PRIMARY RESEARCH PAPER

Is salinity tolerance the key to success for the invasive water bug *Trichocorixa verticalis*?

F. Van De Meutter · H. Trekels · A. J. Green · R. Stoks

Abstract *Trichocorixa verticalis* is a North American water bug (Heteroptera: Corixidae) that occurs in brackish and saline aquatic systems. Recently, it has been found invading three continents including Europe. Its invasive success has been attributed to the capacity of tolerating hypersalinity. We compared both the realized and standardized salinity niche of invasive *T. verticalis* and native Corixidae to verify if *T. verticalis* may fill in an unoccupied niche. We first established the field distribution of *T. verticalis* and native Corixidae along a salinity gradient. Second, we experimentally tested the salinity tolerance of *T. verticalis* and three common native Corixidae species. Of the seven Corixidae species found in the field study, three were positively related to the salinity gradient: *S. selecta*, *S. stagnalis*, and *T. verticalis*. *T. verticalis* showed the highest salinity optimum, however, after correcting for environmental background variation, salinity optima differed little among the three halophilic species. In the salinity tolerance experiment, *S. selecta* outperformed *T. verticalis*, which performed as well as *S. stagnalis*. Based on our experimental results, we cannot support the hypothesis that *T. verticalis*' invasion is mediated by a high salinity tolerance that allows this species to colonize habitats unoccupied by native Corixidae. Although these findings contrast with the field patterns in which *T. verticalis* showed the highest niche optimum and tolerance, these patterns may have been partly due to other environmental factors, particularly anthropogenic disturbance. Our comparative results are for adults only, and it remains possible that relative salinity tolerance patterns for juveniles differ from that for adults, which may add to the observed field pattern.

Keywords Corixidae · *Sigara* · Exotic · Anthropogenic disturbance · Environmental gradient

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Introduction

Invasive species are considered a major and increasing threat for natural ecosystems worldwide (Mack et al., 2000; IUCN, 2008). A fundamental question is why alien invaders can be highly successful in environments previously unknown to them. In general, explanations for invasibility include the invader's traits (e.g.; Kolar & Lodge, 2001; Mack, 2003), the invader's ecological relationships in a new

environment (Keane & Crawley, 2002), and the competitive effects of the native community (Levine, 2001; Kennedy et al., 2002). Relating to the latter hypothesis, some studies have suggested that invasions may concentrate on portions of environmental gradients that are unsaturated because species able to inhabit these habitats have been lost over time or never evolved in the region (Ricklefs, 2004; Gilbert & Lechowicz, 2005).

Trichocorixa verticalis is a small omnivorous water bug (Heteroptera: Corixidae) native to North-American brackish and saline aquatic systems. Recently, it has been found in three other continents; Africa, Europe, and Australia (Jansson, 1982; Jansson & Reavell, 1999; Günther, 2004). Studies on *T. verticalis* in Europe confirm that this species is now established and probably continues to spread (Sala & Boix, 2005; Gómez-Rodríguez et al., 2009), though populations as far as is known remain restricted to coastal areas of the Iberian Peninsula. The ecological impact of alien *T. verticalis* on native aquatic communities at present seems relatively weak (Gómez-Rodríguez et al., 2009). However, some examples of strong trophic cascading effects in its home range suggest that its long-term impact may be substantial (Wurtsbaugh, 1992). The success of *T. verticalis* as an invasive species has been mainly attributed to its capacity to survive droughts in the egg stages and to its exceptional tolerance of high salinity in the juvenile and adult phase (Kelts, 1979). In fact, *T. verticalis* is the only Corixidae species that has ever been found in the open sea (Gunter & Christmass, 1959). Whereas salinity tolerance is well studied in North-American *T. verticalis*, little is known on tolerance ranges of European populations of *T. verticalis* and of native corixids. The question whether colonization and establishment by *T. verticalis* in Europe may be facilitated by the absence of competing corixids at high salinities therefore remained unresolved.

In this study, we aim to study the realized salinity niche and the salinity niche under standardized laboratory conditions of European *T. verticalis* and three common native Corixidae species with which it co-occurs in the study area. This knowledge should add to understanding the mechanism of *T. verticalis*' invasion in Europe. First, we establish the field distribution of *T. verticalis* and native Corixidae in relation to salinity in the area where *T. verticalis* was first reported from in Europe (Günther, 2004).

Second, we expose *T. verticalis* and three co-occurring Corixidae species to a gradient of salinies under laboratory conditions and asses their salinity niche. Finally, we compare salinity niches among species and between field and laboratory assessments and discuss these findings.

Materials and methods

Field sampling

Between 25 November 2008 and 3 December 2008, we sampled 29 lentic open water habitats containing Corixidae in an area of approximately 50×50 km situated between the cities of Huelva, Sevilla, and Cadiz in the marismas del Guadalquivir, South Spain. The majority of sampling sites were within the delta of the Guadalquivir river, which is largely protected as an important wetland area (see, e.g., Rendón et al., 2008). The study area exhibits particularly strong gradients in salinity at a very small geographical scale. We searched for aquatic habitats inhabited by Corixidae representing a maximal salinity gradient and tried to achieve equal representation of sampling sites along this gradient (two sampling sites for each 5 mS/cm interval). We succeeded in finding sites spanning a conductivity gradient from 0.154 to 37.6 mS/cm, but we found only one hypersaline habitat containing Corixidae (conductivity 70.8 mS/cm, 41.8 g/l). Corixidae presences and densities were assessed by taking five standard sweeps; each sweep was taken at a different location in the site. We used a dip net with 500 μm mesh and a sampling surface of 520 cm^2. An average sweep had a length of 1.8 m. At each site, we measured a number of environmental variables upon sampling. pH, electrical conductivity (mS/cm) as a close proxy of salinity and dissolved oxygen (mg/l) were measured using a WTW multi-line F meter (Geotech$^{©}$, Denver, Colorado, USA). Water transparency was measured with a Snell tube (cm). Average water depth at the sampling site was obtained from three measurements with a graduated stick to the nearest 5 cm. Surface area (m^2) of the sampled sites was measured in situ for small sites and from Google Earth (version 4.3.7284.3916, July 2008) for large sites. We estimated cover (%) by submersed vegetation, cover by tall (>5 cm) submersed vegetation, and cover by emergent vegetation

at the sampling location. Substrate type (clay, sand-clay, sand, gravel) was established from repeated bottom samples with a core-sampler. Based on the current use of each site we classified it as either subject to low or high anthropogenic disturbance (0/1). Drainage and irrigation canals, rice fields, fish and shrimp breeding ponds, and salterns were classified as subject to recurrent anthropogenic disturbance (regular input of high levels of nutrients, toxicants, water level manipulations). All ponds and pools, even if they were manmade, that are not currently used for any specific purpose and are not subject to unnatural disturbances are seen as low impacted sites. All adult Corixidae in the sample were identified to species level with the use of Jansson (1986) and Sailer (1976). Due to identification difficulties, juvenile Corixidae were identified to genus level.

Salinity tolerance test

We compared the salinity tolerance of adult *T. verticalis* to that of adults of three other Corixidae species found commonly in the field survey of the Guadalquivir marshes: *Sigara scripta*, *S. selecta* and *S. stagnalis*. *S. selecta*, and *S. stagnalis* are known as typical salinity tolerant European Corixidae species (Savage, 1989). *Sigara scripta* was, next to the two species mentioned above, the commonest *Sigara* occurring in the area where *T. verticalis* was found, yet prefers more freshwater habitats. We collected 50 adult individuals (25 males, 25 females) of all four species from two sample locations of the field survey on 28 November 2008. *S. selecta*, *S. stagnalis*, and *T. verticalis* were collected in a small channel (37°04′18.05″W, 6°21′17.89″W) with brackish water (35.2 mS/cm). *S. scripta* came from a semipermanent pond (37°04′15.92″N, 6°16′20.63″W) that had a much lower salinity (14.05 mS/cm). After acclimatizing for 12 h in their original water to laboratory ambient conditions, Corixidae were placed individually in 55 ml of water that belonged to five salinity concentrations: 0, 2.2, 6.9, 21.2, and 42.5 g/l which corresponds to 0.01, 4, 12, 36, and 72 mS/cm electrical conductivity. This range corresponds to the range of salinities covered in the field survey. We performed no salinity acclimatisation before the experiment, however, similar direct transfer experiments on aquatic invertebrates appeared to yield tolerance values very similar to the field

tolerance (Kefford et al., 2004). The experimental medium was constituted of dechlorinated tap water to which pure sea salt produced in the Guadalquivir estuary (Halendado©, Sanlucar de Barrameda salterns) was added to obtain the required salinity levels. The salinity tolerance experiment had in total 200 test units (4 species × 2 sex × 5 salinity concentrations × 5 replicates). The experiment lasted 72 h and survival was monitored at 12 h intervals. During the experiment, the animals were kept in constant dark and were not fed, following OECD standard guidelines for acute toxicity testing on *Daphnia* sp. (OECD, 2004; no specific protocol is available for Corixidae in the OECD guidelines). During the experiment, we monitored salinity and two other key abiotic variables (dissolved oxygen, water temperature) at each 12 h interval. We noticed small variations in dissolved oxygen (7.23–9.98 mg/l) and water temperature (16.4–17.7°C) through time but these were similar for all species. These values for oxygen and water temperature were within the range of those observed in natural ponds during the field survey (oxygen 5.13–22.8 mg/l, temperature 8.1–18.9°C). Salinity slightly increased for all salinity levels probably due to evaporation and increased to 0.012, 4.15, 12.39, 37 and 73.7 mS/cm, respectively, for the five experimental salinity levels (Fig. 1).

In addition, we also tested 48 h salinity tolerance of *T. verticalis* juveniles. *T. verticalis* juveniles (nymphal stages 3–5) were collected on 2 December 2008 from the same population that was sampled for adult *T. verticalis*. We were not able to test specific salinity tolerances for juveniles of the *Sigara* species
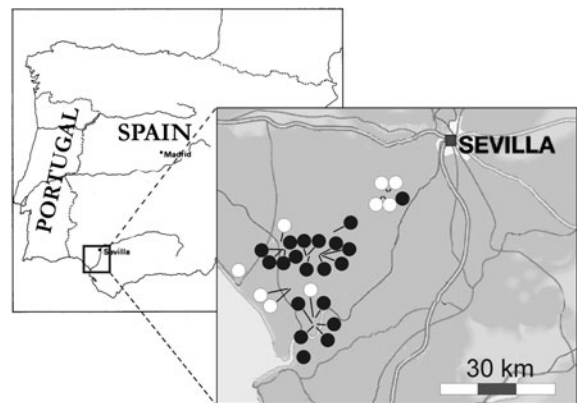

Fig. 1 Map showing the location of the study area. *Circles* indicate sampled pools/ditches; *circles in black* indicate locations where *T. verticalis* was present

since it is extremely difficult to separate *Sigara* juveniles from the studied species in vivo. The experimental set-up was identical to that for adult Corixidae, with the only difference that we could not establish sex for *T. verticalis* juveniles and therefore used half the number of animals ($N_{tot\ juveniles} = 25$; 5 individuals/salinity level) compared to adults. Measurements of abiotic variables during the experiment were all within the above ranges observed for adult Corixidae.

Statistics

To test for the importance of salinity for structuring the Corixidae community in the study area, we performed Redundancy Analysis (RDA) with salinity as the only variable in the predictor variable matrix, and the Corixidae community in the species data matrix. This RDA was repeated with all other environmental variables included as covariables, to partial out their confounding effects. The salinity niche and salinity tolerance of all species was assessed applying Outlying Mean Index (OMI) analysis (Dolédec et al., 2000) using the niche procedure in ADE-4 (Thioulouse et al., 1997). The OMI value indicates the niche position of species relative to the mean habitat conditions of the sampling area. Since we were particularly interested in the niche partitioning along the salinity gradient, the environmental dataset was restricted here to the conductivity data. OMI analysis also calculates niche breadth (named tolerance) that measures the amplitude in the distribution of each species along the sampled gradient. The species counts were logarithmically transformed and all environmental variables were normalized and logarithmically transformed prior to analysis. Multivariate analyses were performed in Canoco 4.5 (ter Braak & Šmilauer, 2002).

Due to either low mortality (*S. selecta*) or to sudden high mortality when moving up one salinity level (*S. scripta*, *S. stagnalis*, *T. verticalis*) in the tolerance experiment, we could not calculate and compare standard tolerance measures (e.g., LC_{50}) among the four Corixidae species. We therefore used survival time as a measure of salinity tolerance. In previous experiments based on pesticide tolerance of Belgian corixids, patterns for survival time and LC_{50} values in Corixidae were shown to render comparable results (Trekels et al., unpublished results). Survival

time values were set to be the last time interval when an individual was seen alive (survival was monitored at 12 h time intervals during 72 h). Individuals that were already dead at the first time interval (12 h) were attributed a survival time of zero. Data from the salinity tolerance laboratory experiment on the four Corixidae species (adults) were analyzed using ANOVA with survival time as the dependent variable and sex, species, and salinity concentration as the categorical variables. The survival time data did not meet the assumptions of standard ANOVA, and we conducted a nonparametric analysis on survival time by first ranking the data and then conducting an ANOVA on the ranks (Relyea & Mills, 2001). To assess differences in performance at high salinities which is one of our main research questions, we performed an ANOVA on the ranked data at the highest tested salinity level (72 mS/cm). Performance of juvenile *T. verticalis* was compared to adult *T. verticalis* by running a similar ANOVA as for adult Corixidae on the ranked survival times. The final model had life stage (juvenile, adult) and salinity concentration as categorical variables, and ranked survival times as the dependent variable. Significant effects were further evaluated using Tuckey HSD post hoc tests. All ANOVAs were done with Statistica 8.0 (Statsoft, 2007).

Results

Field survey

We observed seven Corixidae species in our survey, one of which occurred at one site only (*Micronecta* sp.) and is not further considered. All six other species were relatively common and occurred as adults in at least 25% of all 29 sites: *Corixa affinis* (9 sites), *Sigara lateralis* (12), *S. scripta* (14), *S. selecta* (7), *S. stagnalis* (12) and *T. verticalis* (19). Three species had an observed salinity range extending from freshwater (<200 µS/cm) to brackish sites (*C. affinis*, *S. scripta* and *S. lateralis*) or to saline water (*S. stagnalis*). *S. selecta* and *T. verticalis* were not found at the lowest salinity (freshwater) sites with salinity concentrations lower than 2.23 and <2.99 mS/cm, respectively, but extended to saline (*S. selecta*) and hypersaline water (*T. verticalis*—1 site only). Reproduction of *T. verticalis* as indicated

by the presence of juveniles occurred in the range 4.75–71.4 mS/cm. Juveniles of *Sigara* species were seen in the range 0.154–35.2 mS/cm.

Salinity explained the largest fraction of variation in Corixidae community composition (RDA salinity: 15.5%, $P = 0.001$, all environmental factors: 56.7%, $P = 0.018$, Fig. 2), closely followed by anthropogenic disturbance (14.9%, $P = 0.001$), which was to a large extent collinear with salinity. Salinity remained the most important variable after omitting one highly influential site (site 8, 12.5%, $P = 0.009$) that had exceptionally high densities of all three salinity tolerant species. When corrected for the effect of all other environmental variables, the effect of salinity remained significant (7.1%, $P = 0.048$). Species salinity niches (OMI) and tolerances obtained in the OMI analysis, are summarized in Table 1. *T. verticalis* has the lowest OMI, although differences with the other species were not significant. OMI values are calculated relative to the centre of the environmental factor space. Since we included only salinity as environmental variable that ranged from 0.19 to 71 mS/cm, the niche optimum of *T. verticalis* will be halfway that range. When we considered the OMI corrected for other environmental variables all OMI converged (Table 1).

Salinity tolerance test

The four Corixidae species showed a different survival time during 72 h survival trials to salinity (Table 2, Fig. 3). For *S. selecta* we did not find a significant effect of salinity (all $P > 0.79$). For *S. stagnalis* and *T. verticalis* survival times at the highest salinity level
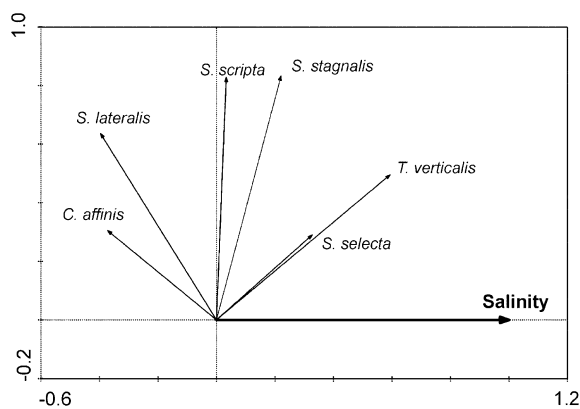
Table 1 Outlying mean index values (OMI) and tolerances (Tol) of six Corixidae species along the sampled salinity gradient for the analysis without (*left*) and with (*right*) correction of salinity for other environmental factors

Species	Without covariables			With covariables		
	OMI	*P* value	Tol	OMI	*P* value	Tol
T. verticalis	<0.001	0.95	0.84	0.004	0.76	0.88
C. affinis	0.46	0.03	0.41	0.004	0.79	0.75
S. lateralis	0.30	0.02	0.52	<0.001	0.96	1.01
S. scripta	0.38	0.01	0.43	0.002	0.84	0.56
S. selecta	0.11	0.43	0.52	0.025	0.77	0.76
S. stagnalis	0.08	0.30	Tol	0.035	0.51	0.49

P values indicate whether the OMI is significantly different from the mean salinity in the dataset. The environmental variables that were used as co-variables are listed in the "Materials and methods" section

Table 2 Results of the ANOVA on the ranked survival times in the salinity tolerance experiment for adult corixids

Effect	df	*F*	*P*
Species	3	8.23	<0.0001
Sex	1	3.28	0.072
Salinity	4	60.00	<0.0001
Species × sex	3	0.13	0.94
Species × salinity	12	6.50	<0.0001
Sex × salinity	4	0.09	0.99
Species × sex × salinity	12	1.25	0.25
Error	160		

Fig. 2 Biplot of the results of the RDA analysis on the Corixidae community as a function of salinity

were strongly decreased compared to all lower salinity levels (both species had all comparisons $P < 0.0001$). Finally, *S. scripta* appeared most sensitive to salinity, with survival times already decreasing strongly and significantly at the second highest tested salinity level (all comparisons with lower levels $P < 0.0001$) and remained low at the highest levels (comparison between highest and second highest level $P = 0.38$). The pattern for survival times at the highest salinity level was in accordance with the general patterns over all levels: *S. selecta* showed a significantly longer survival time than all three other species (all $P > 0.011$) which did not differ among each other (all $P > 0.14$).

The effect of salinity during 48 h exposure trials on survival time differed significantly between adult and juvenile *T. verticalis* ($F_{(4,65)} = 9.495$, $P < 0.0001$). Survival time of adult *T. verticalis* was not affected by
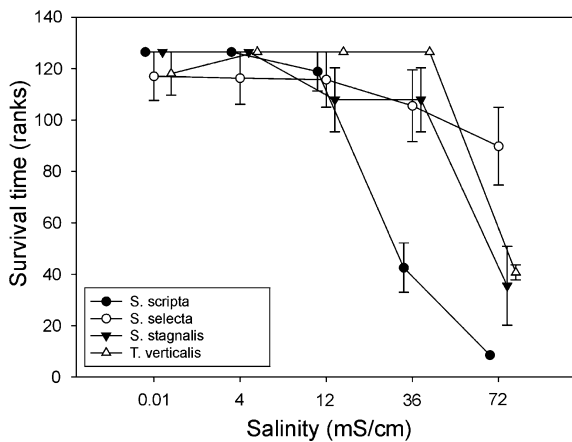
Fig. 3 Average ranked survival time ± 1 SE of adult Corixidae (*S. scripta*, *S. selecta*, *S. stagnalis*, and *T. verticalis*) exposed to a gradient of salinity. Survival was monitored at 12 h intervals during 72 h
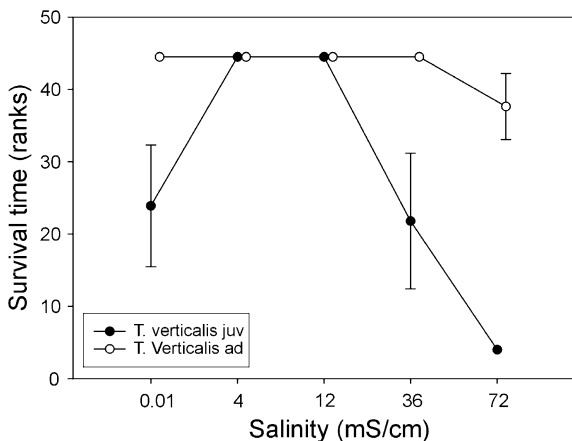

Fig. 4 Average ranked survival time ± 1 SE of adult and juvenile *T. verticalis* exposed to a gradient of salinity. Survival was monitored at 12 h intervals during 48 h

salinity during the 48 h experiment (all post hoc comparisons had $P > 0.77$). *T. verticalis* juveniles, however, showed a significantly decreased survival time at the two highest salinity levels 36 and 72 mS/cm (compared to 4 and 12 mS/cm, all $P < 0.015$), and also tended to have lower survival times at the lowest salinity 0.01 mS/cm (compared to 4 and 12 mS/cm, all $P < 0.063$, Fig. 4).

Discussion

In our field survey we found *T. verticalis* to reproduce at higher salinities than indigenous Corixidae, but overlap of the adult salinity niche was large. To some extent contrasting with the field survey, the laboratory study indicated that native Corixidae performed better (*S. selecta*) or equally well (*S. stagnalis*) at high salinity compared to *T. verticalis*. Both results seem to contradict earlier suggestions that salinity resistance and the ability to fill in an unoccupied salinity niche may be a key mechanism for *T. verticalis*' invasion success (Sala & Boix, 2005, Gómez-Rodríguez et al., 2009). In fact, it may be questioned whether distribution patterns of these corixid species are shaped by antagonistic interactions, as they in most cases occurred together. The autocorrelation between salinity and disturbance observed in the field study indicates that the important role of salinity for the occurrence of *T. verticalis* may be mediated by disturbance. If this is true, dispersal capacity, life-history or reproduction capacity may be more important traits to explain invasion success of *T. verticalis*.

Salinity was a prime determinant of Corixidae community structure in the field study. This was expected, since we deliberately maximized the sampled salinity gradient compared to other environmental variables. *T. verticalis* showed the highest salinity optimum, followed by the two native salinity tolerant species *S. selecta* and *S. stagnalis*, but overlap was high (Table 1). When correcting for other environmental factors the observed high optimum and tolerance to salinity of *T. verticalis* decreased to values comparable to many other species, suggesting other factors, such as anthropogenic disturbance, may have affected the apparently superior salinity tolerance observed in the field.

The patterns obtained in the salinity tolerance experiment differed somewhat from the patterns observed in the field study. During the experiment, *S. selecta* outperformed *T. verticalis* in salinity tolerance, and *T. verticalis* had a similar capacity of coping with salinity as *S. stagnalis*. These differences cannot be due to differences between the populations or their habitats, since all three species originated from the same site. Whereas *T. verticalis* is famed for its high salinity tolerance (e.g.; Gunter & Christmass, 1959; Tones & Hammer, 1975; Kelts, 1979), less is known on salinity tolerance in European species *S. selecta* and *S. stagnalis* that may be found in saline environments. However, *S. selecta* has been found in hypersaline temporary streams at salinities up to

134 g/l and was shown to reproduce at 55 g/l (Velasco et al., 2006). *S. stagnalis* is—together with *T. verticalis*—known capable of hypo-osmoregulation (Kelts, 1979). In combination with our experimental results this seems to indicate that some European Corixidae may have a comparable ability to resist high salinities as *T. verticalis*. *S. scripta* clearly was the least tolerant species to salinity, as expected from the field study. However, experimental individuals of *S. scripta* also originated from a source pond with a lower salinity than the species *S. selecta*, *S. stagnalis*, and *T. verticalis*, such that acclimatisation effects may have added to the observed difference in salinity tolerance.

Remarkable is that Kelts (1979) in his detailed study on the ecology of *T. verticalis* indicates good survival (>70%) of both adult and juvenile *T. verticalis* up to 55 g/l salinity, and a sudden rise in mortality at 65 g/l during 4 day trials. This is a much higher tolerance than reported in this study. Possible explanations are that Kelts continuously fed his individuals, whereas we followed a standard protocol excluding food. Osmoregulation can be an energetically costly process (e.g., Perez-Pinzon & Lutz, 1991; Boeuf & Payan, 2001), but see Sutcliffe (1984). In species where it is energetically costly, it is increasingly costly to balance the osmolarity balance at increasing external osmolarity. This may also explain the observed discrepancy between field and laboratory values of salinity tolerance in *T. verticalis* in this study, but at the same time this would suggest that food stress may play an important role in the distribution of this species. Alternatively, *T. verticalis* studied by Kelts (1979) and *T. verticalis* occurring in Europe may be different ecotypes or subspecies of a species that has an extended range in the nearctic, which may account for some of the differences between the studies.

Both the experimental results and the field study do not corroborate the idea that *T. verticalis* in Europe occupies an empty niche along the salinity gradient, and other traits will be needed to explain its invasive success. Ultimately, this case may resemble the story for gammarid species, where salinity tolerance was a returning but limited part of the ecological profile specific to successful invasive species (Devin & Beisel, 2007).

Although survival of adults was not affected, freshwater conditions appeared to cause mortality in juvenile *T. verticalis* compared to brackish water conditions. In addition, juvenile *T. verticalis* were exceedingly sensitive to high salinity compared to adults. These results are opposite to those of Kelts (1979) where juveniles performed better than adults. Again, the main difference between his and our experiments is in the feeding. Juveniles could be more susceptible than adults to freshwater conditions during food deprivation for several reasons, including their higher surface: volume ratio, their higher investment in growth, or differences in the cuticle.

North-American *Trichocorixa verticalis* have recently established populations on different continents including Europe. Part of its invasive success has been attributed to its high salinity tolerance which makes *T. verticalis* able to colonize habitats previously unoccupied by Corixidae, hence facilitating its establishment (Gunter & Christmass, 1959; Tones & Hammer, 1975; Kelts, 1979). Our study within the current European range of *T. verticalis*, however, indicates that the physiological limits of at least adult *T. verticalis* may not differ much from those of native salinity tolerant Corixidae. In our laboratory assays, *T. verticalis* performed worse than native *S. selecta* at high salinities, but we cannot exclude that these results are affected by food deprivation during the assays. In the field study, *T. verticalis* was particularly successful at high salinities, however, salinity correlated strongly with antropogenic disturbance which may have contributed to the observed patterns. *T. verticalis* indeed may posses unique traits that make it successful in variable environments, such as drought resistant eggs. Together with research on the juvenile phase, this may be the goal of future research trying to understand *T. verticalis* invasions.

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