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Tolerance of *Chironomus riparius* Larvae (Diptera: Chironomidae) to Salinity

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The influence of salinity on freshwater ecosystems is a growing problem in many parts of the world. Freshwater organisms are exposed to increased salinity by seawater intrusion, by saline industrial or municipal effluents, or by irrigation (Hart et al. 1990, Bervoets et al. 1995). Numerous chironomid species such as *Chironomus salinarius* Kieffer, *C. halophilus,* and *Microchironomus deribae* (Kieffer) are tolerant of a wide range of salinities and are a major component of the fauna of brackish waters (Pinder 1995). *M. deribae* was recorded at a salinity of 42 ppt and is an inhabitant of waters subject to seawater infiltration (Laville and Tourenq 1967). The midge larvae of *Tanytarsus barbitursis* Freeman, which normally occupy intertidal marine habitats and salt lakes, exploit waters ranging from 20 to 170 ppt (Kokkin 1986). Parma and Krebs (1977) recorded 13 chironomid species in brackish ditches in the Netherlands, including *Chironomus riparius (thummi),* which may be regarded as a typical inhabitant of freshwater rivers and lakes. This species was recorded at a salinity of 1 ppt (0.5 g Cl 1^{-1}). In Flanders (Belgium), C. *riparius* was found at chloride concentrations up to 3 g 1^{-1} (Bervoets et al. 1994; Bervoets et al. 1995). Unfortunately, these data are rather anecdotal. Studies on the distribution or occurrence of *Chironomus riparius* or other freshwater organisms as a function of salinity or experimental studies on the tolerance to salinity are non-existent (Kilgour et al. 1994). Larvae of *Chironomus riparius* can be found in almost every watercourse in Flanders and are considered as a major food resource for fish. Therefore, knowledge about the salinity effects on this species can be important to the whole aquatic community.

The aim of this study was to make a further contribution to the assessment of salinity effects on freshwater ecosystems. To this purpose, the occurrence of *C. riparius* in Flemish watercourses in relation to the chloride content was examined and laboratory experiments were conducted to study their tolerance to salinity. Field data were compared with experimental data.

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MATERIALS AND METHODS

The field survey consisted of sampling a large number of rivers and streams as part of a survey programme to determine the quality of water in Flanders, Belgium (Wils et al. 1994). The biological water quality of 1228 sampling stations was assessed in a period from June 1988 to May 1994. Sampling sites ranged from unpolluted to extremely organicly polluted sites. Samples were taken with a handnet (500 µm mesh, 200 x 300 mm frame, 500 mm bag depth) fitted to a 1.5 m handle. Sampling procedures were standardized to ensure a semi-quantitative assessment of abundance of organisms. Each sample took 5 minutes and a river stretch of about 20 m was covered. The sample procedures are widely used for qualitative and semi-quantitative evaluations (De Pauw and Vanhooren 1983, Furse *et al.* 1984). On all sampling sites chloride content and conductivity were measured. Larvae of *Chironomus* gr. *thummi* were separated from other benthic invertebrates and preserved in 70 % denaturated ethanol. This species group contains at least 13 different species, which are morphologically very similar (Webb and Scholl 1985). Only with karyological methods can larvae be identified to species. On the sampling sites with chloride concentrations > 1g l^{-1} (> 2.5 ppt) larvae of *C.* gr. *thummi* were karyologically analysed. Samples of at least 10 midge larvae were taken and preserved in ethanol and glacial acetic acid (3:1) for karyotyping at the Institute of Cytology and Genetics at Novosibrisk (Siberia). All individuals were identified as *C. riparius* (Meigen) (Int Panis *et al.* 1994).

For laboratory experiments, fourth instar larvae of *Chironomus* gr. *thummi* (Meigen) were collected live from two sites in the river Jeker (Meuse Basin), an organically polluted river. Since larvae were collected in winter they can be considered as fourth instars (Krantzberg 1989). A subsample of 10 midge larvae was preserved for karyological analysis; larvae were identified as *C. riparius* (Meigen). All organisms were acclimated to laboratory conditions one week in 500 ml plastic beakers containing 200 ml of aerated artificial river water. Larvae were fed with a suspension of ground commercial fish food (Tetravit) and the medium was renewed three times a week. To test the tolerance of larvae to salinity, seven different salinities (i.e. 0.2, 3.0, 5.0, 10.0, 12.0, 15.0, 18.0 ppt) were selected to expose midge larvae. The different solutions were prepared by diluting artificial Sea Water (SW) with artificial River Water (RW). The composition of one litre of the chemically defined SW medium was 23.5 g NaCl, 4.0 g Na_2SO_4, 0.68 g KCl, 0.196 g $NaHCO_3$, 1.47 g $CaC1_2.2H_2O$, 10.78 g $MgC1_26.H_2O$, and 0.026 g H_3BO_3, resulting in a salinity of 35 ppt and a pH of 8.1. The composition of 1 litre chemically defined RW water was 0.096 g $NaHCO_3$, 0.004 g KCl, 0.123 g $MgSO_4.7H_2O$, and 0.06 g $CaSO_4.2H_2O$, resulting in a salinity of 0.2 ppt and a pH of 7.5. The media (SW and RW) were prepared by dissolving the analytical grade products (Merck p.a.) in deionized water. One week before an experiment was conducted larvae were

held at 9 to 11 °C and a 6:18h 1ight:dark regime to retard pupation while maintaining them in a normal physiological state (Bangenter and Fisher 1989). After this period ten larvae were placed individually at each test salinity in a plastic vessel containing 100 ml medium. Shredded paper to a depth of about 1 cm was used as artificial substrate. All beakers were covered with a screen and placed in a thermostated room at 20 to 22 °C and a 18:6h light-dark regime. Salinity was kept constant by adding deionized water daily to compensate for evaporation. The medium was aerated continuously and was renewed three times a week. Larvae were fed 24 hours before renewal of the medium. Dissolved oxygen was measured with a polarographic electrode system (OXI91/EO90, Wissenschäftlich-Technische Werkstätten, Weilheim, Germany) just before renewal of the medium. Survival and eventual emergence was recorded daily during 17 days. The experiment was replicated twice. The relationship between chloride content and salinity was calculated, by measuring both variables at 20 sampling sites and in the above experiment.

Analysis of covariance was used to analyse the data. Statistical methods used are outlined in Sokal and Rohlf (1981).

RESULTS AND DISCUSSION

The empirical relationship between salinity (Sal) and chloride content was : Sal = 0.516 + 0.0023*[Cl^-] (sal in ppt and Cl^- in mg 1^{-1}). *C. riparius* was found in watercourses with chloride contents ranging from 14 to 3040 mg 1^{-1} (7.5 ppt). In concentrations over 1000 mg Cl^-l^{-1} (2.8 ppt), we found *C. riparius* together with typical brackish water species such as the Crustacea *Gammarus zaddachi* and *Palaemonetes varians*. These watercourses had slow flowing water and the brackish conditions were caused by the intrusion of salty groundwater. In one instance, *C. riparius* occurred at chloride concentrations of > 3000 mg $1.^{-1}$ (7.4 ppt) caused by industrial effluent.

In all experimental solutions the water remained saturated with oxygen during the experiment. Mortality started on day 2 and reached 100 % after day 8 at a salinity of 15 ppt. At a salinity of 18 ppt, all larvae died within 4 days. At all other salinities (12 ppt and lower) more than 75 % survived after 17 days of exposure (figure 1). Larvae did not start to pupate before day 8, although experiments started with fourth instars. This long period between fourth instars and pupae at the exposure temperature of 21 °C probably was due to the fact that larvae were in diapause before the start of the experiments and were suddenly placed at higher temperature.

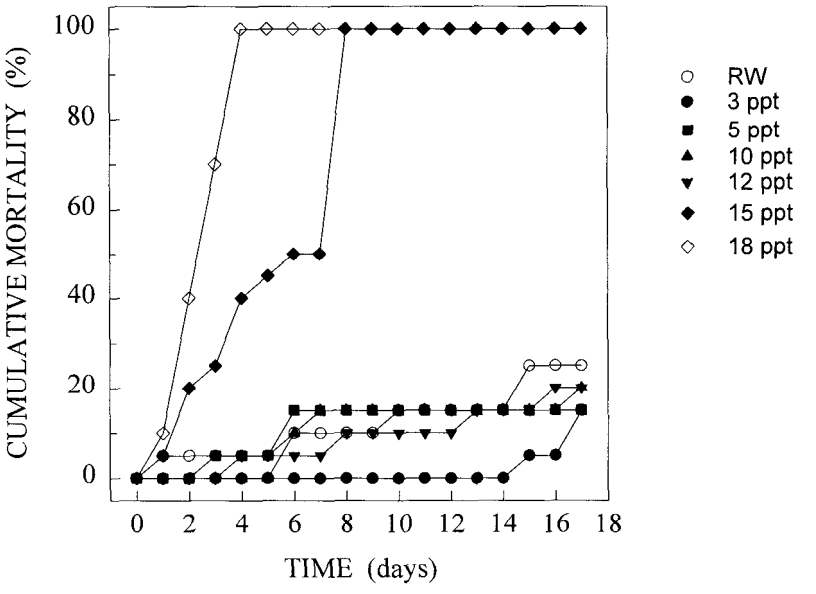

Figure 1. Effect of salinity on mortality of *Chironomus riparius* over time

Emergence of the midges could be followed only in the salinities 0.2, 3, 5 and 10 ppt as no larvae at higher levels emerged. When the overall emergence percentage is calculated (figure 2a), a significant lower emergence was recorded with analysis of covariance for the larvae from the 12 ppt solutions ($F_{1,32}$ slope=7.22, $P < 0.05$). In this figure mortality was taken into account, meaning that percentages represent emergence of the initial number of midge larvae (i.e. 20). When mortality was ignored (figure 2b), giving the percent emergence of the survived midges, no significant differences among the treatment groups were observed (ANCOVA: $F_{4,80}$ slope = 0.42, $P = 0.79$; $F_{4,84}$ elevation = 25.5, $P = 0.97$). This suggests that larvae given time to acclimate to higher salinities (e.g. 12 ppt or even more), the effect on emergence would be lower.

Larvae of *C. riparius* or *C.* gr *thummi* in Flemish watercourses, were recorded at salinities 17.5 ppt, but no effect on survival or emergence was observed when we exposed them in the laboratory to 10 ppt. In nature, tolerance to salinity of fresh water invertebrates will be moderated by several other environmental stress factors including temperature, pollution, and temporal changes in salinity. Fluctuating salinity in a freshwater river generally will have greater effects on the biological communities than constant salinity (Kilgour et al., 1994). In all experiments larvae were transferred suddenly from freshwater to a high salinity, probably causing a salinity shock. A gradual acclimation

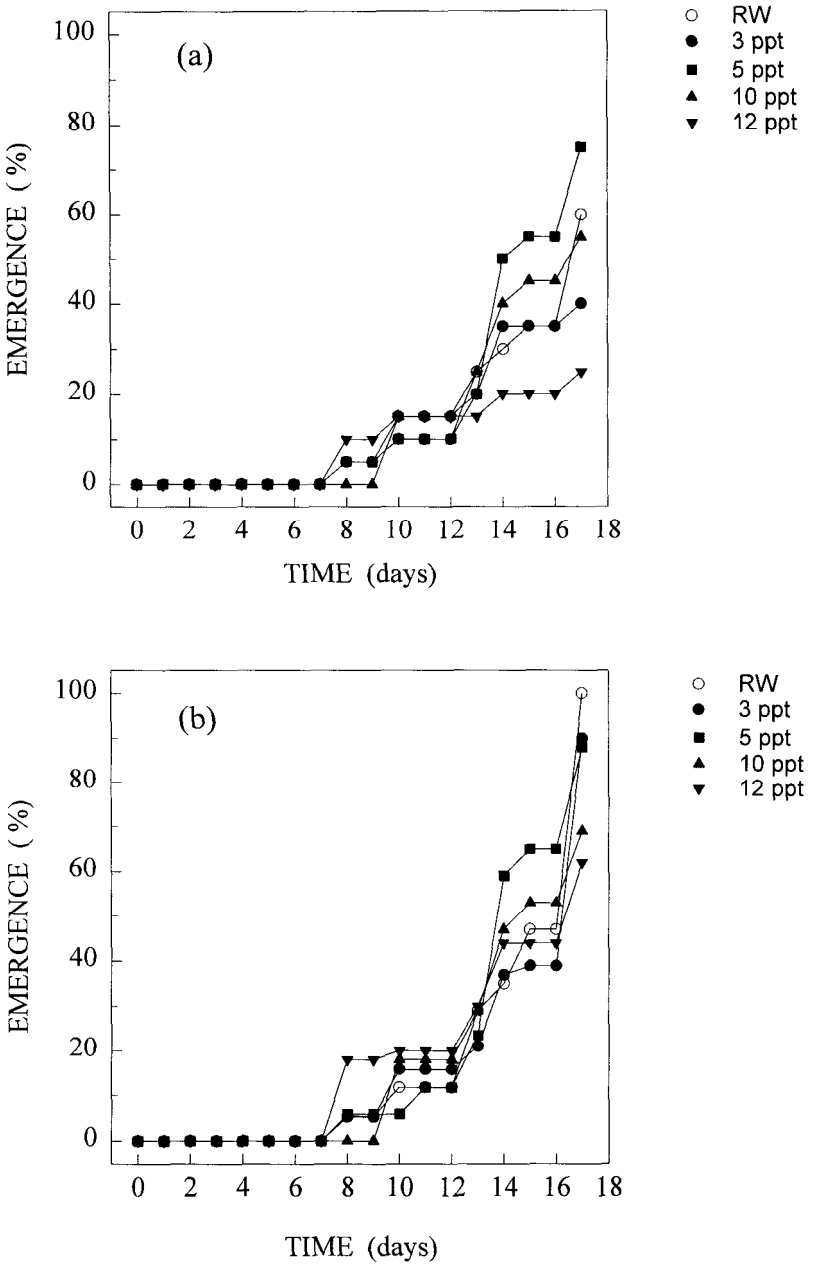

Figure 2. Effect of salinity on the general pattern of emergence of *Chironomus riparius* (a) mortality taken into account; (b) mortality ignored

from freshwater to the highest salinities possibly would have increased their tolerance. Few data are available on the ion- and osmoregulation of freshwater chironomids. The chironomid species *C. plumosus,* which is closely related to *C. riparius,* proved unable to hyporegulate. Its upper limit of salinity tolerance is equal to the concentration of the hemolymph (about 10 g l^{-1} NaCl) (Lauer 1969). This probably means that maximal salinity tolerance will be equal to the internal salinity. To understand the processes influencing the tolerance of *C. riparius* more research on the ion- and osmoregulation of midge larvae at different salinities is necessary.

Compared to the natural salinity of freshwater in Flanders (< 0.5 ppt), larvae of *Chironomus riparius* appear very tolerant to increased salinities. To understand the impact of increased salinity on freshwater ecosystems more research on the tolerance of different freshwater invertebrates and fishes is necessary.

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REFERENCES

Bangenter BR, Fisher F (1989) Different dormancy response in the sympatric Chironomus species Ch. plumosus and Ch. muditarsus. Zoo1 Jb Syst 116:145-150

Bervoets L, Int Panis L, Verheyen R (1994) Trace metal levels in water, sediments and *Chironomus gr. thumni,* from different watercourses in Flanders (Belgium). Chemosphere 29:1591-1601

Bervoets L, Baillieul B, Blust R, Verheyen R (1995) Evaluation of effluent toxicity and ambient toxicity in a polluted lowland river. Environ Pollut 91:333-341

De Pauw N, Vanhooren G (1983) Method for biological quality assessment of watercourses in Belgium. Hydrobiologia 100:153-168

Furse MT, Moss D, Wright JF, Armitage PD (1984) The influence of seasonal and taxonomic factors on the ordination and classification of running-water sites in Great Britain and on the prediction of their macro-invertebrate communities. Freshw Biol 14:257-280

Hart BT, Bailey P, Edwards R Horthe K, James K, McMahon A, Meredith C, Swadling K (1990) Effects of salinity on river, stream and wetland ecosystems in Victoria, Australia. Water Res 24:1103-1117

Int Panis L, Kiknadze I, Bervoets L, Aimanova K (1994) Karyological identification of some species of the genus *Chironomus* MEIGEN, 1803 from

Belgium. Bull Annls Soc r belge Ent 130:135-142
Kilgour BW, Mackie GL, Baker MA, Keppel R (1994) Effects of salinity on the condition and survival of Zebra mussels (*Dreissena polymorpha*). Estuaries 17:385-393
Kokkin MJ (1986) Osmoregulation, salinity tolerance and the site of ion secretion in the halobiont Chironomid, *Tanytarsus barbitarsis* Freeman. Aust J Mar Freshw Res 37:243-250
Krantzberg G (1989) Accumulation of essential and nonessential metals by chironomid larvae in relation to physical and chemical properties of the elements. Can J Fish Aquat Sci 46:1755-1761
Lauer GJ (1969) Osmotic regulation of *Tanypus nubifer, Chironomus plumosus* and *Enalagma clausum* in various concentrations of saline lake water. Physiol Zool 42:381-387
Laville H, Tourenq JN (1967) Contribution à la conaissance de trois chironomides de Camargue et des Marismas du Quadalquivir. Annales Limnol 3:185-204
Parma S, Krebs BPM (1977) The distribution of chironomid larvae in relation to chloride concentration in a brackish water region of the Netherlands. Hydriobiologia 52:117-126
Pinder LCV (1995) Biology of the eggs and first-instar larvae. In : Armitage P, Cranston PS, Pinder LCV (eds) The Chironomidae. The biology and ecology of non-biting midges. Chapman & Hall, New York, pp 86-106
Sokal RR, Rohlf FJ (1981) Biometry. WH Freeman and company, NY
Webb CJ, Scholl A (1985) Identification of larvae of European species of *Chironomus* Meigen (Diptera: Chironomidae) by morphological characters. Syst Ent 10:353-372
Wils C, Schneiders A, Bervoets L, Nagels A, Weiss L, Verheyen RF (1994) Assessment of the ecological value of rivers in Flanders (Belgium). Water Sci Technol 30:37-47