fair, H., Smiley Jr., P.C., Lanno, R., 2021. Determinants of invertebrate community structure in glacial-melt streams of southeast Tibet. Freshw. Biol. 66, 1282–1295.

Finn, D., Khamis, K., Milner, A.M., 2013. Loss of small glaciers will diminish beta diversity in Pyrenean streams at two levels of biological organization. Global Ecol. Biogeogr. 22, 40–51.

Fox, J., Weisberg, H.S., 2019. An R and S-Plus Companion to Applied Regression, third ed. Sage Publications.

Franson, M.A.H., Greenberg, A.E., Clesceri, L.S., Eaton, A.D. (Eds.), 1992. Standard Methods for the Examination of Water and Wastewater, Method 4110B. Anions by Ion Chromatography, 18th Edition of Standard Methods. American Public Health Association, Washington, D.C.

Gislason, G., Adalsteinsson, H., Hansen, I., Olafsson, J., Svavarsdottir, K., 2001. Longitudinal changes in macroinvertebrate assemblages along a glacial river system in central Iceland. Freshw. Biol. 46, 1737–1751.

Griffith, M.B., 2017. Toxicological perspective on the osmoregulation and ionoregulation physiology of major ions by freshwater animals: teleost fish, crustacea, aquatic insects, and Mollusca. Environ. Toxicol. Chem. 36, 576–600.

Griffith, M.B., Norton, S.B., Alexander, L.C., Pollard, A.I., LeDuc, S.D., 2012. The effects of mountaintop mines and valley fills on the physicochemical quality of stream ecosystems in the central Appalachians: a review. Sci. Total Environ. 417–418, 1–12.

Gurnell, A.M., Fenn, C., 1985. Spatial and temporal variations in electrical conductivity in a pro-glacial stream system. J. Glaciol. 31, 108–114.

Hildebrandt, J.-P., Wiesenthal, A., Müller, C., 2018. Phenotypic plasticity in animals exposed to osmotic stress – is it always adaptive? Bioessays 40, 1–9.

Hlina, B.L., Birceanu, O., Robinson, C.S., Dhiyebi, H., Wilkie, M.P., 2021. The relationship between thermal physiology and lampricide sensitivity in larval sea lamprey (Petromyzon marinus). J. Great Lake. Res. 47, S272–S284.

Jacobsen, D., Dangles, O., 2012. Environmental harshness and global richness patterns in glacier-fed streams. Global Ecol. Biogeogr. 21, 647–656.

Kapoor, N.N., 1978. Effect of salinity on the osmoregulatory cells in the tracheal gills of the stonefly nymph, *Paragnetina media* (Plecoptera: Perlidae). Can. J. Zool. 56, 2608–2613.

Kapoor, N.N., 1979. Osmotic regulation and salinity tolerance of the stonefly nymph, Paragnetina media. J. Insect Physiol. 25, 17–20.

Kefford, B.J., Nugegoda, D., Zalizniak, L., Fields, E.J., Hassell, K.L., 2007. The salinity tolerance of freshwater macroinvertebrate eggs and hatchlings in comparison to their older life-stages: a diversity of responses. Aquat. Ecol. 41, 335–348.

Kefford, B.J., Schäfer, R.B., Metzeling, L., 2012. Risk assessment of salinity and turbidity in Victoria (Australia) to stream insects' community structure does not always protect functional traits. Sci. Total Environ. 415, 61–68.

Koch, H.J., 1938. The osmo-regulatory function of anal papillae in Culicidae. J. Exp. Biol. 15, 152–160.

Komnick, H., 1977. Chloride cells and chloride epithelia of aquatic insects. Int. Rev. Cytol. 49, 285–329.

Kuhn, J., Andino, P., Calvez, R., Espinosa, R., Hamerlik, L., Vie, S., Dangles, O., Jacobsen, D., 2011. Spatial variability in macroinvertebrate assemblages along and among neighboring equatorial glacier-fed streams. Freshw. Biol. 56, 2226–2244.

Liu, Q., Liu, S.Y., 2010. Seasonal evolution of the englacial and subglacial drainage systems of a temperate glacier revealed by hydrological analysis. Sci. Cold Arid Reg. 2, 51–58.

Milner, A.M., Petts, G., 1994. Glacial rivers: physical habitat and ecology. Freshw. Biol. 32, 295–307.

Milner, A.M., Taylor, R.C., Winterbourn, M.J., 2001. Longitudinal distribution of macroinvertebrates in two glacier-fed New Zealand rivers. Freshw. Biol. 46, 1765–1775.

Nowghani, F., Jonusaite, S., Watson-Leung, T., Donini, A., Kelly, S.P., 2017. Strategies of ionoregulation in the freshwater nymph of the mayfly *Hexagenia rigida*. Exp. Biol. 220, 3997–4006.

Olson, J.R., Hawkins, C.P., 2017. Effects of total dissolved solids on growth and mortality predict distributions of stream macroinvertebrates. Freshw. Biol. 62, 779–791.

Perry, S., Laurent, P., 1989. Adaptational responses of rainbow trout to lowered external NaCl concentration: contribution of the branchial chloride cell. J. Exp. Biol. 147, 147–168.

R Core Team, 2020. R Version 4.0.2: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Schiebener, S., Conley, J.M., Buchwalter, D., 2017. Sulfate transport kinetics and toxicity are modulated by sodium in aquatic insects. Aquat. Toxicol. 190, 62–69.

Schindelin, J., Arganda-Carreras, I., Frise, E., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682.

Shepard, W., Stewart, K., 1983. A Comparative Study of Nymphal Gills in North American Stonefly (Plecoptera) Genera and a New, Proposed Paradigm of Plecoptera Gill Evolution, vols. 55–58. Entomological Society of America, pp. 1–57.

Stallard, R.F., 1995. Tectonic, environmental, and human aspects of weathering and erosion: a global review using a steady-state perspective. Annu. Rev. Earth Planet Sci. 23, 11–39.

Tiwari, A., Rachlin, J.W., 2018. A review of road salt ecological impacts. Northeast. Nat. 25, 123–142.

USEPA, 2007. Method 6010C (SW-846): Inductively Coupled Plasma-Atomic Emission Spectrometry. Revision 3. United States Environmental Protection Agency, Washington, DC.

Ward, J.V., Malard, F., Tockner, K., Uehlinger, U., 1999. Influence of ground water on surface water conditions in a glacial floodplain of the Swiss Alps. Hydrol. Process. 13, 277–294.
Wheeler, M.W., Park, R.M., Bailey, A.J., 2006. Comparing median lethal concentration values using confidence interval overlap or ratio tests. Environ. Toxicol. Chem. 25, 1441–1444.
Wichard, W., Komnick, H., Abel Jr., J.H., 1972. Typology of ephemerid chloride cells. Z. Zellforsch. Mikrosk. Anat. 132, 533–551.
Wichard, W., Tsui, P.T.P., Komnick, H., 1973. Effect of different salinities on the coniform chloride cells of mayfly larvae. J. Insect Physiol. 19, 1825–1835.
Williams, D.D., Williams, N.E., Cao, Y., 1999. Road salt contamination of groundwater in a major metropolitan area and development of a biological index to monitor its impact. Water Res. 34, 127–138.
Wooton, R.J., 1988. The historical ecology of aquatic insects: an overview. Palaeogeogr., Palaeolimatol., Palaeoecol. 62, 477–492.
YSI, 2009. YSI Professional Plus User Manual. Yellow Springs Instruments, Inc., Yellow Springs.
Zinchenko, T.D., Golovatyuk, L.V., 2013. Salinity tolerance of macroinvertebrates in stream waters (review). Arid Ecosyst. 3, 113–121.
Zwick, P., 2000. Phylogenetic system and zoogeography of the Plecoptera. Annu. Rev. Entomol. 45, 709–746.