



Review

Methods for biological assessment of salt-loaded running waters – fundamentals, current positions and perspectives

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ABSTRACT

Salinisation of running waters is a severe problem in many parts of the world. Monitoring and management of such waters require ecological methods which consider the hydrochemical effects of salinisation on the aquatic communities in order to set targets to protect habitats and biodiversity. Several bioassays have been developed for this purpose and are surveyed here. They are based on the salt sensitivity of the following groups of organisms: diatoms, ciliates and macroinvertebrates. In this paper experiences gained so far are also considered as well as practical applications originating from this research.

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Fundamentals

Chemical basics of salinity of inland rivers

Salinity is a major characteristic of all waters. It is an abiotic factor which markedly influences the living conditions of

aquatic organisms (Schönborn 2003). Fresh waters are typically calcium hydrogen carbonate waters (standard ion combination, Rohde 1949). Depending on the geological conditions prevailing in the catchment area, it is distinguished between (i) siliceous waters which are poor in electrolytes and (ii) carbonate waters rich in electrolytes (Braukmann 1987; Schönborn 2003).

The term “salinity” is usually used for the total concentration of dissolved inorganic ions in the water, namely Na⁺, K⁺, Mg²⁺, Ca²⁺, Cl⁻, SO₄²⁻ and HCO₃⁻. It is specified as mgL⁻¹, gL⁻¹ and gkg⁻¹, respectively. Another summarising measure often used is the elec-

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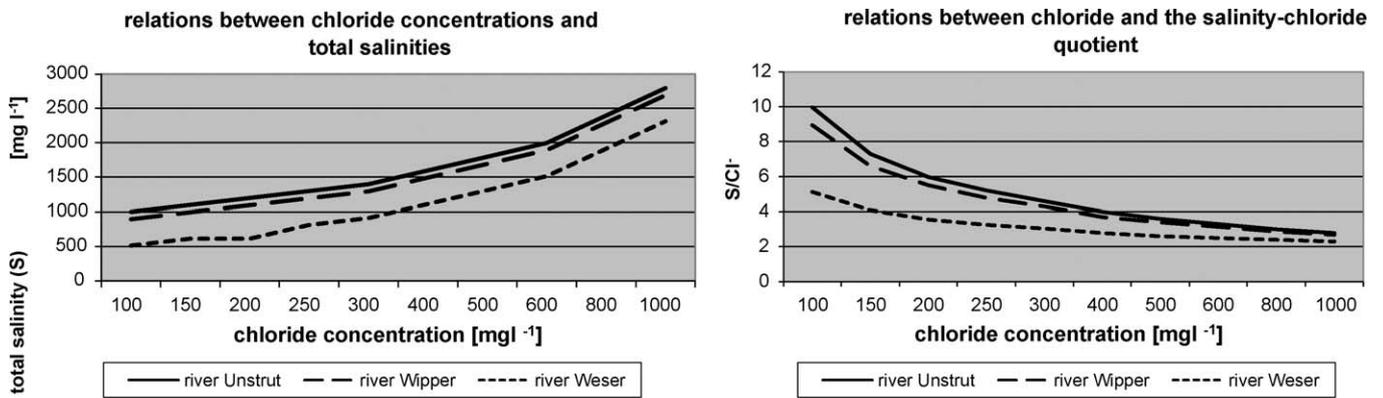


Fig. 1. (a) Relations between chloride concentrations and total salinity (S) as found for the rivers Wipper, Unstrut and Weser (Germany). (b) Relations between chloride concentrations and the total salinity chloride quotient (S/Cl⁻) as found for the rivers Wipper, Unstrut and Weser (Germany).

trical conductivity (EC). It is standardised to 25 °C and expressed as μS cm⁻¹ or mS cm⁻¹. Since the ion proportions of inland salt waters differ considerably (Albrecht 1954; Schmitz 1959), a categorization based on salt concentration only may be problematical. Therefore the different proportions of further ions are sometimes used (Hedgepeth 1959).

Typical salt waters may be characterised as alkaline salt waters. According to the degree of salinisation, ion proportions change continually from fresh to salt water what may influence the biological effect of salinisation (Ziemann 1997a). During this change the chemical character of the water also shifts from calcium-hydrogen-carbonate to alkali-chloride-dominated.

Although the degree of salinisation of inland waters is usually characterised by the chloride concentration, it is not possible to conclude the total salt concentration from the chloride concentration. However, from the ratio between total salinity (or electrical conductivity) and chloride concentration the geochemical type of the water and a given salinisation can be deduced (Table 1, from Ziemann 1997b).

Chloride concentrations exceeding ~50–60 mg L⁻¹ and ratios <12–15 point at types of waters which deviate from the basic limnic type. Fig. 1a and b shows the changes of the salinity/Cl⁻ ratio occurring with increasing salinity when comparing several waters in Thuringia (Central Germany). The ratio decreases with increasing chloride concentrations, reaching a value around 2 in strongly salinised waters independently of the basis. Fig. 1a and b also illustrates the differences in aquatic chemistry between the rivers Unstrut and Wipper (Thuringia, Germany) on the one hand with their high proportions of calcium sulfate in comparison to other rivers of this region (e.g., rivers Werra and Weser) on the other hand. Here, concentrations are considerably lower. As a consequence, in the rivers Werra and Weser sodium chloride dominates already at distinctly lower total salt concentrations than in the rivers Unstrut and Wipper (Ziemann 1967).

The P-value

The so-called “P-value” is a quantification of the combined effect of salt concentration and ion proportion, calculating the product of

Table 1
Ratios between total salinity and conductivity, respectively, and chloride concentrations of some common chemical types of waters according to data from Thuringian running waters (Germany).

Ratio total salinity/Cl ⁻	Ratio conductivity/Cl ⁻	Chemical type of waters
>8...<12	>10...<15	Siliceous waters
>12	>15	Carbonate waters
<4	<6	Salinised waters (alkali-chloride waters)

total salinity (S, in g L⁻¹) and the percentage the alkali metal ions contribute to the sum of alkali metal and alkaline earth metal ions (in mval L⁻¹, Ziemann 1981, 1997a):

$$P = \frac{(\text{Na}^+ + \text{K}^+) \times 100}{\text{Na}^+ + \text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+}} \times S \tag{1}$$

As demonstrated by Ziemann (1981, 1997a), this relation holds good for fresh water and a transition zone up to the border of salinised water where alkaline ions dominate over calcium ions. Their antagonistic activity is thus not effective any more. This is the case if the ratio Na⁺/Ca²⁺ (in mval L⁻¹) is >1, corresponding to a P-value between 80 and 100. A close correlative connection exists between the P-value and the chloride concentrations up to about 1000 mg L⁻¹. Thus it seems to be possible to estimate the ecological impact of salinity by means of the chloride concentration without the time-consuming determination of the P-value (Ziemann 1997b, Fig. 2.)

Biotoxic effects of salinised waters

The biotoxic effects of salinisation are usually due to (i) the osmotic effects of the total salt concentration and (ii) to the ion proportions (Schönborn 2003; Ziemann 1971). In general, the predominating ion concentration is correlated with salinity and changes when salinity increases or decreases. Often salt waters are found to contain comparatively increased proportions of single ions such as Mg²⁺ and SO₄²⁻ which modify the biological effect of these waters in comparison to those of typical alkali-chloride waters (Schönborn 2003; Ziemann 1967). In some cases, special toxic effects may occur due to a surplus of single ions such as K⁺ originating, for example, from brines from potash works or sodium

correlation between chloride concentration and P-value

$$P = 5,044 + 0,131 \text{ Cl}$$

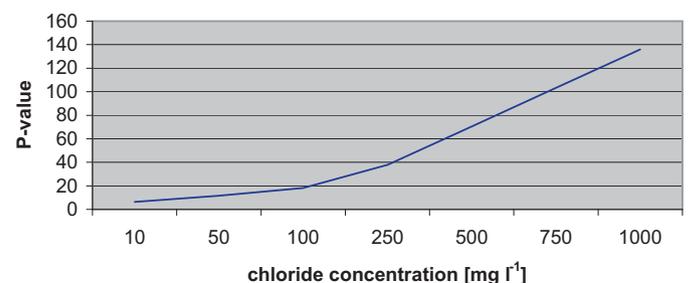


Fig. 2. Correlation between chloride concentrations and P-value.

carbonate waters (Albrecht 1954; Buhse 1989; Ziemann 1971). As recorded by Albrecht (1984b), the $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ratio should be >1 which crucially favours conditions of living. Ratios below this value may cause toxic effects for members of the aquatic community. However, due to the considerably varying ion proportions, no general relation between total salinity (S) and its biological effects can be given with regard to inland waters.

Terminology: primary and secondary salinisation; thalassogenic and athalassogenic waters

In contrast to coastal brackish waters whose salinity is markedly influenced by seawater, salinisation of inland waters may be of a very different origin. In general, it is often distinguished between primary (natural) and secondary (anthropogenic) salinisation (Schönborn 2003). Natural causes of salinisation are, for example, salt springs from salt mines and high irrigation rates in arid areas. In contrast to this, anthropogenic salinisation is due to human activities such as the introduction of salt-containing effluents from salt mining, brine baths, special industries, irrigation and use of road salts. Due to human activities, secondary salinisation has developed to one of the most important anthropogenic surface water contaminations in arid and semi-arid climates (Williams 1999).

Another approach differentiates between thalassogenic (=marine) and athalassogenic (=non-marine) waters. The term "athalassogenic" was introduced by Bayly (1967, 1969) to distinguish inland salt waters from thalassogenic brackish waters based on the fact that due to the various origin of the salts, their composition may differ considerably among different waters. Moreover, besides the various proportions single ions contribute to the total amount of salt, seasonal changes in salinity as well as hydrographic conditions (springs, brooks, rivers, lakes) induce settlement by different aquatic organisms and thus distinct aquatic communities develop which are distinct between marine habitats and those of inland salt waters. For example, freshwater fauna consists primarily of specifically adapted species of limnetic origin which in turn are absent from marine brackish waters (Williams 1981). This has also been found for diatoms (Jahn and Wendker 1987).

Methods measuring the biological effects of salinisation

Salinity is reflected by the composition of the running water aquatic communities (Mayenco 1993; Piscart et al. 2005, 2006; Remane 1971; Schulz 2000; Schulz and Bellstedt 2000; Short et al. 1991; Velasco et al. 2006). To document and to quantify this, several methods have been developed and become part of monitoring programmes. In Australia, e.g., comprehensive investigations on toxicological basis were performed (Kefford et al. 2003, 2004a, 2007a,b). Since many of the salinised waters are NaCl-dominated here, the structures of benthic aquatic communities of many rivers have been analysed and $\text{LC}_{(50)}$ -values have been determined (Kefford et al. 2003). In these cases, factors such as overirrigation and dryland salinisation had led to increasing salinities of rivers by the time. Also a procedure for ascertaining targets for protecting surface waters has been developed (Dunlop and McGregor 2007; Hart et al. 2003; Kefford et al. 2007b; Sim et al. 2007).

In Germany, especially (i) the Halobion Index (Ziemann 1971, 1999) and (ii) the ciliate-based salinity indication method (Nolting and Rustige 1999) were developed. Different from Australia, the main intention of these indications was to evaluate the ecological consequences of introducing salt brines from potash mining works into rivers in order to document the changes in aquatic ecology taking place after termination of potash mining and to set targets for river protection.

Table 2
The Halobion System.

1. Polyhalobic diatom species: salinity $S \geq 30$ (Euryhaline species may undergo $S = 30$)
2. Mesohalobic diatom species:
 - (a) Euryhaline mesohalobic diatom species: $S = 0.2-30$
 - (b) α -Mesohalobic diatom species: minimum NaCl required about $S = 10$
 - (c) β -Mesohalobic diatom species: $S = 0.2-10$
3. Oligohalobic diatom species:
 - (a) Halophilic diatom species
 - (b) Indifferent diatom species
4. Halophobic (haloxenic) diatom species

Diatom-based methods

Sensitivity of diatoms towards salinity and the specific conductance index

Diatom assemblages are well-known to be a suitable basis for the categorization of inland salt waters according to the biological effects of salinity (Hustedt 1957; Ziemann 1971). The close connection between the occurrence of certain diatom species and the salinity of the water body had already been described by Heiden (1902). He divided the diatom taxa into 7 ecologically characterised groups considering also their salinity requirements. Kolbe (1927) established the Halobion System which was developed later on by Hustedt (1957) and Simonsen (1962) see Table 2.

Briefly, this system is based on the finding that there are specific indicative diatom taxa for all levels of salinity (Ziemann 1999). Consequently, different diatom assemblages are to be expected which are adapted to the actual salinity. This was confirmed later by further investigations (Blinn 1993, 1995; Blinn and Bailey 2001; Blinn et al. 2004). On the basis of frequencies of the single species in waters of different salinities, Blinn (1993) proposed an autecological characterisation by means of a so-called "specific conductance index":

$$\text{SCI}_x = \frac{\sum N_x [\text{Log } 10(\text{RA}_i \times 100)] (\text{specific conductance})}{N_x} \quad (2)$$

with RA_i is the relative frequency (%) with which species x occurs at a given sampling location and N is the number of sampling locations where species x was found. The index (SCI) allows calculation of a ranking numeric value for each diatom species indicating the reaction of a diatom species to the salinity of the particular waters.

The Halobion Index

Relating to the sensibility of the diatoms towards salinity, the structure of the diatom community of a given sample can be described by means of the Halobion Index (Ziemann 1971, 1999):

$$H = \frac{\sum h_H - \sum h_x}{\sum h} \times 100 \quad (3)$$

The sums of abundances used in the formula above are those of halobiontic and halophilic diatom species ($\sum h_H$), of halophobic (haloxenic) ($\sum h_x$) and of all species ($\sum h$). The Halobion Index may thus be looked upon as a measure for the complex impact of the salinity on the diatom coenosis and the biological effects of the salinity, respectively. Negative Halobion Indices characterise waters of low salinity and pH-values below 7. Indices around 0 point to typical fresh waters and positive values ($\geq 10-15$) indicate increased salinities.

By means of the Halobion Indices, concentration-dependent changes in the structure of diatom assemblages were presented (i) during an annual period (in Central Europe: spring to autumn, Ziemann et al. 2001), (ii) along the transect of a running water

(Busse et al. 1999) and (iii) over several years (Hofmann 1997; Ziemann 1967, 2005, 2006; Ziemann et al. 2001). Different levels of biological effects of salinisation can be expressed by the occurrence of distinct diatom species and the specific structure of diatom communities. These ranges which are defined exclusively biologically represent halobiotopes and thus form also the basis for a biologically based classification of inland waters (Ziemann 1971, 1997b).

Practical applied aspects

In practice, several modifications to calculate the Halobion Index of a given sampling location have become common. In the latest original version (Ziemann 1999), all diatom species (including rare forms) found in a sample are to be considered. Prior to the final calculation, the numerical frequencies are transformed to abundancies by means of a six-step scale.

Variations of the method may concern for example the limit of a number of frustules to be counted, the usage of a limited catalogue of indicative species (species not listed are neglected), consideration of centric species and changes or the complete omission of the transforming step. E.g., the instruction protocol of the EU Water Framework Directive provides for a total number of 400 frustules to be counted (no separate screening of the slides for rare species, no complete qualitative aspect). It is based on the compilation of a limited number of indicator species and on a five-step scale (Schaumburg et al. 2006). These modifications make the indices of different running waters of one sampling year comparable to each other. However, such changes can influence the results: in the given case, the comparison of data within the same water body of several years does not work (Coring and Bäche 2008). Since in the list of indicator species the different values of indication of the diatom species within the Halobion System is missing, an internal ecological interpretation of the results is impossible. Consequently, this modification cannot be used to document improvements or degradation of the status of salinisation of a river.

Data from Central Europe show that Halobion Index results may depend distinctly on the sampling season (Ziemann et al. 2001): they are strongly influenced by the two factors, hydrological conditions and qualitative composition of the diatom assemblages, both being markedly seasonal. In spring, qualitative proportions of the diatom species may change comparatively quickly, and data from samples taken at that time are thus often of limited value. In summer, late summer and early autumn proportions become more stable and thus are more representative. As also shown by the authors, the substrate (pebbles, stones, phytal, etc.) from which the diatom material is taken may also influence the results. Thus, to get comparable data it is essential that sampling seasons, hydrological conditions and substrates are comparable to each other.

Relations between Halobion Index and water chemistry

The biological effects of salinity on diatom communities can be varied by specific actions of single ions or ion combinations. Within the oligohalobic to lower mesohalobic range, the relations between Halobion Indices and salinity vary due to the antagonistic effect of the calcium as opposed to the alkaline proportion. In stronger salted waters, an elevated proportion of potassium increases the toxic effects (Buhse 1989; Neumann 1962; Ziemann 1967, 1968, 1997a,b). In the same way increasing concentrations of magnesium and/or sulfate as well as increasing pH values may amplify toxic effects of salinity on the diatoms. This holds also good for elevated pH values, especially in combination with raised sodium/calcium ratios in sodium carbonate waters (Blinn 1993; Blinn et al. 2004; Hustedt 1957; Strecker 1997; Ziemann 2002).

Further factors modifying the effects of salinity may be (i) considerable fluctuations in salinity, (ii) nutrient concentrations, (iii) oxygen conditions and organic pollution, (iv) water temperature and light supply, (v) current and substrate conditions, (vi) impacts with toxic effects leading to decimation (Snoeijs 1999; Ziemann 1999).

Under especially extreme or fluctuating conditions when either salt concentrations exceed the maximum tolerance levels of the single species or other toxic effects mask the effects of salinity, the Halobion Index cannot be used (Ziemann 1999). Thus it is always necessary to subject the Halobion Indices to a specific analysis and to check them for plausibility.

Ecotoxicological testing procedures

Use of LC-values and Salinity Index

Salinity tolerance of macroinvertebrates is usually determined in two ways, namely by means of data from field distributions and by lab experiments (Horrigan et al. 2007; Kefford et al. 2003). In the first case, maximum salinity is given at which a species was observed in the field (maximum field distribution, see Rutherford and Kefford 2005). In the latter case, results are given as LC_x values representing that concentration of a compound which induces a specified vital sign for x% (commonly 5, 10, 25 and 50%) of the test organisms after a standard incubation time (commonly 72 or 96 h). Values may relate to very different vital signs such as mortality, changes in reproduction, growth, behaviour, physiology, etc. Salinity thresholds for individual species based on these values can be defined as short-term (acute) or long-term (chronic) effects (Kefford et al. 2007b). The advantage of this kind of tests is that they establish a causal connection between salinity and responses measured. However, care must be taken not to oversimplify the complexity of nature and thus to falsely predict the effects of salinity on organisms in nature (Kefford et al. 2004a).

LC₅₀ values have been measured for a variety of macro- and microinvertebrates, especially from Australian sampling locations (Kefford et al. 2003, 2005, 2007a; Dunlop and McGregor 2007). The data showed that laboratory salinity tolerances of freshwater animals corresponded with their field salinities (Kefford et al. 2004a).

Within one species, salinity tolerance may differ to a certain extent if the sampling locations are located very distant from each other (Dunlop et al. 2008). Younger life stages may be more salinity sensitive than their mature stages, but this is not always true (Kefford et al. 2004b). Moreover, it was demonstrated that the usual assumption of the threshold response to toxic salinities does not hold good for any case: deviations such as inverted U-shaped response occurred also (Kefford et al. 2007b). Due to all of these reasons, LC₅₀ values should not be taken alone when setting targets. For prediction purposes, they are to be multiplied by safety factors (Dunlop and McGregor 2007; Kefford et al. 2007b). Another possibility is to use the Acute to Chronic Ratio (ACR) also incorporating sub-lethal effects of salinity (Kefford et al. 2007b).

Changes in the macroinvertebrate communities associated with a salinity gradient can be expressed by means of the Salinity Index by Horrigan et al. (2005). For this, a salinity sensitivity score is assigned to each macroinvertebrate taxon (1 – very tolerant, 5 – tolerant, 10 – sensitive to salinity). The Salinity Index (SI) based on the cumulative sensitivity scores can be calculated as follows (Horrigan et al. (2005)):

$$\text{Salinity Index (SI)} = \frac{\sum X_i \times \text{SSS}_i}{n}$$

with $X_i = 1$ if taxon i was present, $X_i = 0$ if missing, SSS_i = salt sensitivity score of taxon i and n = the total number of taxa in a given sample. The SI may range from 1 (all taxa of a given sam-

ple are highly tolerant to salinity) to 10 (all taxa are sensitive), indicating the average salinity sensitivity of the macrozoobenthos community of a sampling location. Horrigan et al. (2007) demonstrated that acute toxicity data largely reflect the salinity sensitivity of stream macroinvertebrates derived by using field distributions. They showed that LC₅₀ values from acute toxicity tests were significantly correlated with maximum field conductivities and sensitivity scores.

In toxicity tests being performed with artificial (=equilibrated) seawater, changes in the chemical *in vivo*-composition must be taken into account. In acute toxicity tests, no differences between responses of invertebrates to several ionic compositions tested were found. However, some effects were observed in prolonged tests (Zalizniak et al. 2006): here, low calcium concentrations seem to be detrimental. Schmitz et al. (1967) found that the salt tolerance may be reduced by an increase of the proportions of both magnesium (coinciding with increasing sodium proportions) and potassium whereas rising proportions of calcium enhance salt tolerance. High pH values increase sub-lethal effects of salinity. The effect of water temperature on sub-lethal salinity tolerance is still unclear. Increasing salinity decreases toxicity of most metals; however, it makes organic phosphates more toxic (Kefford et al. 2007b).

Applications of the ecotoxicological approach: a risk assessment framework

Data based on acute toxicity tests of macroinvertebrates can be used to protect particular species of this group. Data from Australian investigations suggest so far that salinity targets for protecting salt-sensitive freshwater macroinvertebrates will protect all biological groups found in freshwater (Kefford et al. 2007b). To protect all biodiversity, a ten-step protocol for deriving thresholds was developed (Dunlop and McGregor 2007). It considers the background of salt concentrations, the taxa requiring protection and their sensitivities to salinity, the salt sensitivity distribution and locally relevant factors.

Various factors may make deviations from this scheme necessary (Kefford et al. 2007b), e.g., (i) temporal changes and/or total duration of salinity, (ii) special ionic compositions and (iii) special chemical properties of saline waters (low or pH values, concomitant presence of metals or organic compounds).

The ciliate assay

Albrecht (1983) described various communities of ciliated protozoa living in salt-polluted mining waters and their value for indication. From this, an assay for salt indication of waters by means of ciliates was developed (Albrecht 1986; Nolting and Rustige 1999). For this, aquatic benthic ciliates are removed from natural substrates such as pebbles, wood, macrophytes, etc. Artificial substrates exposed for 2–4 weeks *in situ* can also be used. All substrates are evaluated by determining the ciliates by use of a microscope. In this way, ciliate coenoses are identified which can be assigned to chloride ranges. Ciliate species are differently sensitive to salinity (Albrecht 1983, 1984a; Mihailowitsch 1989; Nolting and Rustige 1998; Riedel-Lorje 1981; Rustige 1995a,b; Rustige et al. 1997) the occurrence of single species indicates upper and lower limits of the degree of salt concentrations, respectively. Along a salt gradient, characteristic differences within the aspect-forming ciliate taxa become visible.

Although the ciliate assay covers a group of organisms of substantial relevance for the functioning of running waters ecosystems, only a few results obtained from the ciliate assay have been reported so far (Albrecht 1986). A probable reason for this may be that the application of this method requires a strong background

in taxonomy of ciliates by the user which is often lacking. Another aspect is that the results are not reducible to a numerical value and thus are comparatively less precise, e.g., for applied and water management purposes.

Perspectives

Although comprehensive knowledge concerning biological effects of salinisation of freshwater streams is available by now, several problems are subject to future research. In general, it can be stated that despite all progress reported in this paper, there is still neither an assay available for an integrated biologically based assessment of salinised running waters nor a method considering effects of special ions such as K⁺ or Mg⁺⁺ (see part I. 3.). The latter aspect is of special interest for those cases where e.g., effluents from salt mining are introduced into freshwaters resulting in athalassogenic conditions. Further research and development are necessary here.

With regard to the acute toxicity testing approach, Kefford et al. (2007b) have pointed to several questions which are still being clarified. E.g., safety factors must be calculated in such a way that they also include indirect and sub-lethal effects of salinity. It is unclear whether aquatic communities of running waters that have experienced salinisation for a long time are less salt-sensitive in comparison to waters where salinisation is of shorter duration. The effect of temporarily varying salinity on freshwater organisms is still not understood, and this holds also true for the interaction between salinity and other abiotic parameters such as hydrology. Application of the acute toxicity approach outside Australia requires determination of LC₅₀ values of further macroinvertebrate taxa; for water management purposes it would also be desirable to adapt the ten-step protocol framework to non-equilibrated inland salt waters.

With regard to the Halobion Index, results obtained from slight to moderately salinised sampling locations may show an overestimation of halophilic species. A different assessment of the individual indicator species is to be aspired weighting halobiontic species more heavily than halophilic forms. Further steps such as the implementation of a special factor may probably also enhance the precision of the results.

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