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Abstract

The physiological responses of the acid-sensitive amphipod *Gammarus fossarum* exposed *in situ* to acid stress (pH 4.5 and 5.5) and then transferred back to neutral water were investigated. Survival rate and haemolymph [Cl^-] and [Na^+] were assessed after 24, 48 and 72h of exposure in acidic streams and after a recovery period of 12, 24, 36, 48 and 60h. After 24h, exposure to slightly acidic (pH 5.5) and strongly acidic water (pH 4.5) led to a severe and significant depletion in haemolymph [Na^+] and [Cl^-] compared to organisms exposed in circumneutral water (pH 7.3). However, after only a 12h- period of transfer back in neutral water and whatever the previous exposure time (24, 48 and 72h) in both slightly and strongly acidic water, haemolymph [Na^+] and [Cl^-] were equal or superior to the control level without associated mortality. In spite of this fast physiological recovery capacity, populations of *G. fossarum* living in streams undergoing episodic acid stresses were drastically affected thus, demonstrating the high acid-sensitivity of this species. We discuss the possible reasons of population regression and the absence of population recovery.

Keywords: *Gammarus fossarum,* haemolymph, ion loss, acid stress, recovery, *in situ* exposure.

INTRODUCTION

Acidification of freshwater ecosystems related to anthropogenic emissions of SO_2 and NO_X has been one of the most striking ecological problems throughout the northern hemisphere during the 20th century. National and international legislation in the 1980s and 1990s aimed to reduce the emissions of acidifying pollutants (e.g. Clean Air Act in the USA and the Convention on Long-Range Transboundary Air Pollution in Europe) have led to the decline in acidic depositions across wide areas of Europe and North America (Stoddard et al., 1999; Lawrence et al., 2000; Likens et al., 2001). Several recent studies have shown that recovery of alkalinity has occurred in many areas of Europe and North America (Stoddard et al., 1999; Skjelkvale et al., 2001), but acidification of freshwater ecosystems still occurs in many areas (Guérold et al., 2000; Driscoll et al., 2001; Evans et al., 2001). In addition, acidification of aquatic ecosystems is now reported across other large areas of the world where high economic and demographic growth rates occur, such as in China (Thorjørn et al., 1999; Tang et al., 2001) and India (Aggarwal et al., 2001).

Episodic acidification following snowmelt or heavy rainfalls has been well documented (Ormerod & Jenkins, 1994; O'Brien & Eshleman, 1995; Wigington *et al*., 1996). These hydrometeorological events induce a decrease of pH, Acid Neutralizing Capacity (ANC), and base cations concentrations as well as an increase of aluminum concentrations (O'Brien et al., 1993; Soulsby, 1995). The intensity of acid-stress tends to be greater in more acidic environments because low ANC streams can be subject to episodic acidic stress throughout the year (Colin *et al*., 1989). Change in Al speciation accompanying large pH depressions has

been shown to cause stress and mortality in many aquatic species (Weatherley & Ormerod, 1991; Carline et al., 1992; Van Sickle et al., 1996).

One of the most striking consequences of freshwater acidification is the erosion of biodiversity (Muniz, 1991). Numerous studies have clearly demonstrated a failure to regulate blood or haemolymph Na^+ and Cl^- levels in acid-stressed fish, clams (Unionidae) and decapods (Massabuau, 1985; McMahon & Stuart,- 1989; Pynnönen, 1991; Masson et al., 2002). However, most of the studies have focused on large species, and relatively little is known about physiological responses in smaller acid-sensitive species of macroinvertebrates.

Crustaceans contain many of the most acid-sensitive macroinvertebrate species (Sutcliffe & Carrick, 1973; Guerold et al., 2000). In previous studies, Felten & Guérold (2001, 2004) showed that *Gammarus fossarum* (Crustacea: Amphipoda) also suffered a severe depletion of haemolymph Na^+ and Cl^- ions when exposed to acidic conditions. Conjointly, we determined relationship between acidification level and haemolymph ion losses. Thus, we proposed that haemolymph ion concentrations in the acid-sensitive species *G. fossarum* could represent effective biomarkers for monitoring acidification of running waters.

The present study aims to investigate the recovery of haemolymph [Na^+] and [Cl^-] in *G. fossarum* previously exposed to different magnitudes of acid stresses. In this context we assessed *in situ* the short-term response of haemolymph [Na^+] and [Cl^-] in *G. fossarum* transferred to 3 headwater streams providing 3 different acidification levels (defined by: pH, ANC, [Mg^{2+}], [Ca^{2+}] and [Al_{tot}]). For each stream and each exposure time, organisms were transferred back to their native circumneutral stream not only to test for *G. fossarum* recovery capacity but also to evaluate the effect of an episodic acid stress.

MATERIALS AND METHODS

Study organisms. Experiments were performed on *Gammarus fossarum* because this species presents several interesting characteristics for ecotoxicological investigations : 1) it is an acid-sensitive species (Guérold *et al.*, 2000), 2) widespread and common in West Paleartica (Barnard & Barnard, 1983), 3) often occuring in high density, 4) easy to identify to species level, 5) characterised by a sexual dimorphism and (6) playing a major role in the leaf litter breakdown process and consequently in the entire food web (Pöckl, 1995).

Experimental design. The study was conducted in the Vosges Mountains (north-eastern France) in 3 headwater streams draining sandstone bedrock and providing exposure to a range of acidification levels (**Fig.1.**, neutral : La Maix; Ravine: slightly acidified; Gentil Sapin: strongly acidified). The 3 sites were located in the same area, the highest distance between 2 sites being less that 10 km representing a 20 mn transport of the organisms.

Males *G. fossarum* with 8-10mm body size were collected from the neutral stream, La Maix. The experimental design we used is shown in **Fig. 2.**. Each Plexiglas flow-through enclosure (experimental unit) contained 144 *G. fossarum*. Enclosures were divided in 18 compartments with 8 individual each. Two enclosures like this were placed in each river. Enclosures were then transferred to each of the 3 streams including the neutral streams. The total number of *G. fossarum* collected for this experiment was 2592.

For each stream, one enclosure was used for haemolymph analyses and the other for the assessment of survival. For each stream, there were 3 pairs of enclosure each corresponding to one exposure time (24, 48 and 72 h).

In order to evaluate the initial concentrations of haemolymph Cl^- and Na^+ in *G. fossarum*, 12 organisms were sampled in the neutral "native" stream just before the onset of the experiments (T_o, control). At 24, 48 and 72 h of exposure, survival was assessed and samples of haemolymph from 8 organisms were randomly collected in each stream for analysis (in

101 enclosure corresponding to exposure time). For more details concerning the enclosure
102 protocol see Felten & Guérold (2004).
103 After each exposure time, the sampling enclosures were transferred to the circumneutral
104 stream (La Maix) in order to initiate the recovery experiment (**Fig 2.b.**); the latest values of
105 haemolymph [Cl^-] and [Na^+] recorded before the transfer to circumneutral water were referred
106 as t_o for the recovery experiment. After 12, 24, 36, 48 and 60 h of recovery, the survival was
107 assessed and samples of haemolymph from 8 organisms were randomly collected for analysis
108 in each enclosure corresponding to each stream exposure time (**Fig 2.b.**).
109 Survival rates calculated for the recovery period are based on the number of organisms
110 remaining (in the enclosure) at the time of transfer to the circumneutral stream. Thus, there
111 was a 60 h-recovery kinetic for each exposure type (circumneutral, slightly acid and strongly
112 acid).
113 **Survival, haemolymph sampling and analysis.** For each acid exposure time and recovery
114 time, the survival was assessed in each stream (3 replicates of 48 organisms). Samples of
115 haemolymph (0.8 to 1.2 µl) were taken from the telson of each individual (n = 8) using a
116 microsyringe, transferred to a gauged 5-µl microcapillary tube and centrifuged for 10 min at
117 6596 *g*. After centrifugation the liquid phase was diluted in 2 ml of Nanopur water to
118 determine chloride and sodium concentrations in haemolymph by ionic chromatography
119 (Dionex 4500i with Ion Pac AS4A column) and atomic absorption spectrophotometry (AAS)
120 (Perkin Elmer Analyst 100), respectively.
121 **Water analysis.** Water samples were collected at the initiation of the experiment (T_o) and at
122 each time of acid exposure (24, 48 and 72 h) and recovery (12, 24, 36, 48 and 60 h). Cations
123 were analysed by flame AAS and anions by ionic chromatography as described previously.
124 Total aluminium was determined by graphite furnace AAS (Varian Spectraa 300) after
125 acidification with 0.25% HNO_3. Acid neutralising capacity (ANC) was measured by Gran's

titration and pH (glass electrode), and conductivity with multi-parametric equipment (WTW). Chemical characteristics of water from each stream are given in **Table 1**.

Statistical analysis. All data are reported as mean ± SD. Statistical comparisons of experimental data were performed by two-way analysis of variance (ANOVA) and Ficher's Least Significant Difference test (LSD). The analyses were carried out using STATISTICA (Microsoft), with a probability limit of $p \leq 0.05$ considered as significant.

RESULTS

Acid exposure

Acidified streams were characterized by low pH, low ANC, low [Mg^{2+}] and low [Ca^{2+}] and high [Al_{tot}] (**Table 1**). The baseline levels of haemolymph [Cl^-] and [Na^+] prior to the exposure were 54.1 ± 8.1 and 90.4 ± 15.9 mmol l^{-1}, respectively, in control organisms (**Fig. 2.a.-b.**).

The 2-way analysis of variance (ANOVA) indicated that stream acidity, exposure time and the interaction between them (Stream × Exposure Time) exerted a significant effect on haemolymph parameters ([Na^+], [Cl^-]) and survival (**Table 2.a.**).

Haemolymph [Na^+] and [Cl^-] in *G. fossarum* exposed to circumneutral stream remained constant over a 72-h exposure period, but decreased significantly in organisms exposed to slightly acidic (Ravine, mean pH = 5.5) and strongly acidic streams (Gentil Sapin, mean pH = 4.5) during the first 24 h (**Fig. 3.a.-b.**). Indeed, after 24 h of exposure, the loss of haemolymph Cl^- ranged from 22.5% in the slightly acidic stream (mean haemolymph [Cl^-] = 41.9 ± 6.7 mmol l^{-1}) to 48.8% in the strongly acidic one (mean haemolymph Cl^- = 27.7 ± 5.5 mmol l^{-1}) compared with the control (mean haemolymph Cl^- = 54.1 ± 8.1 mmol l^{-1}) (**Fig. 3.a.**). The same trend was observed for haemolymph [Na^+]. After 24 h of exposure, the loss of haemolymph Na^+ ranged from 19.9% in Ravine (mean haemolymph Na^+ = 72.4 ± 13.6 mmol

151 l^{-1}) to 53.9% in Gentil Sapin (mean haemolymph Na^+ = 41.7 ± 9.6 mmol l^{-1}) compared with
152 the control (mean haemolymph Na^+ = 90.4 ± 15.9 mmol l^{-1}) (**Fig. 3.b.**).

153 After 48 h of exposure, the haemolymph [Na^+] and [Cl^-] of the organisms exposed to slightly
154 and strongly acidic streams were close to 24h-exposure values and remained constant until the
155 end of the experiment. Individuals transferred for 72 h to Ravine had a mean haemolymph
156 [Cl^-] of 42 ± 11.5 mmol l^{-1} and [Na^+] of 70.8 ± 20 mmol l^{-1}, representing a significant 22.3%
157 loss of chloride and a significant 21.7% loss of sodium ($p<0.05$). On the other hand,
158 individuals transferred for 72 h to Gentil Sapin had a haemolymph [Cl^-] of 25.6 ± 4.6 mmol l^{-1}
159 and [Na^+] of 44.7 ± 6.9 mmol l^{-1}, representing a chloride loss of 45.3% and a sodium loss of
160 50.5 ($p<0.001$).

161 For each exposure time, the survival rate in *G. fossarum* transferred to the native
162 circumneutral stream (La Maix) remained very high and above 99%. After 72h of exposure,
163 the survival rates in organisms exposed to slightly and strongly acidic streams were
164 significantly different from mean control values for the same exposure time ($p<0.05$),
165 reaching 89.6 ± 7.5% and 42.4 ± 11.9% respectively. On the contrary the survival rates after
166 24 and 48h of exposure in slightly acidified streams (Ravine) were not significantly different
167 from those observed in the neutral stream whereas significant differences were measured in
168 organisms transferred to strongly acidified stream (Gentil sapin). Thus, survival rates of
169 gammarids transferred to Gentil sapin reached 79.9 ± 11.8% and 51.7 ± 10.8% after a 24h and
170 a 48h-exposure time respectively (**Fig. 3.c.**).
171
172 **Recovery**
173 The 2-way analysis of variance (ANOVA) indicated that Exposure Time and/or Recovery
174 Time as well as the interaction term had a significant influence on haemolymph [Na^+],
175 haemolymph [Cl^-] and survival (**Table 2.b.**).

We observed a rapid and total recovery of haemolymph [Cl^-] and [Na^+] in organisms previously exposed to slightly and strongly acidic stream. After only 12h transferred back in the native circumneutral stream, the mean values of haemolymph parameters were similar or significantly higher than those measured at To (**Fig. 4.a.-b.** and **Fig. 5. a.-b.**).

After a 12h-recovery in La Maix following a 72h-exposure in Gentil sapin (the longest and most intense acid stress tested), the mean haemolymph [Cl^-] increased from 29.6 ± 4.3 mmol l^{-1} to 63.8 ± 13.9 mmol l^{-1}, representing an increase of 115.6%. Similarly the mean haemolymph [Na^+] increased from 44.7 ± 6.9 mmol l^{-1} to 85.2 ± 8.5 mmol l^{-1}, representing an increase of 90.6%.

In all enclosures, the survival rates remained high throughout the recovery experiment (>93%, **Fig. 4.c.** and **Fig. 5 .c.**).

DISCUSSION

Acid-exposure

Several studies have shown that crustaceans exposed to water-borne pollutants, environmental stressors and pathological agents usually exhibit disruption of ionic regulation (Lignot et al., 2000). Different causes include alterations in the structure and ultrastructure of the branchial and excretory organs, and changes in Na^+, K^+-ATPase activity, ion fluxes and surface permeability (Lignot et al., 2000). Ion-regulation failure leading to a severe deficiency of extracellular ions (i.e. Na^+ and Cl^-) has been recognised to be the major response in fish to acid stress (McDonald et al., 1989; Potts & McWilliams, 1989; Wood, 1989). Similar results have been reported in crayfish (Appelberg, 1985; Fjeld et al., 1988; McMahon & Stuart, 1989; Jensen & Malte, 1990), gammarids (Rupprecht, 1992; Felten & Guérold, 2001, 2004) and molluscs (Pynnönen, 1991). Surprisingly, and despite the numerous papers reporting detrimental effects of acidification on invertebrate communities, few studies have been

201 performed on the ecophysiology of smaller acid-sensitive macroinvertebrate species
202 (Herrmann, 1987; Herrmann & Andersson, 1986).
203 In the present study we showed that exposure of *G. fossarum* to strongly acidic or slightly
204 acidic water induced early significant losses of haemolymph [Na^+] and [Cl^-]. Moreover,
205 failure in ion-regulation was accompanied by a significant mortality. These results were in
206 agreement with those obtained in previous studies (Felten & Guérold, 2001, 2004) which
207 permitted us to demonstrate that ion losses were significantly correlated to pH.
208

209 **Recovery from acid stress**

210 Despite drastic ion losses (Cl^- and Na^+) following exposures to slightly and strongly acidic
211 stresses, organisms transferred back to the native circumneutral stream were surprisingly able
212 to recover rapidly from acid stress (as soon as 12h). Moreover, survival rates associated with
213 the recovery period were very high (>93.4%). Consequently, the ion-regulation failure
214 evidenced during the exposure under acidic conditions appears reversible even when the loss
215 of ions was severe (>50%).
216 These results are in accordance with those of several studies which have highlighted the
217 recovery capacity of several acid-sensitive species following their transfer from an acidified
218 medium to a circumneutral medium. Favrel (1998) observed a total recovery of haemolymph
219 [Cl^-] and [Na^+] in *Dinocras cephalotes* (Plecoptera) after 16 days of recovery in a
220 circumneutral water (pH = 6.6, [Al] = 10 µg l^{-1}) following a 8 days exposure in an acidified
221 water (pH = 4.6, [Al] = 540 µg l^{-1}). Comparable results had been also reported for *Anodonta*
222 *anatina* (Mollusc) for a 8 day exposure to pH 4.0-5.0 followed by a 8 days transfer in a water
223 with a pH 7.2-7.4 (Pynnönen, 1994). Similary, Kroglund & Staurnes (1999) conducted an
224 experiment showing that acid-exposed (pH 6.0 and 5.6) *Salmo salar* smolts were able to re-
225 establish their plasma chloride levels within 3 days in pH 6.28 water, but the authors did not

sample organisms until the end of the 3 days. In our study, the recovery observed for *G. fossarum* was definitely faster than those observed in the species mentioned above.

Moreover, these results are in accordance with our previous observation (Felten & Guérold, 2004) showing the physiological adaptation of *G. fossarum* exposed to slightly acidic stress (pH 5.7, $[Al_{tot}] = 2.5$ µmol l^{-1}, $[Ca^{2+}] = 26.8$ µmol l^{-1}). After 24h of exposure, *G. fossarum* had undergone significant ion losses but following 96h, no differences were observed in haemolymph $[Cl^-]$ and $[Na^+]$ (compared to the control) without any additional mortality.

Several assumptions may be advanced to explain the fast recovery of haemolymph $[Cl^-]$ and $[Na^+]$. On the one hand Chamier et al. (1989) reported that Na^+ turnover is very fast in *G. pulex* (65% of total body in Na^+ per day at 9°C). In addition, Wood & Ronago (1986) observed an increase in Cl^- and Na^+ uptake during the recovery following an exposure to pH 4. This can be due to a reduction of the $[H^+]/[Na^+]$ ratio in water, consequently decreasing the competition between these two ions at ionic transport sites, leading to an increase in Na^+ uptake. In addition organisms transferred back to circumneutral waters were then facing lower passive ion losses and water uptake because of the increase in $[Ca^{2+}]/[Al]$ and $[Ca^{2+}]/[H^+]$ ratios. Thus, the easier access to Ca^{2+} allowed the reinforcement of cellular junctions allowing reduced permeability. Finally, different studies have shown an increased number of chloride cells in gills as well as morphological changes in the apical surface of these chloride cells, in various species of fish exposed to acid water (Chevalier et al., 1985; Karlsson-Norrgren et al., 1986; Jagoe & Haines, 1990, 1997). For example, chloride cells can present apical wells (Leino & McCormick, 1984; Leino et al., 1987a, 1987b) or apical evaginations (Chevalier et al., 1985; Leino et al., 1987a, 1987b) increasing exchange surface. This kind of change which can limit and/or offset ion losses under acidic conditions, could explain the fast recovery of haemolymph Na^+ and Cl^- concentrations after a transfer back to circumneutral water.

250 However, to our knowledge, no study has highlighted such modifications in small
251 macroinvertebrate species.

252

253 **Extrapolation for populations**

254 In a previous study (Felten, 2003), populations of *G. fossarum* were sampled using a surber
255 sampler, on 10 occasions and in 3 sites of the same circumneutral stream (La Maix). This
256 stream was characterized by a decreasing gradient of acidification from upstream to
257 downstream (the most distant sites were 2.7 km apart). The most downstream site, studied in
258 this paper, was circumneutral throughout the year (mean pH : 7; from 6.7 to 7.4; n = 25). The
259 most upstream site was affected by episodic strong acidification events (mean pH: 6.0; from
260 4.3 to 6.80; n = 25) whereas slightly episodic stresses occurred in the intermediate site (mean
261 pH : 6.6; from 5.7 to 7; n = 25). As a result, *G. fossarum* was totally absent from the upper
262 site and, at the intermediate site, the density of the species was 10 times lower (443 ind./m^2)
263 that those observed downstream (4317 ind./m^2). Consequently, although *G. fossarum* was
264 able to rapidly compensate high ion losses caused by acid stress, gammarid population was
265 drastically affected by repeated episodic acid stress meaning that the rapid compensation of
266 high ion loss seems not to help the *G. fossarum* population in surviving acid episodes if these
267 are repeated.
268 Episodic acidification has been recognized to exert a drastic impact on macroinvertebrate and
269 fish populations and communities (Weatherley & Osmerod, 1991 ; Baker *et al*., 1996 ; Van
270 Sickle *et al*., 1996; Lepori *et al*., 2003). McCahon & Poulton (1991) and Merrett *et al.* (1991)
271 showed the cumulative effect of multiple acid stresses on macroinvertebrate mortality.
272 Finally, several studies conclude that acid episodes can restrict or offset the recovery process
273 of acid sensitive invertebrates (Kowalik *et al.*, 2006) and fish (Kroglund et al., 2001).

According to Davies *et al*. (1992), episodic acidifications take place over hours (rainfall) to months (snowmelt). In a previous experiment, Felten & Guérold (2005) showed that a 168h-exposure of *G. fossarum* to Gentil sapin streamwater led to 80 % of mortality which can explain the total absence of the specie in the upper site.

Several non exclusive hypotheses can conjointly explain the decrease of density or the loss of population in streams subjected to episodic acid stresses, such as *i*) an increase of drift-response and mortality of sensitive stages (juveniles) (McCahon & Poulton, 1991; Taylor *et al.*, 1994), *ii*) a lower food quality (Willoughby, 1988; Willoughby & Mappin, 1988; Sutcliffe & Hildrew, 1989) or/and a lower conversion efficiency of food to growth (Lee *et al.*, 1983; Hargeby & Petersen, 1988), *iii*) a lower food intake (Lemly & Smith, 1985; Tierney & Atema, 1986), *iv*) an enhanced energetic cost associated with osmoregulation, ion retention and respiration (Økland & Økland, 1986) leading to a decrease of growth and reproduction (Maltby, 1994; Seiler & Turner, 2004).

Moult is known to be a critical phase for crustaceans (Wright & Frain, 1981; McCahon & Pascoe, 1988; Wheatly & Gannon, 1995) requiring a lot of energy (Maltby, 1994; Wheatly & Gannon, 1995). According to Pöckl (1992), in younger gammarid stages, moult occurred close together. Consequently, a higher acid-sensitivity of younger stages (Naylor *et al*., 1990) explained by the moult frequency could partly account for population regression in acidified streams. Moreover, small-sized gammarids affected by an acid stress may be unable to resist to the associated hydrological stress.

Conclusion

G. fossarum facing acid stress fail to regulate ion losses, leading thus to an important mortality when the environmental stress is intense. This failure is rapidly reversible when the stress ceases. However, despite the recovery capacity of *G. fossarum* from important ion

losses following acid stress, populations have been severely reduced or have totally disappeared from numerous headwater streams draining catchments subject to acidification. Thus, it is suggested that both the frequency and the intensity of stresses clearly structure gammarid population. In this context, we recommend to conduct further studies dealing with the effects of episodic acidification on population structure, in relation to physiological parameters (e.g. energetic cost, growth) to better understand population regression and recovery. This kind of study is particularly important since the decline of base cations (mainly Ca^{2+} and Mg^{2+}) in soils and surface waters has been reported in most areas where high rates of sulphur depositions occurred previously. These trends should indeed lead to an increase of episodic acid stress frequency and intensity.

Acknowledgements. We would like to thank Crane Rogers for linguistic corrections of this manuscript.

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541 **Table 1.** Mean and SD values of chemical parameters of each exposure stream (n = 4). ANC:
542 Acid Neutralizing Capacity.

	La Maix		Ravine		Gentil Sapin	
	Mean	SD	Mean	SD	Mean	SD
pH	7,3	0,0	5,5	0,1	4,5	0,1
ANC (µeq l^{-1})	599,3	10,4	5,5	3,5	-19,4	6,5
conductivity (µS cm^{-1})	78,6	2,2	30,5	2,0	29,9	1,0
Temperature (°C)	10,3	0,3	11,9	0,5	12,9	0,5
Ca^{2+} (µmol l^{-1})	281,7	5,2	55,9	5,6	38,8	0,8
Mg^{2+} (µmol l^{-1})	187,1	4,7	35,7	2,4	25,9	1,3
Na^{+} (µmol l^{-1})	47,6	0,0	52,0	4,8	37,0	2,0
K^{+} (µmol l^{-1})	45,7	0,6	42,5	2,7	25,7	0,8
SO_4^{2-} (µmol l^{-1})	63,4	0,4	72,5	5,1	50,7	2,6
NO_3^{-} (µmol l^{-1})	62,8	1,0	49,6	5,5	80,3	3,4
Cl^{-} (µmol l^{-1})	38,7	1,2	42,2	2,5	32,1	1,4
Total Al (µmol l^{-1})	0,4	0,2	4,4	4,8	15,9	1,3

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558 **Table 2.** Summary of 2-way analysis of variance. F, F ratio; p, probability; d.f., degrees of
559 freedom; n.s., not significant.
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	[Cl^-] hemolymph			[Na^+] hemolymph			Survival		
	d.f.	F	p	d.f.	F	p	d.f.	F	p
a) Exposure effect									
Factor a (Stream)	2	34,1	**$< 10^{-3}$**	2	46,2	**$< 10^{-3}$**	2	110,4	**$< 10^{-3}$**
Factor b (Exposure time)	3	18,0	**$< 10^{-3}$**	3	23,0	**$< 10^{-3}$**	3	29,8	**$< 10^{-3}$**
Interaction	6	4,6	**$< 10^{-3}$**	6	6,7	**$< 10^{-3}$**	6	20,0	**$< 10^{-3}$**
b) Recovery effect									
Slightly acid exposure (Ravine)									
Factor a (Exposure time)	3	3,2	**$< 5*10^{-2}$**	3	1,7	**n.s.**	2	0,3	**n.s.**
Factor b (Recovery time)	5	14,1	**$< 10^{-3}$**	5	17,5	**$< 10^{-3}$**	5	2,9	**$< 5*10^{-2}$**
Interaction	15	2,0	**$< 5*10^{-2}$**	15	3,2	**$< 10^{-3}$**	10	0,3	**n.s.**
Strongly acid exposure (Gentil sapin)									
Factor a (Exposure time)	3	3,9	**$< 5*10^{-2}$**	3	0,5	**n.s.**	2	11,7	**$< 10^{-3}$**
Factor b (Recovery time)	5	37,3	**$< 10^{-3}$**	5	53,6	**$< 10^{-3}$**	5	0,7	**n.s.**
Interaction	15	5,2	**$< 10^{-3}$**	15	7,9	**$< 10^{-3}$**	10	0,7	**n.s.**

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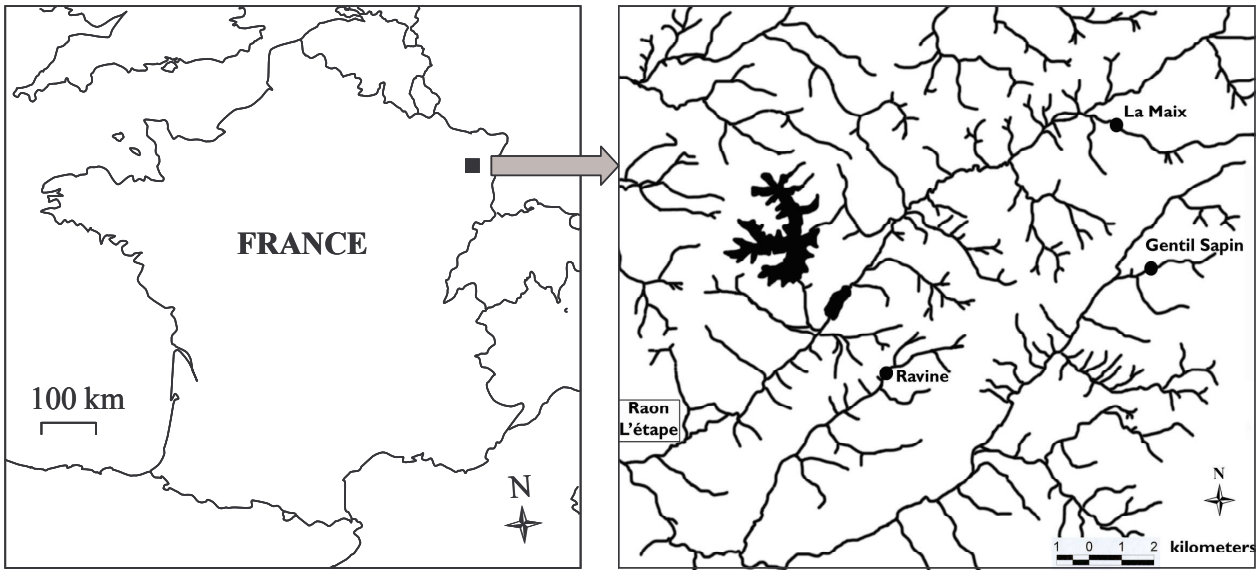

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574 **Figure 1.** Location of the 3 study sites in north-eastern France.

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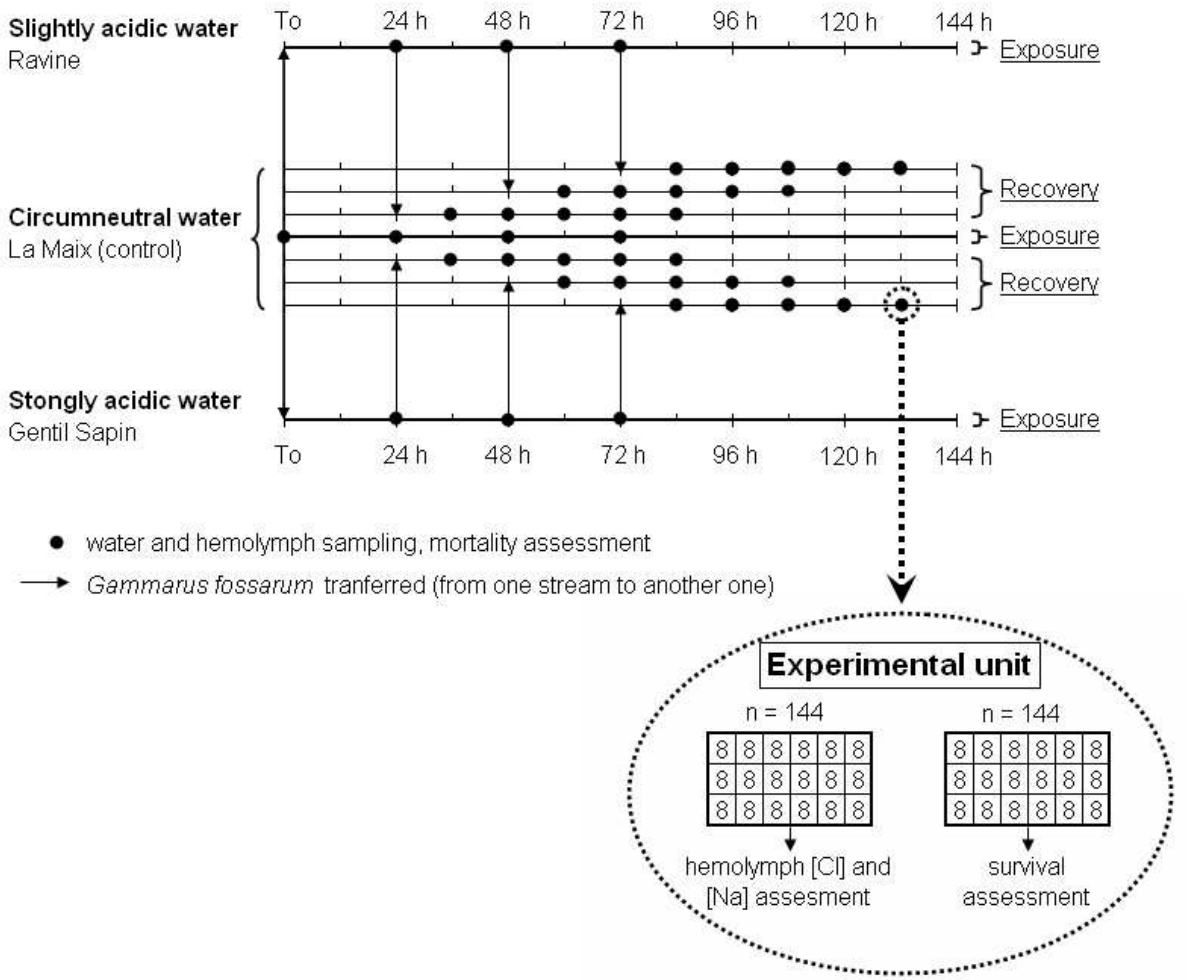

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591 **Figure 2.** Experimental design of the exposure and recovery experiment

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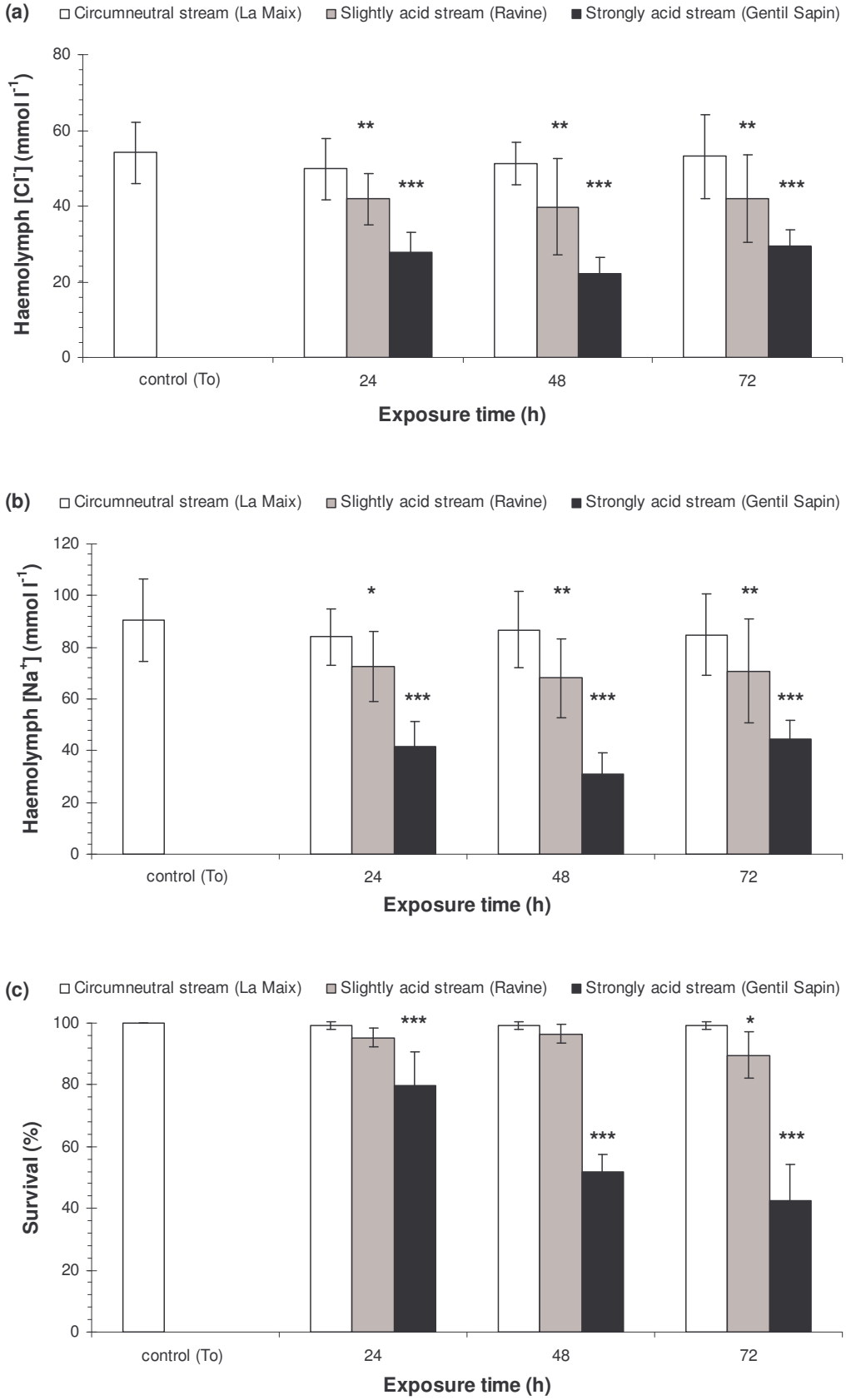

Figure 3. Mean (±SD) [Cl^-] haemolymph (a), [Na^+] haemolymph (b), and survival (c) of *Gammarus fossarum* exposed to circumneutral, slightly and strongly acidic streams waters. Significant differences against T_o are indicated by asterisks (Ficher's Least Significant Difference test; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$).

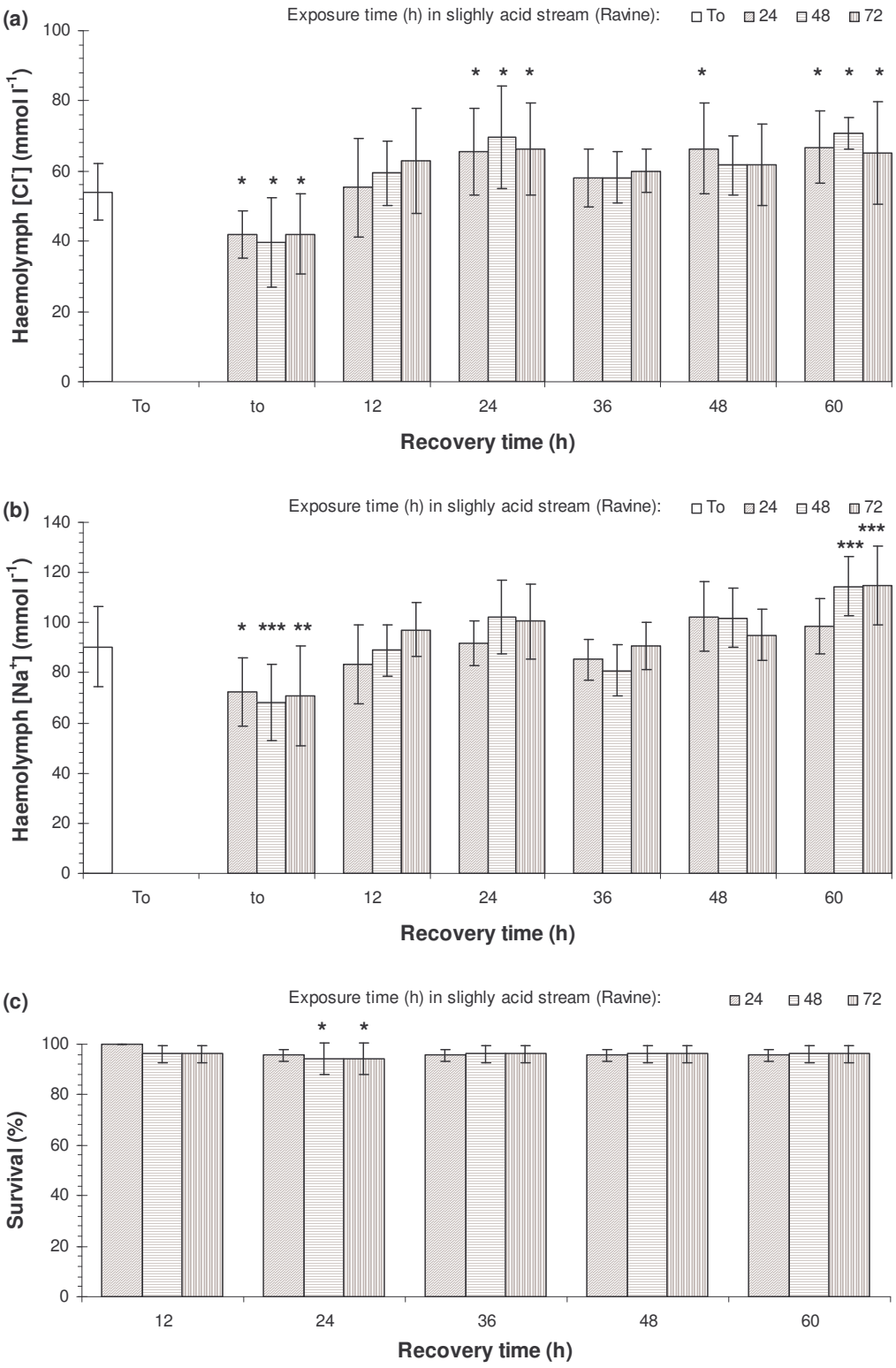

Figure 4. Mean (±SD) [Cl^-] haemolymph (a) and [Na^+] haemolymph, (b) and survival (c) of *Gammarus fossarum* exposed to slightly acidic waters (Ravine) and transferred in the circumneutral stream (La Maix) to test recovery capacity. Significant differences against T_o are indicated by asterisks (Ficher's Least Significant Difference test; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$). t_o represents the last exposure time before organism transfer in circumneutral stream.

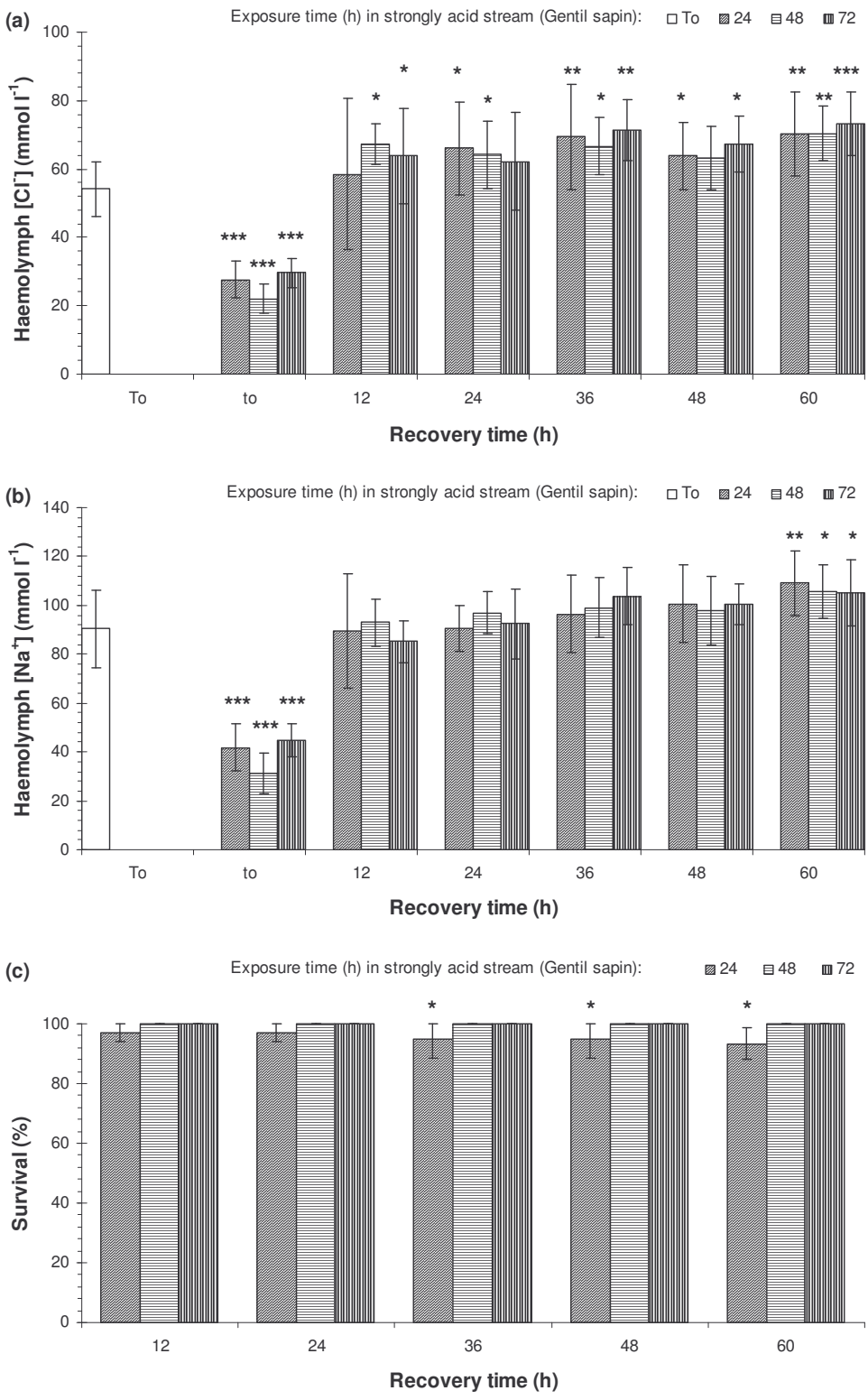

Figure 5. Mean (±SD) [Cl^-] haemolymph (a) and [Na^+] haemolymph, (b) and survival (c) of *Gammarus fossarum* exposed to strongly acidic waters (Gentil sapin) and transferred in the circumneutral stream (La Maix) to test recovery capacity. Significant differences against T_o are indicated by asterisks (Ficher's Least Significant Difference test; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$). t_o represents the last exposure time before organism transfer in circumneutral stream.