RESEARCH ARTICLE

How does salinity tolerance influence the distributions of *Brachionus plicatilis* sibling species?

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Abstract Environmental salinity is important in defining *Brachionus plicatilis* sibling species distributions. However, while salinity influences distributions, sibling species often co-exist. Three different mechanisms potentially account for the partial co-occurrence of sibling species: (1) siblings have differing salinity tolerances that partially overlap; (2) siblings physiological tolerances may be commonly broad, but relatively small differences in tolerances differentiate distributions via interactions e.g. competition; or (3) siblings distributions may be influenced by physical factors other than salinity. Here, we assess the extent of salinity tolerance in three *B. plicatilis* sibling species (*B. plicatilis* 6TUR, *B. plicatilis* IOM and *B. rotundiformis* 6TOS) by measuring population growth rate (μ, day^{-1}) and egg development time in response to salinity (5–60‰) and salinity fluctuations ($\leq \Delta 40$‰). Sibling species were identified by analysis of the mitochondrial COI gene, and salinity responses were compared by regression analysis. Responses differed significantly between siblings, although the broad trends were similar. Positive growth occurred at all salinities, and highest growth rates ranged between 0.93 and 1.08 day^{-1} at 16–18‰. Rapid changes in salinity reduced growth rates, but net mortality occurred only in one treatment (100% mortality on transfer from 10 to 40‰). Egg development time was largely invariant with salinity except for *B. plicatilis* IOM and where rotifers were transferred from 30 to 60‰. We indicate that several siblings are similarly euryhaline and tolerate salinity fluctuations. Undoubtedly, wide tolerances in *B. plicatilis* are adaptations to ephemeral and seasonally variable habitats. Given common broad salinity tolerances, it is unlikely that the differential distributions of sibling species are a direct result of physiological constraints. Instead, we illustrate using a simple model that subtle differences in physiological tolerances may have important impacts on interactions between sibling species, which may in turn influence distributions.

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Introduction

Biodiversity is an integral component of ecosystem function. However, a large proportion of biodiversity is potentially unaccounted for as it is now recognised that cryptic species complexes are common for many taxa (Knowlton 1993). For instance, the rotifer *Brachionus plicatilis*, an important component of microbial food-webs in continental aquatic systems (Walker 1981), which was considered a cosmopolitan euryoecious species, is now recognised as a species complex (Ciros-Pérez et al. 2001b; Gomez et al. 2002). The existence of such complexes raises important questions regarding the extent of niche separation required to maintain highly similar species and the mechanisms that result in cryptic species formation (Knowlton 1993).

The *B. plicatilis* complex consists of at least seven sibling species (Ortells et al. 2003), three of which are

currently described as separate siblings (*B. plicatilis*, *B. rotundiformis*, *B. ibericus*; Ciros-Perez et al. 2001b). *B. plicatilis* sibling species are common in ephemeral and highly variable brackish coastal marshes and inland salt lakes (Gomez and Snell 1996; Walker 1981). Field based studies have indicated that the distributions of siblings are differentially influenced by a small number of environmental variables, primarily salinity (Gomez et al. 1995), such that different physiological tolerances confine siblings to different spatial and temporal distributions, allowing sibling species co-existence (Gomez et al. 1997). However, while it is clear that distributions are strongly dependent on salinity subsequent studies have demonstrated that sibling species distributions commonly overlap (Ortells et al. 2003).

These more recent data raise important ecological questions concerning the role of environmental variables in determining the distributions and co-existence of sibling species. Broadly three potential scenarios may explain the observed patterns of sibling species distributions in natural habitats: (1) sibling species may have widely differing salinity tolerances that only partially overlap, (2) the physiological tolerances of sibling species may be similarly broad, and relatively small differences in physiological responses account for partially overlapping distributions via biological interactions, e.g. local competitive exclusion, or (3) sibling species distributions may be influenced by factors other than salinity.

Current studies indicate that numerous environmental factors influence sibling species distributions (e.g. Gomez et al. 1995; Ortells et al. 2003) and provide evidence of competitive exclusion between siblings (e.g. Ciros-Pérez 2001a), thus it is becoming clear that a potentially complex series of interactions may ultimately determine distributions in natural environments. However, details of how underlying ecophysiological responses (e.g. growth rate) and environmental tolerances (e.g. salinity) differ between siblings are scarce, this is partially because such details cannot easily be inferred from field studies and partially because a large number of the experimental studies assessing physiological tolerances in *B. plicatilis* predate the discovery of cryptic species in this taxon (e.g. Miracle and Serra 1989, but see Gomez et al. 1997; Lowe et al. 2005a, b). As a result, our knowledge of the extent of differentiation in physiological tolerances between sibling species is limited. Such information is critical to understand the relative roles of environmental factors and sibling species interactions in determining distributions. If, for example, physiological tolerances overlap extensively, interactions between sibling species may be common, such a scenario raises important questions regarding sibling species co-occurrence and sympatric speciation.

Here we focus on rigorously characterising salinity tolerance in three defined sibling species, to assess the extent of overlap in physiological tolerances. We also extend studies of salinity tolerance in these organisms, by assessing the potential effect of fluctuations in environmental salinity on the ecology of *B. plicatilis* sibling species. Such fluctuations, and episodic changes, in environmental variables are potentially important parameters shaping the distributions of, and interactions between, organisms (Chesson and Huntley 1997); this may be especially important in ephemeral and high disturbance environments, such as the shallow brackish water habitats where *B. plicatilis* sibling species are common and salinity may fluctuate widely (Miracle and Serra 1989).

In this study, we use an experimental approach to compare ecophysiological responses of three *B. plicatilis* sibling species to both a broad range of salinities (5–60‰) and to rapid salinity changes (Δ10–40‰). First, sibling species identities were confirmed by analysing fragments of the mitochondrial COI gene. Then, differences in salinity responses were identified using estimates of population growth rate and egg development time. Finally, we illustrate, using our data and a simple population growth model, the potential influence of small differences in physiological tolerances on the occurrence of sibling species in natural habitats.

Materials and methods

Maintenance of rotifer cultures

Three monoclonal isolates from the *Brachionus plicatilis* complex were cultured. *B. plicatilis* IOM is used in commercial aquaculture and was provided by N. Fullerton (Larval rearing centre, Port Erin marine laboratory, Isle of man). *B. plicatilis* and *B. rotundiformis* were environmental isolates provided by M. Serra, University of Valencia, Spain. All isolates were maintained on a diet of the flagellate *Dunaliella salina*, as previously described (Lowe et al. 2005a).

Mitochondrial COI sequencing and analysis

The identity and phylogenetic positions of the three isolates were examined using sequence data for a fragment of the mitochondrial cytochrome oxidase one gene (following Gomez et al. 2002). For DNA extraction, rotifers were washed on Nitex mesh with sterile saline and left for 12 h to process ingested food. Rotifers were then washed again, concentrated into 2 ml screw top cryotubes (Nalgene), and pelleted by

centrifugation. Pellets were stored at $-80\ ^{\circ}\text{C}$ until processed. Genomic DNA was extracted using a high salt protocol (Sambrook and Russell 2001) and a fragment of the mitochondrial COI gene amplified by PCR using the following primers: forward GGT CAA CAA TCA TAA AGA TAT TGG, and reverse TAA ACT TCA GGG TGA CCA AAA AAT CA. PCR products were sequenced using the BigDye Terminator v3.1 sequencing kit on the ABI 3,100 genetic analysis system.

Sequences were aligned using DNA Star and subjected to maximum likelihood analyses (PAUP, V4.0b10). An optimal model ($P = 0.05$) was determined using Modeltest V3.06 (Posada and Crandall 1998). The best-fit model was the transversional model of evolution with estimated base frequencies and a substitution rate model; the proportion of invariant sites was 0.5542, and a gamma distribution (shape parameter = 2.0307) was invoked on the remaining sites. Parameters were added prior to maximum likelihood runs, and heuristic searches were performed. To examine robustness, bootstrap values were calculated by stepwise addition (1,000 replicates).

The effect of salinity on growth rate

For acclimated growth experiments, rotifers were maintained at one of 12 salinities between 5 and 60‰ for > 2 weeks. Experiments were conducted as described by Lowe et al. (2005a). Specific growth rate (μ, day^{-1}) was calculated, from the change in total number of females as $\mu = \ln(N_t/N_0)/t$, where N_0 is the number of females at the start of the growth incubation, N_t is the number of females after 3 days, and t is time in days. During all acclimation and experimental incubations food remained replete.

To examine the response of rotifers when transferred between different salinities, three un-acclimated growth responses were examined. Rotifers acclimated at 10‰ were transferred directly to increasing salinities (20, 30, 40‰); rotifers acclimated at 50‰ were transferred to decreasing salinities (40, 30, 10‰); finally, rotifers acclimated at 30‰ were transferred to increasing and decreasing salinities (20, 10, 40, 50, 60‰). Experiments on un-acclimated rotifers were otherwise identical to the acclimated experiments. For each of the three transfer experiments an aliquot of rotifers was transferred to the salinity, at which it was acclimated, to test for the effects of the transfer procedure.

Statistical analysis

Predictive models were fit to the growth rate data to examine responses to salinity and to compare responses between siblings. Responses for all three siblings were characterised by fitting polynomial models, using the non-linear curve fitting function in SAS (v8.02). The complexity (e.g. linear, 2nd, 3rd or 4th order polynomial) of each model for each sibling was determined by sequentially adding parameters; whether the addition of a parameter significantly improved the fit was then tested using a sum of squares reduction test (Zar 1999).

Comparisons between growth rate responses for each sibling were made in a similar fashion. Growth responses for the three siblings were combined and two models were fitted to the data: (1). a full regression model, in which each component of the model was allowed a separate parameter for each sibling (e.g. $y = b_1x^2 + b_2x^2 + b_3x^2$, where b_{1-3} are separate parameters for each sibling, for the component bx^2); and (2). a reduced regression model, in which each component of the model was constrained to a single parameter (e.g. $y = b_nX^2$ where b_n is a single parameter for all siblings, for the component bx^2). Thus, if all responses are the same, no difference between the full and reduced regression models occurs. Differences between models were again analysed using the sum of squares reduction test.
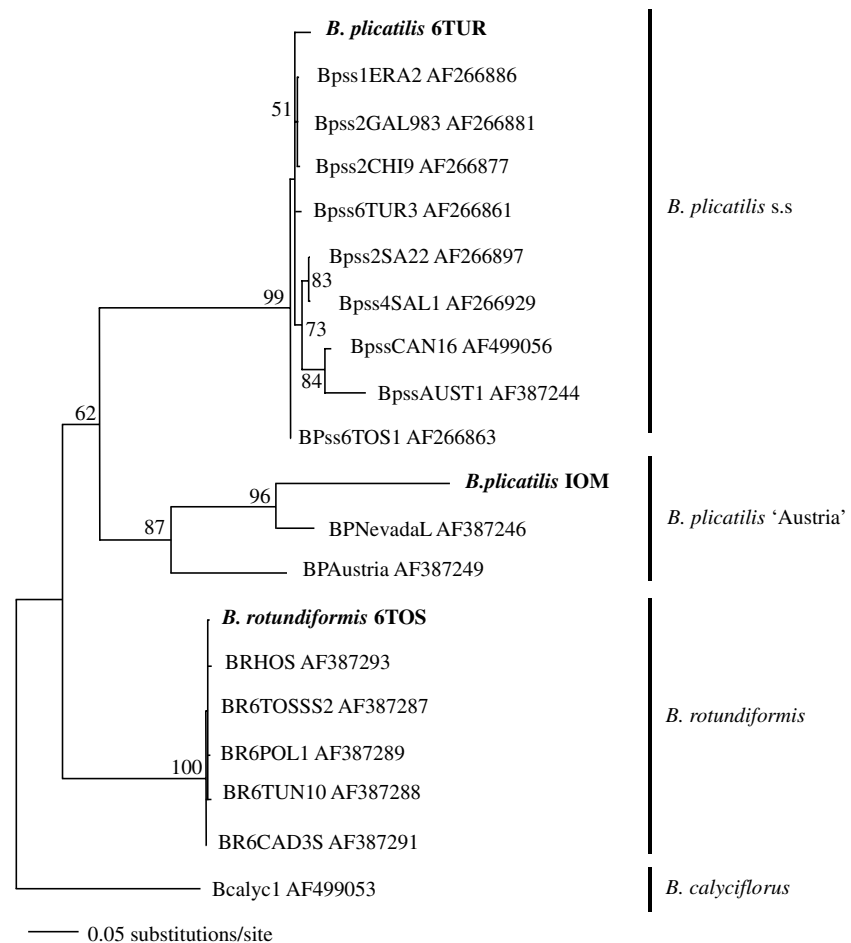
Egg development time was calculated, for both acclimated and transfer treatments, using model II linear regression (Sokal and Rohlf 1995), as the slope of growth rate vs. mean number of eggs female^{-1} as $\mu = \ln(E + 1)/\text{EDT}$, where EDT is egg development time, and E is the average number of amictic eggs female^{-1}.

Results

Phylogeny

The three *Brachionus plicatilis* isolates used in this study were placed in a phylogeny of the *B. plicatilis* species complex (Fig. 1). Phylogenetic analysis and nucleotide blast searches (Altschul et al. 1997) supported *B. plicatilis* to be an isolate from Estany den Turies, Spain (isolate shared 96% identity with Genbank entries AF266853, AF266854, and AF266855 from Estany den Turies) and *B. rotundiformis* as an isolate from Poza Sur (isolate shared 99% identity with Genbank entries AY785221 and AY785222, from Poza Sur). From here on we refer to these isolates as *B. plicatilis* 6TUR and *B. rotundiformis* 6TOS (following Gomez et al. 2000). The identity of the *B. plicatilis* isolate provided by the larval rearing centre, Isle of Man (referred to as *B. plicatilis* IOM) was unknown; the mitochondrial COI data place this isolate within the 'Austria' clade of *B. plicatilis*, which is likely a discrete sibling species (Gomez et al. 2002).

Fig. 1 The phylogenetic positions of the *Brachionus plicatilis* sibling species used in this study based on maximum likelihood analysis of a fragment the mitochondrial COI gene. The phylogeny presented is a subset of the *B. plicatilis* species complex and does not include all strains or sibling species for which sequences are available (e.g. *B. ibericus*). Numbers above selected nodes are bootstrap values (1,000 replicates) and are given where support was > 50%. Designations are as defined by Gomez et al. (2002). The GenBank accession number for the previously unstudied *B. plicatilis* IOM is DQ140386

B. plicatilis 6TUR
Bpss1ERA2 AF266886
Bpss2GAL983 AF266881
Bpss2CHI9 AF266877
Bpss6TUR3 AF266861
Bpss2SA22 AF266897
Bpss4SAL1 AF266929
BpssCAN16 AF499056
BpssAUST1 AF387244
BPss6TOS1 AF266863

B. plicatilis s.s

B.plicatilis IOM
BPNevadaL AF387246
BPAustria AF387249

B. plicatilis 'Austria'

B. rotundiformis 6TOS
BRHOS AF387293
BR6TOSSS2 AF387287
BR6POL1 AF387289
BR6TUN10 AF387288
BR6CAD3S AF387291

B. rotundiformis

Bcalyc1 AF499053

B. calyciflorus

0.05 substitutions/site

The effect of salinity on growth rate and egg development time

Over the range of salinities tested (5–60‰), all three acclimated isolates achieved positive population growth. The modelled responses are given in Fig. 2 and the parameters summarised in Table 1. The growth rate responses were significantly different between the three siblings ($F_{7,95} = 12.05$, $P < 0.001$). At all salinities *B. rotundiformis* 6TOS achieved the highest growth rate. *B. plicatilis* 6TUR exhibited the lowest growth rate (0.26 day^{-1}) at a salinity of 60‰. The growth rate responses for *B. plicatilis* 6TUR and *B. plicatilis* IOM intersected; at low salinities *B. plicatilis* 6TUR achieved higher growth rates, above 10‰ the relationship was reversed.

The shapes of the growth rate responses differed for each sibling as indicated by the complexity of the polynomial regressions fitted to each response (Fig. 2; Table 1). Growth rate for *B. plicatilis* 6TUR was relatively constant at low salinities; maximum growth rate (0.93 day^{-1}) occurred at 16‰ and decreased rapidly at high salinity. For *B. plicatilis* IOM and *B. rotundifor-*

mis 6TOS distinct decreases in growth rate occurred below 15‰; growth maximum occurred at 16‰ for *B. plicatilis* IOM (1.05 day^{-1}) and 18‰ for *B. rotundiformis* 6TOS (1.08 day^{-1}), followed by a less pronounced, relative to *B. plicatilis* 6TUR, reduction in growth rate with increasing salinity

A strong positive relationship between $\ln(E + 1)$ and μ for *B. plicatilis* 6TUR and *B. rotundiformis* 6TOS ($R^2 = 0.61$ and 0.76 respectively) indicates that salinity had only a small affect on egg development time (calculated as the slope of $\ln(E + 1)$ vs. μ, Fig. 2). For *B. plicatilis* IOM the relationship between $\ln(E + 1)$ and μ was weak ($R^2 = 0.23$) suggesting that egg development time varied with salinity. Shortest egg development time (0.6 day) occurred at 20‰ and longest development at 60‰ (average 1.0 day, data not shown).

The effect of rapid changes in salinity on growth rate

In all cases changes in salinity significantly altered growth rate relative to acclimated responses (Fig. 3; Tables 2, 3). Both the direction of the transfer and the starting salinity influenced growth rate. When

Fig. 2 The response of growth rate (μ, day^{-1}) to salinity for *B. plicatilis* 6TUR (*a*), *B. plicatilis* IOM (*b*), and *B. rotundiformis* 6TOS (*c*). Polynomial models (*solid lines*) were fitted to each response. The parameters for each model are given in Table 1 and a summary of statistical comparisons in Table 3. The relationship between μ and $\ln(E + 1)$ for *B. plicatilis* 6TUR (*d*), *B. plicatilis* IOM (*e*), and *B. rotundiformis* 6TOS (*f*). Egg development time is given as the slope of μ versus $\ln(E + 1)$
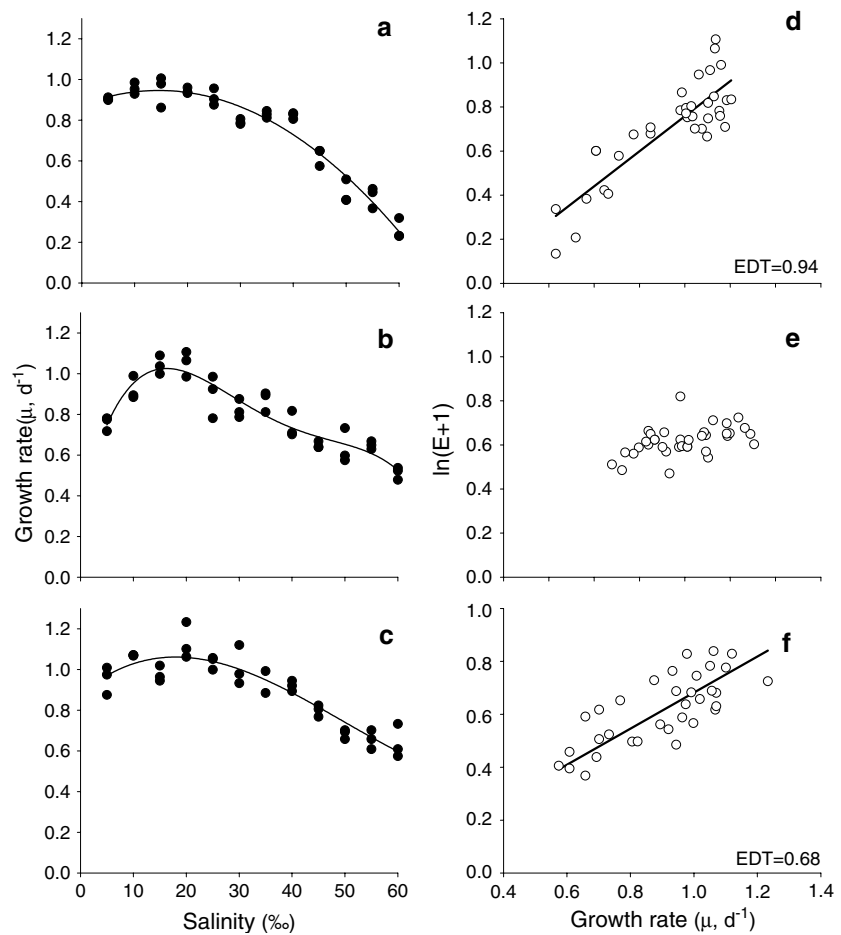

Table 1 Parameters for polynomial models of the acclimated growth rate responses to salinity for *B. plicatilis* 6TUR, *B. plicatilis* IOM, and *B. rotundiformis* 6TOS (see Fig. 2 for responses)

	B. plicatilis 6TUR		*B. plicatilis* IOM		*B. rotundiformis* 6TOS	
	Value	SE	Value	SE	Value	SE
y_0	0.87	0.034	0.35	0.098	0.82	0.063
A	9.8×10^{-3}	2.4×10^{-3}	0.103	0.019	0.031	8.1×10^{-3}
B	-3.0×10^{-4}	3.9×10^{-5}	-5.3×10^{-3}	1.1×10^{-3}	-1.1×10^{-3}	3.0×10^{-4}
C			9.6×10^{-5}	2.4×10^{-5}	8.3×10^{-6}	2.6×10^{-6}
D			-6.3×10^{-7}	1.8×10^{-7}		
R^2	0.94		0.87		0.85	

transferred upward, from low salinities (10‰) growth rate decreased (Fig. 3), and at 40‰ all individuals died immediately on transfer. Transfers upward from 30‰ also resulted in a decreased growth rate in comparison to acclimated responses, although the effect was smaller than that for transfers upward from 10‰. In contrast, decreases in salinity had a lesser impact on growth rates. When rotifers were transferred to decreasing salinities (from 30 to 50‰), growth rates increased (from 30‰) or did not significantly decrease (from 50‰).

Responses to salinity transfers were similar, for all three siblings. No significant difference in growth rate responses between *B. plicatilis* IOM and *B. rotundiformis* 6TOS occurred for any of the transfer treatments (Table 3). In all transfer experiments *B. plicatilis* 6TUR achieved lowest growth rates (Fig. 3; Table 3).

In contrast to growth rate, there was no pronounced relationship between changes in salinity and egg development time. For all siblings and treatments egg development time was constant with salinity (Fig. 4a–c), and the relationship between μ and $\ln(E + 1)$ was linear
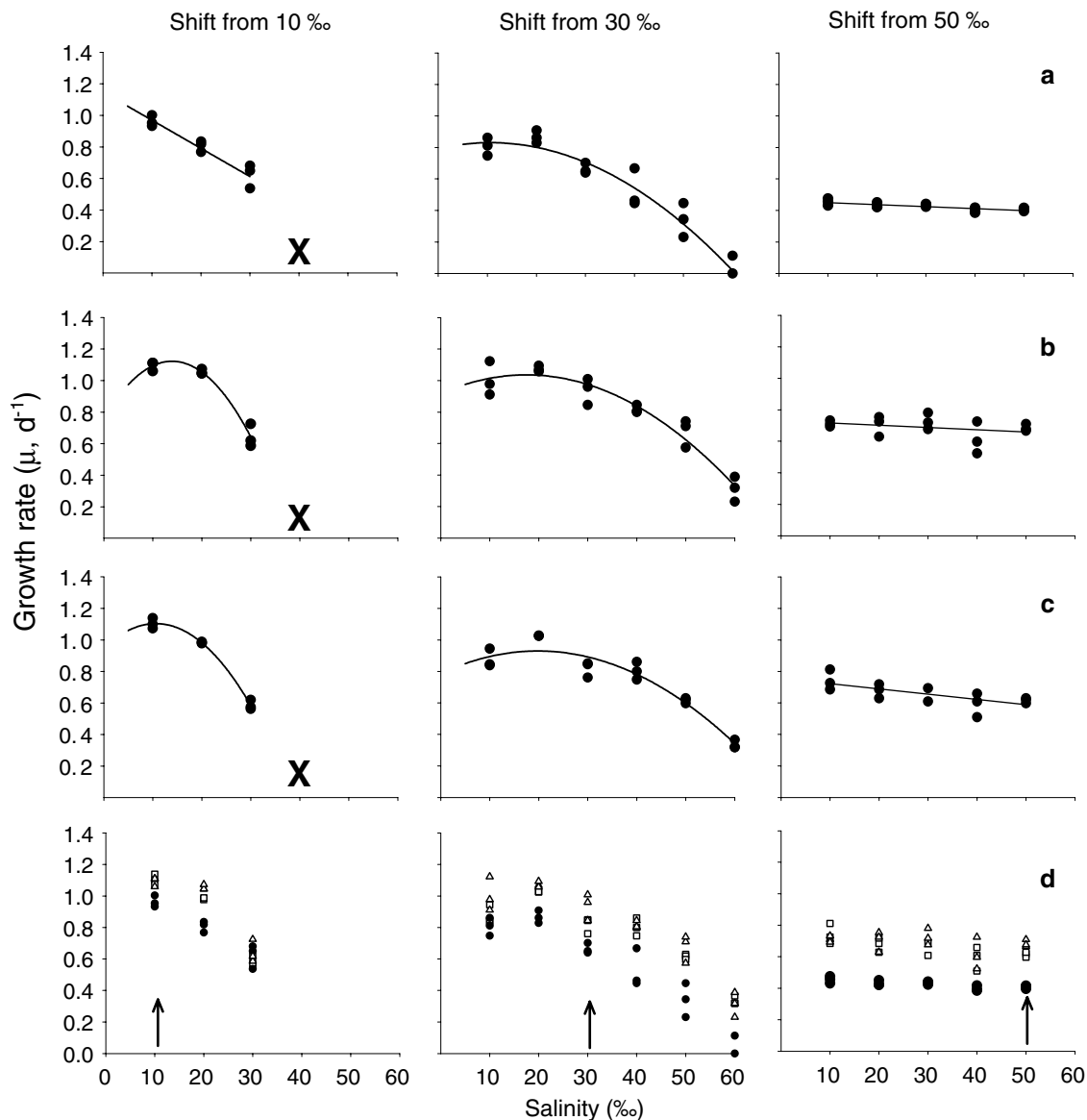
Fig. 3 The effects of transfer between salinities on population growth rates for *B. plicatilis* 6TUR (row *a*), *B. plicatilis* IOM (row *b*), and *B. rotundiformis* 6TOS (row *c*). Data for each isolate, for transfer experiment, are also plotted together for comparison (row *d*). Polynomial or linear (*B. plicatilis* 6TUR shift from 10‰) models (*solid lines*) were fitted to each response. The parameters for each model are given in Table 2 and a summary of statistical comparisons in Table 3. Cases where 100% mortality occurred on transfer to a new salinity are indicated (*x*)

(data not shown). One exception occurred, for all three siblings transfers from 30 to 60‰ resulted in a 2.4×, 3.4× and 2.5× increase in egg development time for *B. plicatilis* 6TUR, *B. plicatilis* IOM, and *B. rotundiformis* 6TOS, respectively.

Discussion

Sibling species of the *Brachionus plicatilis* complex are common brackish water organisms (King and Serra 1998), and field studies indicate that different sibling spe-

cies display differing temporal and spatial distributions, which correlate with salinity variation (Ortells et al. 2003). Initial field observations suggested that unique adaptations partition siblings and facilitate their co-occurrence (Gomez et al. 1995, 1997). However, more recent field and ecophysiological data indicate substantial overlap in the ecological tolerances of siblings, forcing a re-examination of the evolutionary mechanisms allowing sibling species coexistence (Ciros-Pérez et al. 2001a; Lapesa et al. 2004; Ortells et al. 2003).

Here we compared salinity tolerance in three sibling species by measuring growth rate and egg development

Table 2 Parameters for polynomial models of growth rate responses to salinity transfer for *B. plicatilis* 6TUR, *B. plicatilis* IOM, and *B. rotundiformis* 6TOS (see Fig. 3 for responses)

B. plicatilis 6TUR

	10‰		30‰		50‰	
	Value	SE	Value	SE	Value	SE
y_0	1.14	0.049	0.79	0.069	0.46	0.030
a	−0.017	2.3×10^{-3}	7.1×10^{-3}	4.5×10^{-3}	-1.3×10^{-3}	9.0×10^{-4}
b			-3.3×10^{-4}	6.3×10^{-5}		
B. plicatilis IOM						
Y_0	0.76	0.12	0.917	0.071	0.73	0.039
A	0.052	0.014	0.014	4.6×10^{-3}	-1.4×10^{-3}	1.2×10^{-3}
B	-1.9×10^{-3}	3.4×10^{-4}	-3.9×10^{-4}	6.5×10^{-5}		
B. rotundiformis 6TOS						
Y_0	0.95	0.094	0.79	0.068	0.76	0.038
A	0.030	0.011	0.014	4.4×10^{-3}	-3.4×10^{-3}	1.1×10^{-3}
B	-1.4×10^{-3}	2.7×10^{-4}	-3.6×10^{-4}	6.2×10^{-5}		

Numbers 10, 30 and 50 refer to the starting salinities (‰) for the unacclimated growth experiments

Table 3 Pair-wise statistical comparisons between growth rate responses to salinity treatments (* $P < 0.05$, ** $P < 0.001$). 'Ac' refers to acclimated growth responses

Numbers 10, 30 and 50 refer to the starting salinities (‰) of the unacclimated growth experiments

time over salinities between 5 and 60‰ and compared the effects of sudden transfer between salinities on these life history parameters. To our knowledge this is the broadest range of salinities over which the tolerances of defined *B. plicatilis* sibling species have been assessed (for previous studies, see Miracle and Serra 1989; Lubzens et al. 2001; Serra et al. 1998). In addition to providing a rigorous characterisation of ecophyiosological responses, the use of a broad range of salinities and the statistical comparison of salinity tolerances, using predictive models, provides useful data for future studies modelling the effects of environmental variation on sibling species distributions.

In nearly all cases, the shape and magnitude of salinity responses significantly differed between sibling species. However, the overall trends in responses to salinity were similar: all were tolerant to a broad salinities range and were robust to sudden salinity changes. In acclimated experiments, all three sibling species achieved maximum growth between 16 and 18‰ and maintained positive growth between 5 and 60‰. When transferred between salinities, sibling species maintained net positive growth over large decreases (Δ 40‰) and increases (Δ 30‰) in salinity. Net mortality occurred only in one case (transfer from 10 to 40‰) where all individuals died on transfer. Further, egg development time was invariant with changes in salinity except for *B. plicatilis* IOM in acclimated experiments and for all three sibling species where rotifers were transferred from 30 to 60‰. Below, we assess the likely relevance of the observed similarities and differences in salinity tolerance between siblings in determining their distributions.

Broad, but varying, salinity tolerances may be common in the *B. plicatilis* complex-common, broad salinity responses, and tolerance to salinity fluctuations, suggest that narrow non-overlapping tolerances as a mechanism for isolating different sibling species is unlikely. Admittedly, we have examined only a single isolate from each of three sibling species, and ideally more isolates should be assessed to support our results. Nevertheless, the isolates examined were from phyliogenetically diverse sibling species suggesting that broad salinity tolerances, and tolerance to fluctuating salinities, might be common traits in the *B. plicatilis* species complex.

Undoubtedly, there are strong selection pressures to maintain wide physiological tolerances. *B. plicatilis* rotifers are common in continental brackish water
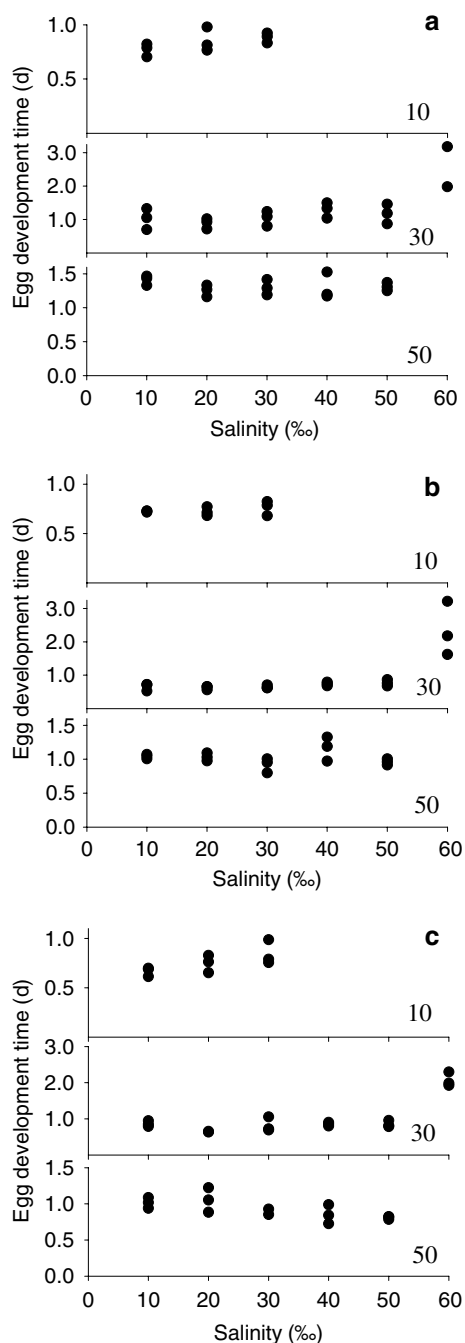
Fig. 4 The effect of salinity transfer on egg development time for *B. plicatilis* 6TUR (*a*), *B. plicatilis* IOM (*b*), and *B. rotundiformis* 6TOS (*c*). In each case the relationship between μ and ln(*E* + 1) was linear (see Materials and methods), thus egg development time was assumed to be constant with salinity. Numbers 10, 30 and 50 refer to the starting salinities (‰) for the three transfer experiments

environments, which experience broad seasonal changes in physical factors (King and Serra 1998). In such ephemeral habitats, long-term survival of rotifers depends on the production of resting eggs via sexual reproduction (Gomez et al. 1995; King and Serra

1998). However, short term survival and colonisation of new environments is likely to depend on physiological tolerance. For example, in small aquatic invertebrates, such as rotifers, the rapid establishment of large populations following dispersal to novel environments potentially "buffers" habitats from further colonisation by competitors (De Meester et al. 2002). As such, wide environmental tolerances, allowing rapid growth in new environments, are likely to be important components of dispersal and colonisation and in determining sibling species distributions.

Information on how wide salinity tolerances, and tolerances to salinity fluctuations, are maintained by *B. plicatilis* rotifers is limited, but understanding how physiological adaptations differ between siblings may elucidate the causes of their distributions. It was previously held that *B. plicatilis* was an osmoconformer with limited ability to adapt to salinity changes (Epp and Winston 1977). However, we have previously indicated that *B. plicatilis* sibling species are likely to be osmoregulators, based on the activity of the osmoregulatory enzyme Na^+/K^+ ATPase (Lowe et al. 2005a). We have further indicated that Na^+/K^+ ATPase activity correlates with differences in growth rate responses to salinity in siblings, and that the differences in ion regulatory activity were associated with variation in the expression of Na^+/K^+ ATPase gene transcripts that were highly similar between siblings (> 99% similarity, Lowe et al. 2005b). These data illustrate that differences in ecologically important adaptations between sibling species may be accounted for by relatively minor genetic variation.

How regulatory mechanisms associated with salinity tolerance differ in response to fluctuations in salinity has not been assessed, though here we indicate that growth rate differences between sibling species may be reduced by salinity fluctuations. For example, *B. rotundiformis* 6TUR achieved comparatively high-acclimated growth rates, but in all salinity transfer experiments growth rates for *B. rotundiformis* 6TOS and *B. plicatilis* IOM were the same; thus, *B. rotundiformis* was more susceptible to salinity fluctuation. Whether differences in responses to fluctuating environmental variables are ecologically important is, at this point, difficult to assess. In a general context, the potential consequences of environmental fluctuations for ecological communities have proved contentious (May and MacArthur 1972; Hebert and Crease 1980; Chesson and Huntley 1997). Nevertheless, many of the habitats in which *B. plicatilis* rotifers occur are ephemeral, and there is growing evidence suggesting strong interactions and competition between *B. plcatilis* sibling species (Ciros-Pérez et al.

2001a; Lapesa et al. 2004). Given these conditions, the reductions in growth rate associated with salinity changes, and the subsequent rate of recovery, are likely to influence the distributions of sibling species in natural environments.

Small physiological differences potentially affect sibling species distributions

Given the common broad salinity tolerances displayed by the sibling species examined in this study, the differences in the shapes of the growth rate responses, though significant, appear comparatively minor (Fig. 1). However, specific growth rate (μ) is an exponent of population growth, and as a result small differences in growth rates can result in large differences in subsequent population sizes over time. Thus, the apparently minor differences in growth rate responses to salinity assessed here have potentially significant ecological consequences. We illustrate this using a simple population model based on logistic growth, assuming that two sibling species have the same density-dependent effect on growth (i.e. a shared carrying capacity). Using STELLA 8 (High performance systems), population growth of *B. plicatilis* 6TUR and *B. plicatilis* IOM was simulated using the coupled equations $dN_{TUR}/dt = N_{TUR}\mu_{TUR}$ $[1-(N_{TUR}+N_{IOM}/K)]-$ $N_{TUR}m$ and $dN_{IOM}/dt = N_{IOM}\mu_{IOM}$ $[1-(N_{IOM}+N_{TUR}/K)]-N_{IOM}m$ where N is population density (initially 1 for both sibling species; subscripts refer to rotifer siblings), μ is the specific growth rate (determined as a function of salinity, again subscripts refer to siblings), K is the carrying capacity (100 for both siblings) and m is a density dependant loss rate (akin to losses due to e.g. predation and set at 0.1, for both sibling species, Brandl 2005). The model was applied at three salinities (Fig. 5), using the growth rate response parameters given in Table 2.

The model outputs (Fig. 5) illustrate both the potentially large differences in population size resulting from relatively small differences in salinity tolerance and how different sibling species might dominate under differing conditions. Although these are simple simulations, they highlight an important point: the distribution of a sibling species may be constrained well within its potential distribution based on physiological tolerances by, for example, interactions with other sibling species. Notably, we have not tested for competition between siblings in this study, and the inclusion of an interaction between sibling species (i.e. shared density dependence) in the model is speculative. However, as sibling species are by definition highly similar and are likely to share many ecological
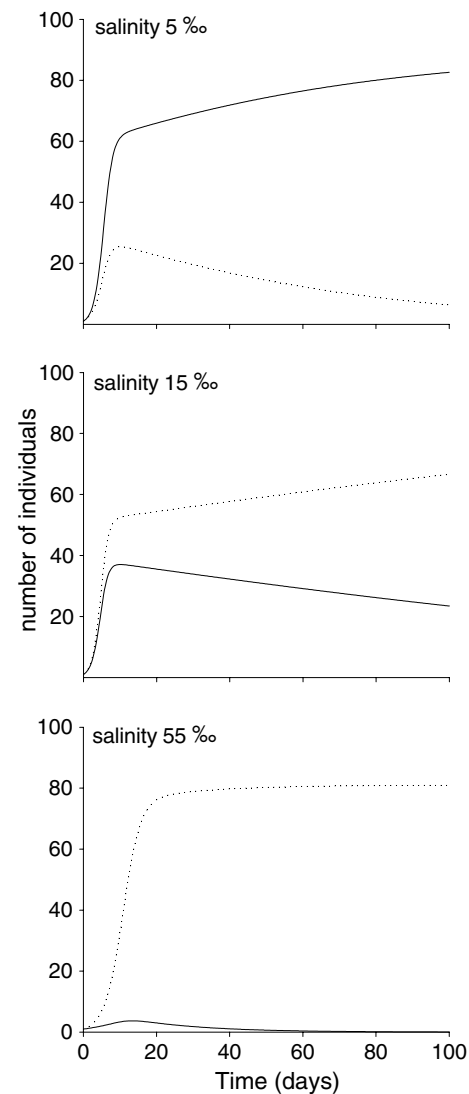

Fig. 5 The modeled population dynamics of *Brachionus plicatilis* IOM (*solid lines*) and *B. plicatilis* 6TUR (*dotted lines*) at salinities of 5, 15 and 55‰. Specific growth rate responses to salinity were determined using parameters from Table 1

niche parameters (Ortells et al. 2003; Serra et al. 1998), the assumption of competition between sibling species seems reasonable. Indeed, there is now strong evidence that *B. plicatilis* sibling species compete for resources and are capable of out-competing each other (Ciros-Pérez et al. 2001a). In this context, subtle differences in physiological tolerances (e.g. to salinity) might significantly influence sibling species distributions and abundance by mediating biological interactions. Future direct competition experiments to fully parameterise the impact of salinity and other key factors on sibling species interactions will undoubtedly provide information regarding the mechanisms for sibling species isolation.

The role of multiple environmental variables
in determining distributions

Although salinity is undoubtedly a major factor influenc-
ing *B. plicatilis* sibling species distributions, ecological
niches are potentially defined by a number of environ-
mental variables. Multivariate analyses of sibling species
abundances in natural habitats highlight temperature,
alkalinity and oxygen concentrations (in addition to salin-
ity) as principle parameters differentiating distributions
(Gomez et al. 1995; Ortells et al. 2003), though it is now
recognised that overlaps in sibling species distributions
are common and substantial (Ortells et al. 2003). These
field investigations require parallel multifactor experi-
mental studies to provide precise ecophysiological
responses to asses the impacts of both direct physiological
limitation and potential biological interactions on distri-
butions. Some multifactor experimental approaches have
been attempted. For instance, Gomez et al. (1997) dem-
onstrated that salinity and temperature have an interac-
tive effect on the growth rates of *B. plicatilis* with
interactive effects differing between sibling species. As
Gomez et al. (1997) assessed interactions using only three
salinities and three temperatures the data are insufficient
to parameterise limits to physiological tolerance or build
multifactor interactions into potential ecosystem models.
Clearly the next step will be to combine field data with
multifactor experimental approaches to assess the inter-
active effects of key environmental variables on sibling
species distributions (e.g. Kimmance et al. 2006).

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