Is all salinity the same? I. The effect of ionic compositions on the salinity tolerance of five species of freshwater invertebrates.

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Abstract

Salts of marine origin, predominantly consisting of Na^+ and Cl^- ions are dominant in most Australian inland saline waters. The proportions of other ions, Ca^{2+}, Mg^{2+}, SO_4^{2-}, HCO_3^- and CO_3^{2-}, in the water may influence salinity tolerance of freshwater organisms and thus the effect of increasing salinity may vary with difference in ionic proportions. We exposed freshwater invertebrates to different concentrations of four ionic compositions and compared them to the commercial sea salt, Ocean Nature. They were: synthetic Ocean Nature (ONS) and three saline water types (ONS but without [1]: SO_4^{2-}, HCO_3^- and CO_3^{2-}, [2]: Ca^{2+}, HCO_3^- and CO_3^{2-}, [3]: Ca^{2+}, Mg^{2+}) which are considered to be the predominant saline water types in southeastern Australia and the Western Australian wheatbelt. The 96-h LC_{50} values for the five media were determined for six invertebrate species and sub-lethal responses were observed for two species. There were no differences between responses of invertebrates to various ionic compositions in acute toxicity tests. However in prolonged sub-lethal tests animals reacted differently in the various ionic compositions. The greatest effect was observed in water types lacking Ca for which plausible physiological mechanisms exist. Variation in ionic proportions should be taken into account when considering sub-lethal effects of salinity on freshwater invertebrates.

Keywords: salinity, ionic compositions, freshwater invertebrates, toxicity

Introduction

The salinization of freshwaters is a major environmental concern in all continents with large arid and semiarid regions, including Australia (Williams 1987). Recently attention has been given to the lethal (Berezina 2003, Kefford *et al*. 2003) and sub-lethal tolerance (Kefford and Nugegoda 2005a) of freshwater invertebrates to increased salinity, while other studies have experimentally considered effects of salinity on freshwater invertebrate communities (Neilsen *et al*. 2003, Marshall and Bailey 2004). All of these studies used artificial sea salts, the ionic proportion of which approximates seawater, because it is the most common composition of saline water bodies of southeastern Australia (Bayly and Williams 1973), which are sodium chloride (NaCl) dominated. However recently it has been acknowledged that there is some variation in the ionic proportion of NaCl-dominated inland saline waters of southeastern Australia (Radke *et al*. 2002, 2003). The three major saline water types existing in southeastern Australia (Radke *et al.* 2002), and the wheatbelt region of Western Australia (Pinder *et al*. 2005), were proposed by Drever (1982) and occur due to precipitation out of solution of specific minerals during evapoconcentration of saline waters and result in reductions in the relative concentrations of specific ions. If variations in ionic proportions in NaCl-dominated inland saline waters result in differing biological effects, then studies investigating the effects of saline water with a particular ionic proportions (such as seawater) may not accurately describe the effects of changes in salinity with differing ionic proportions. Consequently, we investigated whether these three common ionic proportions and artificial seawater altered lethal and sub-lethal effects of salinity on freshwater invertebrates. For the common ionic proportions we used the most extreme cases where specific ions are eliminated from a

saline water source, and therefore refer to the ionic compositions (presence/absence of specific ions), because if the absence of specific ions do not affect salinity tolerance then it is very unlikely that a reduction in the proportions of these ions would affect salinity tolerance.

Materials and methods

Test animals

Six species of freshwater invertebrates were used for acute 96-h LC_{50} toxicity testing (LC_{50} is the concentration of a toxicant lethal to 50% of a population). The protozoan *Paramecium caudatum* Ehrenberg and hydrozoan *Hydra oligactis* Pallas were purchased from Southern Biological, Nunawading, Victoria, Australia. Other species, collected from central Victoria, in the southern end of the Murray-Darling Basin were: gastropod *Physa acuta* Draparnaud (Campaspe River, at the Kyneton-Heathcote Rd. ($37^{o}23$'S $144^{o}31$'E)), caddis fly *Notalina fulva* Kimmins, water bug *Micronecta robusta* Hale and mayfly *Centroptilum* sp. (King Parrot Creek, a tributary of the Goulburn River, at Flowerdale ($37^{o}23$'S, $145^{o}16$'E). These specific species were chosen because they represent a wide range of different taxonomic groups found in freshwaters and were obtainable in sufficient numbers to experimentally expose to varying salinity and ionic composition treatments. Previous experiments where macroinvertebrate species have been collected from different sites or from the same site on different dates have shown no detectable difference in acute lethal salinity tolerance (Kefford *et al*. 2003, 2005, unpublished data). This is despite large differences in the acute lethal salinity tolerance between species. We thus assume that any difference in salinity tolerance or response to the different ionic compositions of

species obtained from different sources represent differences between the species tested rather than differing responses of animals collected from different sites.

Water quality data from collection sites are given in an auxiliary publication, Table 1.

Three species, *P. acuta, P. caudatum* and *H. oligactis* were used in chronic toxicity tests. The results for *P. acuta* will be presented elsewhere (Zalizniak *et al.* in prep). For hydra and paramecia the culture growth in different types of treatments was determined as the measure of sub-lethal toxicity and EC_{50} values calculated (EC_{50} being the concentration of a toxicant that produced the effect in 50% of population). For *H. oligactis* another sub-lethal end point, tentacle retraction, was used.

Preparation of solutions

Five different solutions were tested. Concentrated stock solution of around 40 mS/cm of Ocean Nature artificial sea salt (ON) (Aquasonic, Wauchope, NSW) was prepared in Milli-Q water and used in preparation of dilutions. Based on both the manufacturers claimed elemental composition and elemental analysis (ICP-MS) of Ocean Nature, 'Ocean Nature Synthesized' (ONS) was prepared from analytical grade reagents. Major ions and trace elements were considered (22 total), and their quantities calculated (see auxiliary publication, Table 2). ON was used as a standard to compare with previous investigations using this salt (Kefford *et al*. 2003, 2004a,b, 2005a,b), and ONS was used as control for possible effects of synthesized various ionic compositions. Based on ONS preparation three different ionic compositions were derived to reproduce the three major saline water types described in Radke *et al.* (2002): S1 had the same ionic composition as ONS except there was no sulphates (SO_4^{2-}) and no carbonates (HCO_3^- and CO_3^{2-}, referred to as alkalinity (Alk)); S2 was

without calcium (Ca^{2+}) and Alk, and S3 had Ca^{2+} and magnesium (Mg^{2+}) excluded (Fig. 1; also see auxiliary publication Table 2). Natural S1, S2 and S3 waters have some levels of the elements (see Radke 2002), which we excluded. We excluded them in the stock solutions to represent a worst-case scenario. The control and dilution water had enough of these excluded ions to allow high (>85%) survival. Where possible we tried to use carbon filtered Melbourne tap water (WLW) as our dilution water and control. However, lab cultures required specific media for their maintenance. For paramecia we used Lozina-Lozinsky medium (Lozina-Lozinsky 1931), and for hydras – M4 medium (Elendt and Bias 1990). Though M4 medium was designed for daphnids, prolonged culturing of hydra (over several months) using this medium was successful. These media served as culture media, dilution water and control in corresponding experiments. The analysis of major ions for some of these media is presented in auxiliary publication, Table 3.

Animal cultures

Brown hydra *H. oligactis* was fed daily with brine shrimps or juvenile *Daphnia carinata* (whatever available, since previous observations showed that the cultures survive equally well with either) *ad lib*. Medium was replaced three times a week.

For paramecia *P. caudatum* culturing technique was per Sazonova *et al.* (1997). Lozina-Lozinsky medium was boiled with 0.4 g L^{-1} of dry yeasts and cooled, and then inoculate of culture was introduced. After two days of acclimatising, animals were used for the experiment. Medium was replaced weekly or as necessary.

Animals collected from the field were transported from the site to the laboratory and transferred to the testing solutions as quickly as possible (as per Kefford *et al*. 2003, 2004a, 2005b).

Acute toxicity testing experimental protocols

There was no water replacement or feeding.

Hydras

The protocol published by Pollino and Holdway (1999) was used and is only briefly described. Non-budding hydras were used. To achieve this hydra were not fed for 1-2 days. Five concentrations of each salt type were used: 4, 6, 8, 10 and 12 mS cm^{-1} replicated 4 times in Petri dishes (∅ 54 mm), with 5 animals per replicate and 15 mL of test solution. Observations were made daily for 96 hours; deaths and tentacle retraction of hydras were recorded. For the tentacle retraction only two rankings were used: 'unaffected' being normal, and 'affected', which is any degree of shortening or disintegration (Pollino and Holdway 1999). It is not certain in the tulip stage if animals are truly dead; consequently at the end of experiment tulip stage animals were transferred to control solution for 48 hours. If animals recovered from the tulip stage, they were counted as alive.

Paramecia

Five concentrations of each salt type were used: 2, 4, 6, 8, and 10 mS cm^{-1} each with 10 animals per concentration held individually in 2-mL wells. Paramecia were fed with the suspension of yeasts (10 g L^{-1}) in Lozina-Lozinsky medium every second day (0.02 mL per well). Mortality and numbers present was recorded and LC_{50} was determined after 24, 48 and 96 hours of exposure.

Insects

The rapid toxicity testing method was used (Kefford *et al.* 2003, 2005b, in press). There were 10 animals of each species per treatment of 3L of water. Exposure concentrations were: *Centroptilum* sp.: WLW (EC≈0.13±0.01 mS cm^{-1}), 5, 10, 15 and 20 mS cm^{-1}, and other species: WLW, 10, 15, 20, 25 and 30 mS cm^{-1}. Observations were made daily for 96 hours.

Sub-lethal toxicity testing experimental protocols

Hydra

Experimental procedure was as per acute test (Pollino and Holdway 1999) and is only briefly described. However, budding hydras were used. To achieve this hydra were fed in excess for 4-6 days. Three concentrations of each salt type were used: 1, 2 and 4 mS cm^{-1}. After counting animals and observing tentacle retraction, animals were fed in excess with brine shrimps (0.2 mL per dish). After 1 hour all solutions were changed. All parameters for each day were calculated as the geometrical mean between new and old medium.

The mean relative growth rate of hydra for each treatment concentration was calculated as follows (Pollino and Holdway 1999):

$$K=(lnN_t-lnN_{t-1})/\Delta t$$

Where N_t is the number of animals at time *t,*

N_{t-1} the number of animals at time of previous observation

Δt time between two observations.

Paramecia

The experimental protocol is as per acute toxicity testing with paramecia. The culture growth rate (for individual animals) was calculated using a standard formula:

$$\mu = lnN/T$$

where N is number of animals in the well at time T

and T is time from the start of the experiment (days).

Statistics

For each species and treatment type, Probit regression models (see Agresti 1990) were fitted with the x-variable being EC and the y-variable the response variable (survival, population growth or tentacle contraction). From these regressions LC_{50} and EC_{50} values and their 95% confidence intervals were calculated for each treatment type. Post hoc comparison of EC_{50} values was performed using a paired t-test assuming unequal variances.

Results

Acute tests

For all species examined there were no statistically significant differences in their 96-h LC_{50} values for the different types of treatments (Table 1). The results for *Centroptilum* sp. (Table 1) are, however, somewhat inconclusive. For treatments other than ON over 96 hours of exposure, they had partial but < 50 % mortality at the lowest salinity treatment, 5 mS cm^{-1}, consequently their 96-h LC_{50} value is below 5 mS cm^{-1} for all types of treatments except ON. Since concentrations below 5 mS cm^{-1} were not tested in this experiment, the error in LC_{50} calculation is higher than for the other species and thus there is a greater probability of a type 2 error. Across the three species, however, there would appear to be no detectable effect of the different saline water types on acute survival of freshwater invertebrates tested.

Sub-lethal tests

Though there are differences in tentacle retraction of hydra at 24 hours, they were eliminated at 72 hours (Fig. 2). The EC_{50} for S3 salt type initially increased then later decreased. Thus the hydras appear to adapt to their environment when the initial shock is reduced, and they can return to their 'normal' condition. Interestingly 24- and 48-h EC_{50} for S1 seemed higher (though not statistically significant) than all the others. It may be that sulphates are more toxic to hydra than chlorides and eliminating them results in a marginally reduced overall toxicity.

Hydra culture growth was partially affected by the variation in ionic compositions (Fig. 3). Ninety six-hour EC_{50} value for S2 treatment was significantly lower than for the ONS and S3 types of treatments.

The population growth of the paramecia was significantly reduced (Fig. 4), when Ca was eliminated from the media (S2 and S3 types).

Discussion

General observations

There were no significant differences in toxicity between ON, ONS and S1 (no sulphates and alkalinity) treatments in any of the experiments. While it was expected with ON and ONS, it also indicated that removal of SO_4^{2-} and Alk did not change the toxicity of salinity in any detectable way. The proportion of these anions is around 13% of the total anions load in ONS, the rest being mostly Cl^-. When S1 and S2 treatments were prepared these anions were replaced with Cl^-, thus increasing its load. Kefford *et al.* (2004a) observed that ON was less toxic to freshwater invertebrates than pure NaCl. The lack of a difference in toxicity between ON, ONS and S1 may

indicate that the difference in toxicity between ON and NaCl is not because of Cl^- toxicity, but rather lack or difficulty in extraction at high salinity of essential and trace elements, such as calcium, potassium, copper, selenium etc. 24- and 48-h EC_{50} for hydra's tentacle retraction in S1 were slightly higher than in other treatments. We did not specifically test toxicity of Cl^- against SO_4^{2-}, but other studies with a range of freshwater invertebrate taxa indicate that Na_2SO_4 is more toxic than NaCl (Goetsch and Palmer 1987; Kefford *et al*. 2004a; Palmer *et al*. 2004) and that NaCl is more toxic than ON (Kefford *et al.* 2004a). It would therefore appear that SO_4^{2-} is more toxic than Cl^-. The replacing of SO_4^{2-} with Cl^- could thus have slightly reduced the overall toxicity to hydra.

The results regarding treatments with Ca deficiencies are discussed in detail below.

Acute tests

Short-term acute toxicity testing is usually conducted in sub-optimal conditions for animals tested: static water regime and no food supply. Though these tests convey very useful information on the range of tolerance of the animals to a particular toxicant, which can be very useful in modelling and management on a wider scale, they give very little information on the mechanisms of action or the effects of a toxicant to organisms subject to long exposures and low sub-lethal concentrations. These experiments are therefore usually regarded as a starting point for more detailed long-term sub-lethal exposures, from which one can get more definite information on the effects of a particular toxicant. Though both species were clearly affected by the different ionic compositions in our sub-lethal experiments, it was not so in the acute tests (Table 1). In a short-term exposure with lethal concentrations of salinity, the

different ionic compositions had no detectable effect. Osmoregulatory mechanisms may have played a major part in combating the effects of high salinity, rather than fine-tuned biochemical and physiological interactions. Chapman *et al.* (2000) found that there were no differences in the survival or swim-up fry toxicity tests (96-h exposure) of rainbow trout embryos in two saline effluents with different ionic proportions. However they found that chironomid larvae grew differently in the different effluent (10-d exposure). The same results were obtained for sulphates-dominated saline lakes in the USA (Dickerson *et al.* 1996). Though the researchers stated that undiluted lake water was toxic to *Ceriodaphnia dubia* and attributed this to the differences in ionic composition of major ions, when we recalculated LC_{50} (% of dilution) provided by the authors, the LC_{50} in terms of electrical conductivities were surprisingly similar and not significantly different for *C. dubia* (except in very saline waters) and fathead minnows. These studies and our results consistently indicate that the short-term lethal toxicity of saline solutions found in nature is not generally affected by different ionic proportion/composition, but longer exposures or sub-lethal effects can reveal the differences. Salinity produced from pure salts (e.g. NaCl, Na_2SO_4) and one to one ratio of pure salts, neither of which occur in nature, however, do have differing toxicity to that of mixtures of salts (Mount *et al*. 1997, Kefford *et al*. 2004a, Palmer *et al*. 2004).

Sub-lethal tests

There could be several explanations regarding the chronic sub-lethal effects of varying ionic compositions:

(1) Direct effect of deficiency of the essential element Ca.

(2) Indirect effect of hardness cations (Ca^{2+}, Mg^{2+}) and carbonates on the biochemistry of the trace-metals.

Direct effects of deficiencies in Ca

Effects on paramecia

Paramecia have around 5000 cilia. Movement of the cilia is controlled by their membrane potential. Stimulation of cilia (chemically or physically) activates a voltage-sensitive Ca^{2+} current associated with the ciliary membrane (Preston and Hammond 1998). This results in avoidance behaviour, making paramecia swim backward (Preston *et al.* 1992). Nakaoka and Ooi (1985) found that in the presence of ATP as a stimulus in the medium, paramecia swim forward if Ca^{2+} concentration is below 10^{-6} M (40 μg L^{-1}) and backward if it is higher than 10^{-6} M. This suggests that, though directional swimming is governed by the *intracellular* Ca^{2+} concentration, a minimum amount of calcium in medium is required to maintain normal responses to stimuli. Slightly proportionally higher concentrations of trace metals in S2 and S3 (especially at higher salinities) might have affected animals, but lack of calcium in these media did not allow them to respond adequately. In the case of acute toxicity (Table 1) the differences between various ionic composition types were not evident possibly because short-term effect of higher salinity *per se* was greater than the effect of ionic composition of media, making osmoregulatory mechanism primarily responsible for mortality. At lower salinities in sub-lethal exposures calcium deficiencies might play a greater part in paramecia swimming behaviour, thus making animals in Ca^{2+}-lacking media more prone to abnormal behaviour, and consequently expending more energy. In addition morphogenesis of the complex cell surface during mitosis involves transcellular wave signal, which involves cortical alveoli that

act as Ca reservoir in the cell (Laurent and Fleury 1995). Presumably if there were not enough Ca to initiate the signal, mitosis would be abnormal.

Effect on hydra

External Ca^{2+} ions play a major role in the nematocyst discharge in hydrozoans (Salleo *et al.* 1994a,b, Yanagita 1973, McKay and Anderson 1988; cited in Kawaii *et al.* 1999). Santoro and Salleo (1991) observed that nematocytes do not discharge in Ca^{2+}-free medium, and that La^{3+}, Cd^{2+} and Co^{2+} prevented discharge by blocking the Ca^{2+} channel even when some Ca^{2+} is present. Gitter *et al.* (1994) found that discharge of the stenoteles (a type of nematocyst) in *Hydra vulgaris* is regulated by a mechanism, allowing intake of Ca^{2+} from ambient solution. This may explain why hydras were not affected in the acute toxicity test (involving no feeding and therefore no nematocysts discharge) (Table 1), but were growing slower in sub-lethal test (where nematocysts were discharged to capture their prey) in the S2 treatment compared to ONS control (Fig. 3). As there was some Ca^{2+} present in the M4 medium, which was used as control and dilution water for the range of salinities prepared, at higher salinities the effect of blocking Ca^{2+} by increasing concentrations of Co^{2+} and Ni^{2+} (see Auxiliary publication, Table 2) may have begun to play a role. Kawaii *et al*. (1999) reported that Mg^{2+} also had an inhibitory effect on *atrichous isorhiza* (a type of nematocyst) discharge, and that the inhibitory effect of Mg^{2+} increased when the external concentration of Ca^{2+} was lowered. This might explain why the S2 type affected sub-lethal salinity tolerance in hydra. S3 type medium, though lacking Ca^{2+}, may not affect hydra as much as an S2 type (Fig. 3) because it also lacked a powerful Ca^{2+} blocker i.e. Mg^{2+}.

Freshwater hydras reproduce by means of forming buds and developing a foot at the base of a bud and then detaching from the parent. A separated bud was counted as a new animal in our experiments. Zeretzke *at al.* (2002) found that in *Hydra vulgaris* (Zurich strain) foot formation was prevented by lowered concentrations of ambient Ca^{2+}, making animals form branches, that persisted on parent's body instead. It would definitely affect the culture growth in our study, as the number of separate individuals has not increased.

Increased toxicity of trace metals

Water quality parameters such as hardness and alkalinity can influence the interactions of ions in the ambient solution. Increases in hardness have shown to result in decreased copper toxicity to fish (Pagenkopf 1983) and cladocerans *Daphnia magna* (Schamphelaere and Janssen 2002) as a result of competition between the hardness metals (Ca, Mg) and trace-metal species for interaction sites. Welsh *et al.* (2000) also showed that acute copper toxicity was lower in waters containing proportionately more Ca. They also indicated that Ca is more important than Mg in modifying the toxicity of Cu in rainbow trout and chinook salmon. The same applies to uptake of zinc by rainbow trout (Alsop and Wood 1999) and *D. magna* (Heijerick *et al.* 2001), cadmium by *D. magna* (Penttinen *et al.* 1998) and the amphipod *Hyalella azteca* (Jackson *et al.* 2000), and manganese by brown trout (Stubblefield *et al.* 1997) in the presence of competing Ca^{2+} ions. All water types used in our study contained essential and trace metals Fe, Mn, Cu, Zn, Mo, Se, Li, Sr, Br, Rb, Co, V, Ni (auxiliary publication, Table 2) that at elevated concentrations can be toxic to aquatic invertebrates. Though the concentration of each trace element was very low, a combined load might be significant in the absence of calcium. Elimination of Ca

and/or Mg out of the solution can result, first, in the relative increase of concentrations of trace elements, especially at higher salinities, and second, in increased toxicity of these elements because in the absence of Ca and/or Mg more sites are available for binding at the organism-water interface. The hypothesis of increased trace metals toxicity in Ca^{2+} lacking media remains to be tested.

Metal toxicity can also be reduced by complexation with carbonate, thus decreasing the activity of free hydrated metal ions (Barata *et al.* 1998).

Conclusions

Variation in ionic compositions common in saline inland waters of southeastern Australia did not affect acute lethal salinity tolerance of any species investigated. However the different ionic compositions affected the three sub-lethal responses of investigated species. The water types lacking Ca had sub-lethally most deleterious effects on the animals. The different responses of invertebrates to various ionic composition types in combination with the sub-lethal range of salinity may be governed by deficiencies of Ca, the chemical interaction of hardness cations, alkalinity and trace metal uptake and toxicity.

In assessing the effects of salinity on freshwater invertebrates the ionic proportions should be considered in salinity exposures that are likely to induce sub-lethal effects.

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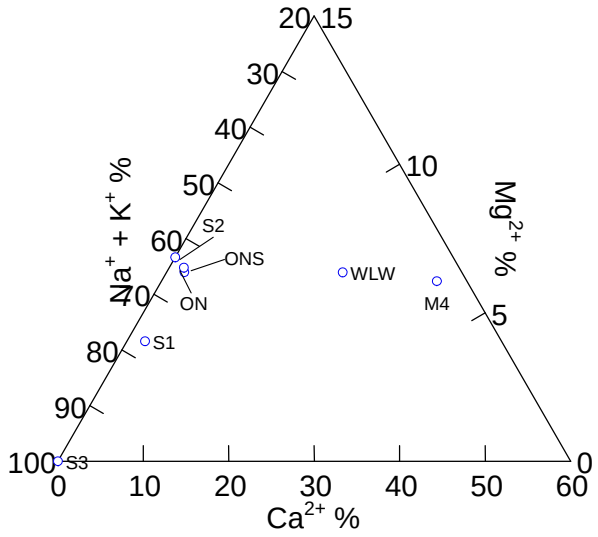
Table 1. The LC_{50} values for animal species tested in acute 96-h experiments.

Species	Type of treatment	LC_{50} values (95% confidence intervals)			
		24-h	48-h	72-h	96-h
P. caudatum	ON	8.70 (7.81-9.67)	8.70 (7.81-9.67)	NM	8.70 (7.81-9.67)
	ONS	8.66 (7.77-9.62)	8.66 (7.77-9.62)	NM	8.66 (7.77-9.62)
	S1	9.10 (8.17-10.17)	8.85 (7.93-9.88)	NM	8.85 (7.93-9.88)
	S2	7.24 (6.24-7.82)	7.24 (6.24-7.82)	NM	7.24 (6.24-7.82)
	S3	7.58 (6.65-8.40)	7.38 (6.47-8.15)	NM	7.38 (6.47-8.15)
H. oligactis	ON	8.95 (8.50-9.48)	8.75 (8.33-9.32)	8.56 (8.15-9.22)	8.37 (7.91-9.21)
	ONS	9.08 (8.60-9.57)	8.79 (8.37-9.30)	8.61 (8.20-9.15)	8.35 (7.90-8.96)
	S1	9.09 (8.63-9.57)	9.09 (8.63-9.57)	8.90 (8.47-9.40)	8.81 (8.39-9.32)
	S2	9.12 (8.66-9.58)	8.86 (8.43-9.32)	8.86 (8.43-9.32)	8.53 (8.07-9.00)
	S3	9.10 (8.63-9.57)	9.10 (8.63-9.57)	8.92 (8.49-9.39)	8.33 (7.76-8.84)
N. fulva	ON	NC	40.55	22.96 (20.32-25.56)	18.46 (16.10-20.94)
	ONS	NC	33.03 (28.91-119.74)	28.17 (24.32-37.90)	19.58 (7.93-23.90)
	S1	NC	32.83	22.55 (19.85-25.55)	18.64 (15.89-21.14)
	S2	60.18	29.51 (26.74-38.27)	24.20	17.97 (14.18-21.54)
	S3	97.46	23.66 (21.28-26.91)	18.27 (15.64-21.04)	15.69 (13.21-17.92)
M. robusta	ON	21.44	19.08	14.51 (-8.27-24.92)	13.44 (-99.73-32.06)
	ONS	25.15 (23.17-27.54)	21.45	18.70	10.51
	S1	29.71 (25.42-45.73)	23.67	17.90	15.78 (2.96-26.14)
	S2	27.61 (25.57-29.67)	23.76	18.02	16.46
	S3	24.15 (15.81-83.88)	19.01	14.11	11.22 (6.65-14.60)
Centroptilum sp.	ON	14.94 (12.58-16.97)	9.25	6.60	5.58
	ONS	13.61	6.33 (2.70-9.26)	2.46 (-3.49-5.33)	1.75 (-3.58-4.21)
	S1	14.37 (12.42-16.76)	10.24	6.57	4.63
	S2	11.32 (8.91-13.49)	7.89	5.17	3.79
	S3	10.19 (7.75-12.53)	6.38 (3.71-8.94)	4.11 (0.07-6.89)	3.57 (-0.95-6.36)

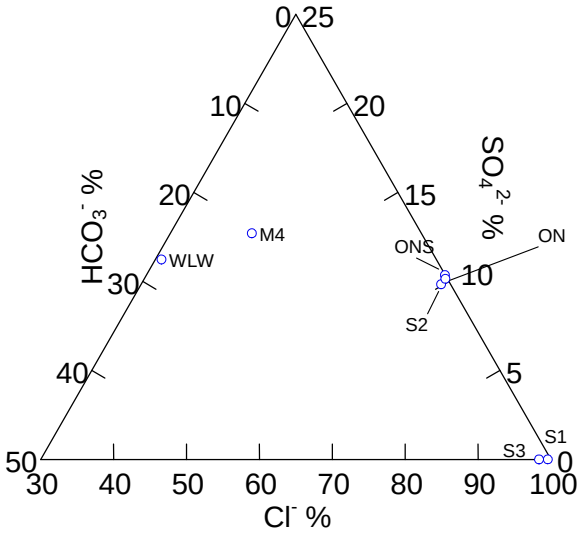
NM – not measured, NC – not calculated (100% survival in all concentrations). For some values CI could not be calculated.

1 a)



2

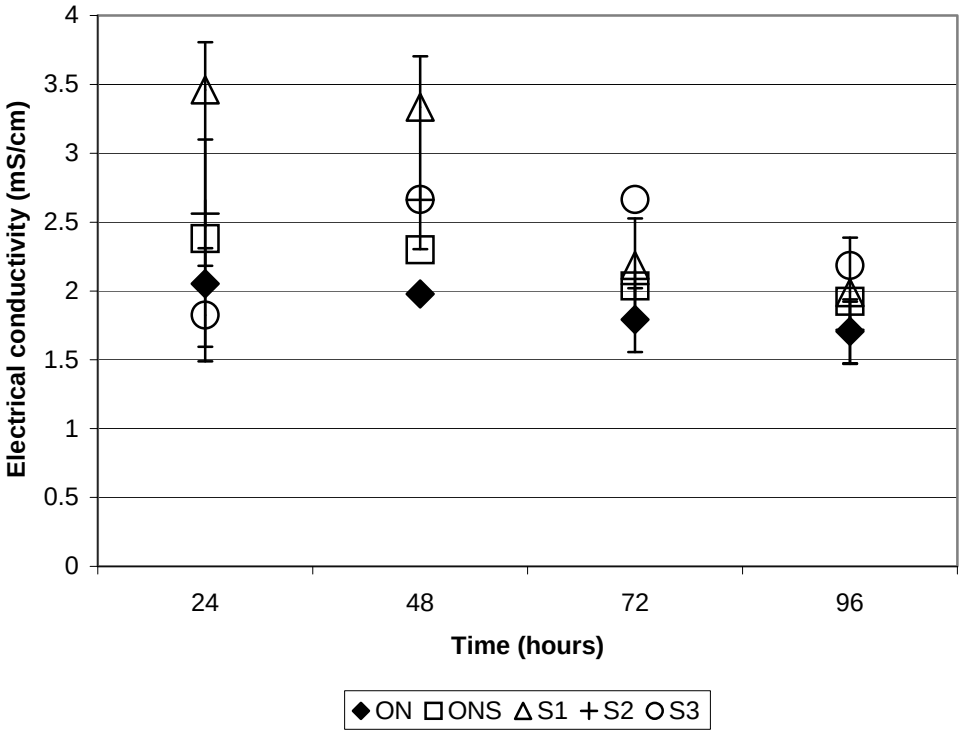
3 b)



4

5 Fig. 1. Measured ionic proportions of the various saline water types, media (M4) and
6 WLW (see auxiliary publication Table 3 for raw data): a) cations and b) anions as a
7 percentage of the total major cations/anions on a mass to volume basis.

1



2 Fig. 2. Values of EC_{50} (tentacle retraction) for *H. oligactis* in different types of

3 treatment (error bars indicate 95% CI).

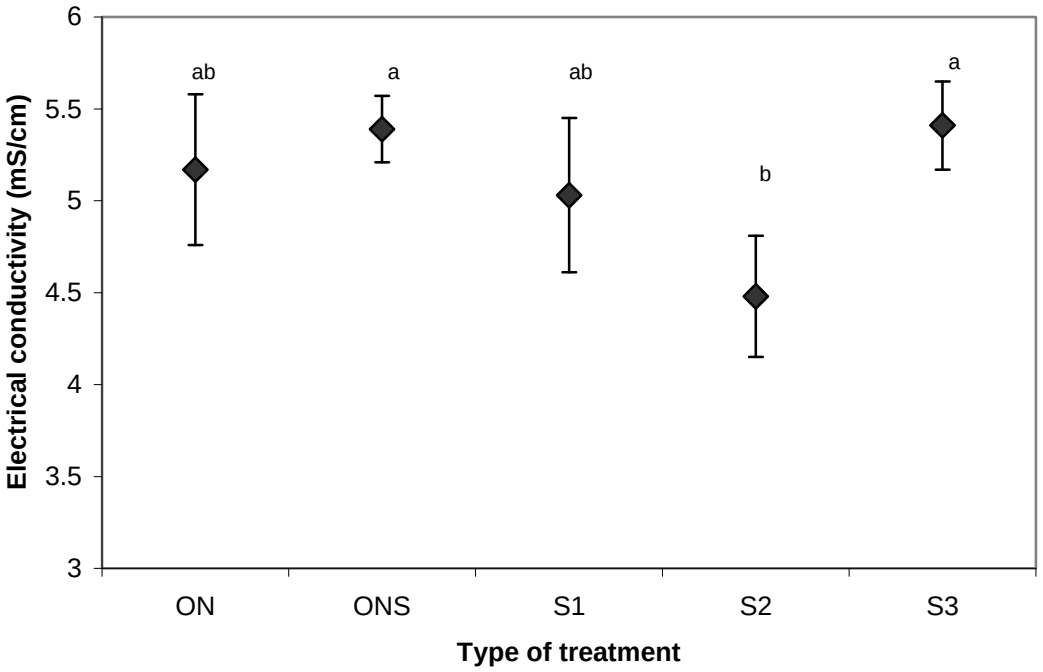
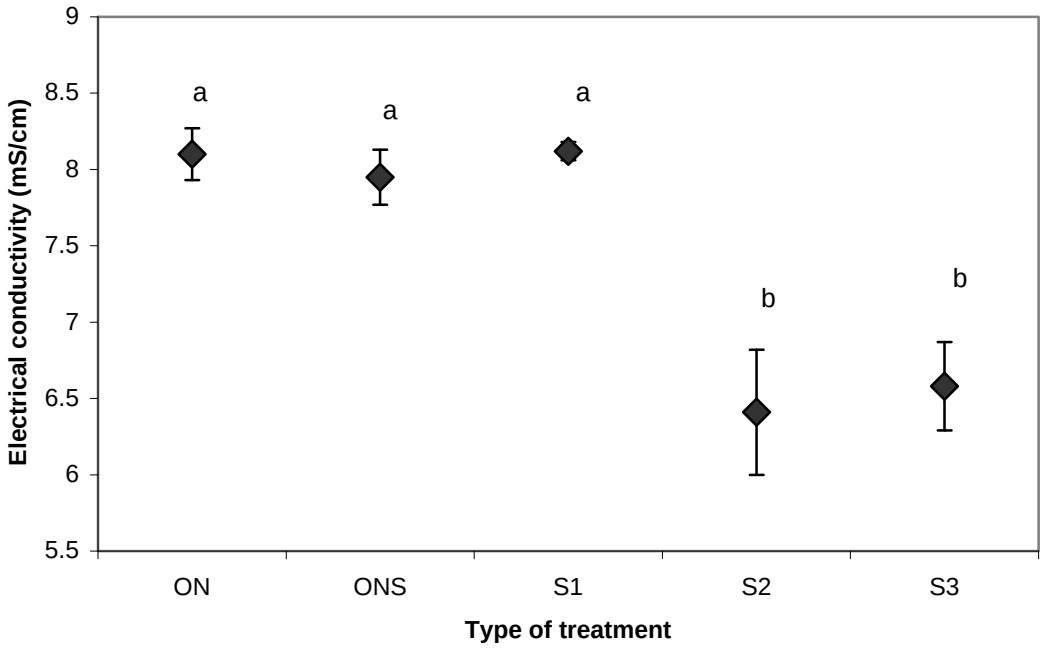


Fig. 3. Ninety six-hour EC_{50} values (culture growth) for *H. oligactis* in different types of treatment (Mean±SE, N=4). Different letters represent significantly different results.



1 Fig.4. Ninety six-hour EC_{50} values (culture growth) for *P. caudatum* in different types
2 of treatment (Mean±SE, N=10). Different letters represent significantly different
3 results.