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Long-term effects of salinity and disturbance regime on active and dormant crustacean communities

Aline Waterkeyn,[a,b,*] Bram Vanschoenwinkel,[a] Hanne Vercampt,[a] Patrick Grillas,[b] and Luc Brendonck[a]

[a] Laboratory of Aquatic Ecology and Evolutionary Biology, Katholieke Universiteit Leuven, Leuven, Belgium
[b] Research Center for Mediterranean Wetlands Tour du Valat, Arles, France

Abstract

In a large-scale and long-term outdoor mesocosm (400-liter) experiment, we studied the interacting effects of salinity and disturbance (hydrological) regime on the active and dormant crustacean communities of Mediterranean temporary wetlands (Camargue, southern France). Sixty-four mesocosms, inoculated with a regional species pool (mixed dormant egg banks), were exposed to a full factorial treatment combination of four salinity levels and four disturbance regimes during three consecutive years. Both in the active and dormant community component, considerable shifts in community composition occurred because of direct and probably also second-order effects of the treatments. All large branchiopod species had low long-term salinity tolerances and showed species-specific preferences for disturbance regimes according to their life cycle strategy. The highest salinity (5) was not limiting for cladocerans and ostracods, which thrived in the absence of the competitively stronger, predatory, and bioturbating large branchiopods. Copepods were negatively associated with salinity and coped better with the imposed biotic pressure. Zooplankton diversity and density peaked in intermediate-disturbance regimes, probably because only specialized species survived the high-disturbance regimes, whereas at low-disturbance frequencies high densities of predatory *Triops* controlled zooplankton communities. Although crustacean dormant egg banks can temporarily buffer against unfavorable conditions, persisting bad conditions may lead to their exhaustion within 4 to 10 yr. Predicted aridification (leading to more intense disturbance regimes) may result in the loss of late successional species (chydorids and ostracods), whereas salinization may wipe out sensitive freshwater species such as large branchiopods.

Global climate change is recognized as a major threat for species survival and health of natural ecosystems worldwide (Hulme 2005). Wetlands in general and temporary wetlands in particular are among the ecosystems most threatened by climate change, since they are very sensitive to changes in quantity and quality of their water supply (Pyke 2005; Erwin 2009). Climate change can result in changes in precipitation patterns, runoff, temperature, and sea level; parameters that affect wetlands in two fundamental ways: quantitatively, the number of functioning wetlands may decline, and qualitatively, the remaining wetlands may undergo shifts in their hydrological cycles and water quality (including salinity) (IPCC 2007; Erwin 2009; Nielsen and Brock 2009). In addition, the growing demands for irrigation and drinking water imply increased anthropogenic stress that may alter the water balance and water quality of wetlands (Angeler 2007; IPCC 2007; Nielsen and Brock 2009). Any hydrological modification or change in salinity is predicted to strongly affect species composition and ecosystem functioning of wetlands and result in shifting species distributions and extinctions, especially for species that are already at the edge of their ecological range (Poff et al. 2002; Nielsen and Brock 2009). This is particularly true across more fragmented landscapes because of the continuous decline in wetland number and anthropogenic alteration of the environment, limiting the ability of species to reach suitable habitats.

Vulnerability to climate change will be highest in semiarid and arid regions, where precipitation is concentrated in a few months and year-to-year variation is high (IPCC 2007). This is also the case in the Mediterranean region, where many wetlands are temporary, with a recurrent dry phase in summer. Because of their shallow depth and small volume, Mediterranean temporary wetlands in general are poorly buffered against changes in hydrology and are thus exceptionally susceptible to climate change, whose adverse effects may aggravate the effect of other anthropogenic stressors, such as destruction or change of the original habitat, pollution, irrigation, and drainage (EPCN 2008). Several field surveys already demonstrated that both salinity and hydroregime (also indicated as "disturbance regime" because of recurring drought acting as a disturbance; Wellborn et al. 1996; Vanschoenwinkel et al. 2010) are the most important factors shaping plant and invertebrate communities and influencing diversity in Mediterranean temporary wetlands (Boix et al. 2008; Waterkeyn et al. 2008). Hydrological modifications and changes in salinity therefore constitute important threats to the unique biodiversity supported by this priority habitat for conservation (Habitat Directive, Natura code 3170, 92/43/CEE, 21 May 1992) (Zacharias et al. 2007).

In general, increased salinity has a negative effect on biodiversity, with predicted greatest diversity reduction in wetlands that were originally fresh (Nielsen and Brock 2009). Changes in disturbance regimes, on the other hand, are predicted to cause shifts in species composition toward

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* Corresponding author: aline.waterkeyn@bio.kuleuven.be

species with different life cycles or species from different successional stages (Pyke 2005; Nielsen and Brock 2009). Since modified hydroregimes and salinities often go along with changes in other wetland characteristics, such as water quality, turbidity, or vegetation structure, or with biotic pressure by adding or removing species (Wellborn et al. 1996; Nielsen et al. 2003*a*; Boven et al. 2008), there may also be second-order effects. Even more, entire regime shifts (Scheffer et al. 1993) can be triggered by the salinity-mediated presence or absence of keystone species (Jeppesen et al. 2007; Waterkeyn et al. 2010).

For temporary wetlands, there is a particular interest in understanding the effects of environmental change on the permanent inhabitants (mainly crustacean large branchiopods and zooplankton), as they are probably most affected by changes to their habitat, not being able to actively escape (Angeler et al. 2008) and since they fulfill an important function in the habitat as dominant primary consumers. Permanent residents mostly rely on their dormant egg banks to persist under unsuitable conditions (Brendonck and De Meester 2003) and these biotic reservoirs consequently play a crucial role in their resilience to disturbance (Angeler and Garcia 2005). However, for this to be a successful survival strategy, species need to hatch fractionally corresponding with chances for successful recruitment (Cohen 1966; Brown and Venable 1986), grow in time to maturity, and produce sufficient numbers of dormant eggs to replenish the dormant egg bank to compensate for losses due to hatching and mortality. Under more saline conditions, freshwater organisms are unlikely to hatch optimally and to survive until reproduction (Nielsen and Brock 2009; Vanschoenwinkel et al. 2010). Failure to reproduce (abortive hatching) during several consecutive seasons may cause depletion of the dormant egg bank and eventually extinction of the local populations.

The interacting effects of disturbance regime and salinity on the dormant and active communities of permanent inhabitants of temporary wetlands remain to be studied. Also, since dormant egg banks have the capacity to conceal the first signs of deterioration because of their buffering capacity (partial hatching) there is a need to investigate the effect of stressors over several seasons and multiple generations. In this study, we attempt to experimentally investigate the effect of interacting stressors on a community level over a long term. We exposed a regional species pool of permanent wetland inhabitants, starting from dormant eggs, to different salinity levels and disturbance regimes in mesocosms for three consecutive years. Our experiment was designed to investigate community differentiation (community trajectories) over time and to compare long-term responses in active and dormant community components. We hypothesize that high disturbance regimes (i.e., with short unpredictable hydroperiods) and more saline conditions will result in a lower diversity and lower egg bank size, particularly in typical freshwater species (such as many large branchiopods) and species with long life cycles or from late successional stages. On the other hand, it is also possible that increased salinity will modify biotic interactions by excluding the salt-sensitive large branchiopods (including the predator *Triops*), potentially promoting diversity of prey species (second-order effects). Results will be discussed in the light of predicted scenarios of climate change and water management.

This experiment is a follow-up of a previous study in which the effect of salinity on the succession of permanent inhabitants of temporary wetlands was assessed during only one inundation cycle (Waterkeyn et al. 2010).

Methods

Study site—This study was carried out in the Camargue, a Mediterranean wetland area situated in the Rhône delta (southern France) consisting of a mosaic of salt pans, lakes, lagoons, saline and freshwater marshes, and temporary ponds. The wetlands in this region are naturally characterized by variable and sometimes elevated salinity levels due to the proximity of an underlying saline aquifer. The Camargue is a region of high ecological value, where wetlands fulfill important ecosystem services and play an important role in maintaining biodiversity. Nevertheless, this region is also a heavily managed complex hydrosystem where massive amounts of freshwater from the Rhône River are pumped into the deltaic plain via a dense network of irrigation channels, mainly for agricultural purposes (flooded rice cultivation).

Predicted climate change effects in the Camargue are, on the one hand, intensified drought due to increased temperatures (enhancing evaporation) and less evenly spread more intense rainfall. Sea-level rise, on the other hand, will cause more seawater intrusion and a rise of saline groundwater tables (Pont et al. 2002). Depending on the relative contributions of future water management schemes and climate change, different scenarios of environmental effect are possible. Aridification may lead to loss of ephemeral wetlands and a general shift toward shorter, more unpredictable hydroperiods (more intense disturbance regimes) and more saline conditions. On the other hand, rising groundwater tables may induce more saline, longer hydroperiods (low-intensity disturbance regimes) in wetlands near the coast. Conversely, intensifying agriculture could lead to even more irrigation, desalinizing wetlands and altering disturbance regimes.

The experiment was conducted on the experimental field (1200 m^2) of the research center Tour du Valat. The estate of Tour du Valat encloses approximately 50 temporary and 6 semipermanent wetlands of varying salinities and disturbance regimes. Most wetlands are filled during the wet autumn and winter seasons and dry up in late winter to early summer, often with a strong salinity rise at the end of the inundation. Both flooding and drying dates fluctuate considerably between years, depending on rainfall pattern. The wetlands are not subjected to any direct water management and occur on extensive pasture land for local breeds of horses and cattle. For a more detailed description of the study site *see* Waterkeyn et al. (2008).

Experimental design—During the summer of 2006, dry surface sediment (top 3 cm) was collected from 15 pristine temporary freshwater (< 2.0 when fully inundated)

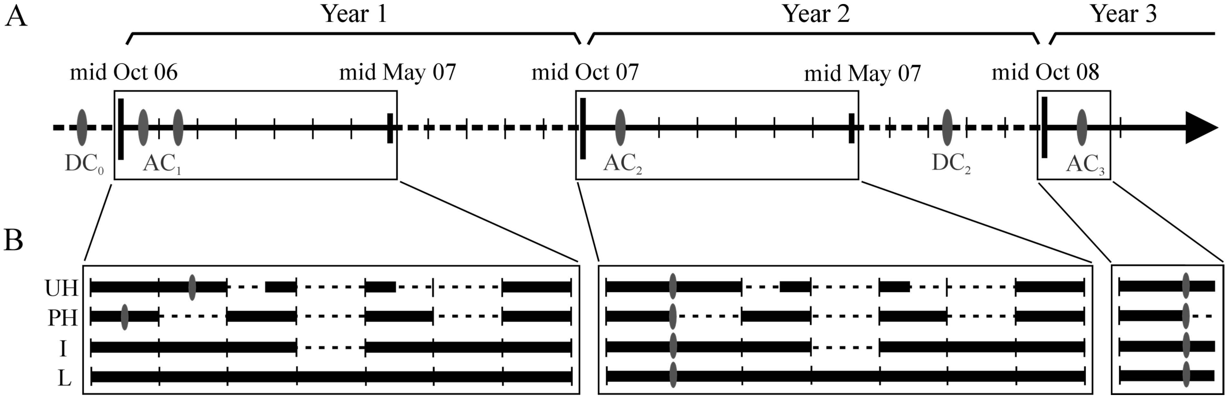

Fig. 1. (A) Time line (vertical lines indicate months) of the mesocosm study, with indication of the natural flooded (full horizontal line) and dry phases (dotted horizontal line); gray vertical marks indicate sampling events of the entire active (AC, with the number indicating in which year the sample was taken) or dormant (DC, with the number indicating in which year the sample was taken) crustacean community. (B) Detailed scheme of each of the three flooded phases with indication of the different experimental disturbance regimes (predictable high-disturbance regime = PH, unpredictable high-disturbance regime = UH, intermediate-disturbance regime = I, and low-disturbance regime = L) applied during each flooded phase (full horizontal line = inundated; dotted horizontal line = dry). Gray vertical marks indicate different sampling events of the entire active community.

wetlands varying in disturbance regime (hydroperiods ranging from 3 to 9 months). From each wetland, 28 sediment samples of 0.25 m^2 (upper 3 cm) were collected according to a grid (14 from the marginal and 14 from the central zone), resulting in a total of 7 m^2 of sediment per wetland. The sediment of all wetlands was mixed using a concrete mixer. In total, 64 mesocosms (1.0-m^2 tanks; 600 liters) were inoculated with a 3-cm layer of pooled sediment, assigned to 1 of 16 treatments arranged in a randomized block design (four blocks; one replica per block). Each treatment was a combination of one of four salinity levels (0.5, 1.0, 2.5, and 5.0) with one of four disturbance regimes (predictable high-disturbance regime = PH, unpredictable high-disturbance regime = UH, intermediate-disturbance regime = I, and low-disturbance regime = L). The mesocosms were exposed to the treatments from October 2006 to November 2008 (Fig. 1).

Mesocosms were inundated during the seven "flooded" months of each year (from mid-October to mid-May), and kept dry during each dry phase (mid-May to mid-October) (Fig. 1). Each year, mesocosms with high-disturbance regimes (H) were inundated four times for on average 1 month; mesocosms with intermediate-disturbance regimes two times for 3 months; mesocosms with low-disturbance regimes stayed inundated throughout the 7 months (Fig. 1). The two high-disturbance regime treatments differed in length of individual inundations and dry periods, separating them to mimic predictable (PH) and unpredictable (UH) hydroperiods.

Mesocosms were filled to a depth of 40 cm (400 liters) using local nonchlorinated tap water. Salinity levels were created using sea salt from local salt works in the Camargue (Salin de Giraud). In the lowest salinity level (0.5) no salt was added. In the third year, treatments were stopped after all samples had been taken (5 weeks after the start of the flooded phase). To keep the water levels and salinity values constant during inundations or to keep the sediment dry when needed, mesocosms were covered with a transparent plastic lid during rains, while water was added to compensate for evaporation. All tanks were covered with a 500-μm net to prevent colonization by flying invertebrate predators, amphibians, or birds. To simulate the gradual process of drying out at the end of each inundation, water level was lowered by gradually removing water and increasing salinity during the three last weeks of the inundation. Twice a week 6.5 cm of water was removed using a pump fitted with a 64-μm net to avoid loss of zooplankton. Loss of salt due to removal of water was compensated by adding a corresponding amount of salt in the remaining mesocosm water.

Sampling—Both the active and dormant crustacean communities were sampled. Active communities were sampled during the first month of inundation in each of the three flooded phases (Fig. 1). Dormant communities (dormant egg banks) were sampled before the start of the experiment and during the dry phase following the second series of inundations.

The active communities of the rapidly developing large branchiopod communities were monitored more closely by sampling them weekly during the first month of inundation. They were caught with a 1-mm aquarium net (15 sweeps, catch area 450 cm^2), counted, and identified according to Defaye et al. (1998) and afterward returned to the tanks.

The active zooplankton communities needed more time to develop and were therefore only sampled at the end of the first month of the first inundation of each of the three flooded phases, together with the large branchiopods (active communities AC_1, AC_2, and AC_3 in Fig. 1). As no samples were taken at comparable times during the first flooded phase, we used mean zooplankton densities of samples taken after weeks 2 and 6 of the first inundation (Fig. 1). Since in the first year disturbance regime could not have an effect yet, we did not sample all mesocosms; only four replicates per salinity treatment were used to test for the effect of salinity. Before taking zooplankton samples, the water in the tanks was mixed to homogenize the community. Afterward, a sample of 40 liters was taken by submerging a 5-liter beaker at different places in the mesocosm. The water was filtered over a 64-μm net and samples were stored in 70% ethanol. Zooplankton was counted and identified under a stereomicroscope, or a higher-resolution microscope when needed. Cladocerans were identified according to Alonso (1996) and Flößner (2000). *Simocephalus* and *Ceriodaphnia* specimens were identified to genus level, all others down to species level. We included Calanoida, Cyclopoida (Copepoda), and Ostracoda as additional taxa. For zooplankton, subsamples of at least 300 cladocerans were counted. The densities of the different zooplankton taxa were expressed as number of individuals per liter.

During each sampling, standard environmental variables were assessed. Conductivity (mS cm^{-1}), pH, and oxygen concentration (mg L^{-1}) were measured using Wissenschaftlich-Technische Werkstätten meters (WTW conductivity meter 330i, oxygen meter 315i, pH meter 340). Chlorophyll *a* concentration (mg L^{-1}) was determined using the methanol extraction method (Talling and Driver 1963) with a portable spectrophotometer Hach DR2400. Water transparency was determined using a Snell's tube (cm visibility). Total submerged vegetation cover (%) was estimated.

The dormant crustacean community was sampled before the start of the experiment (dormant community: DC_0 in Fig. 1) by taking four sediment samples (500 g) from the pooled sediment that was used to inoculate the mesocosms. Dormant egg banks were also sampled after the second flooded phase (DC_2 in Fig. 1) by taking three dry sediment samples using a core sampler (diameter 5.2 cm, depth 3 cm) along one diagonal cross-section in each mesocosm. In the laboratory, dormant eggs were isolated from the sediment using the sugar floatation method (Onbé 1978; Marcus 1990) and stored in 70% ethanol. Afterward, they were counted and identified using available literature (for Cladocera: Flößner 2000; Vandekerckhove et al. 2004; for large branchiopods: Thiéry and Gasc 1991; Defaye et al. 1998). Only propagules without external signs of degradation were deemed viable and used in our analyses. Finally, dormant egg bank densities were calculated as number of viable dormant eggs per 100 g of dry sediment. These densities were compared with dormant egg bank densities in the sediment before the start of the experiment to determine the loss or gain of dormant eggs in the sediment. Unfortunately, dormant egg bank densities of copepods and ostracods could not be identified because of the small size or lack of distinguishing characteristics of their dormant eggs.

Data analyses—To investigate the effect of salinity, disturbance regime, and the interaction between both factors on the active and dormant crustacean communities, we used both multivariate (community structure) and univariate (taxon richness and abundances) statistics.

To study the effect of the treatments and their interaction on the community structure of each of the sampled active (AC_1, AC_2, and AC_3) and dormant (DC_2) crustacean communities, we opted for redundancy analyses (RDA) since detrended correspondence analyses indicated a dominance of linear gradients (CANOCO 4.5; Microcomputer Power). The statistical significance of different constructed models was assessed using Monte Carlo permutation tests ($n = 999$). Abundance data of all species were logarithmically transformed. To next visualize the community trajectories (changes in active community structure) over the three consecutive years in the different salinities, treatments, and disturbance regimes, ordination diagrams of principal components analyses (PCA) of the species data were made with the interaction factor between salinity or disturbance regime and year plotted as supplementary variables. Similarly, the effect of the applied treatments on the measured environmental variables during the three seasons was tested using RDA.

Since we focused on the long-term effects of exposure to the salinity and disturbance regime treatments, we then focused on the response of the dormant community after 2 yr (DC_2) and the hatching community in the subsequent year (AC_3) using univariate analyses. Analyses were performed separately for large branchiopods and zooplankton. Factorial two-way ANOVA was used to investigate the effect of both experimental treatments and their interaction on cumulative large branchiopod species richness and maximum abundance of each species reached during the first 4 weeks of the flooded phase. The same was done for total zooplankton taxon richness and abundance of separate groups (nonchydorids, chydorids, copepods, and ostracods) after 4 weeks of inundation (STATISTICA 8.0; Statsoft). Similarly, we tested for the change in dormant egg bank size during the two first years of the experiment (DC_2–DC_0). All abundances were logarithmically transformed to remove heteroscedasticity. The effect of the treatments on the environmental factors in the mesocosms in the third year was also analyzed using factorial two-way ANOVA.

To finally test for the potential role of second-order effects, especially through biotic interactions between large branchiopods and zooplankton, we used variation partitioning to disentangle the effect of large branchiopod density from the applied treatments on the zooplankton community. Variation partitioning was applied using RDA models of zooplankton community structure in year three (AC_3), with large branchiopod density as explanatory variable and the interaction term between salinity and disturbance regime as a covariable. Similarly, we tested for the effect of large branchiopod density on the environmental variables in the mesocosms.

Results

General patterns—Temporary wetland crustaceans that hatched from the sediment over all treatments included five species of large branchiopods (with *Triops cancriformis* being the most dominant one: relative abundance of 71% over the whole experiment), 14 cladoceran taxa (with *Daphnia magna* being the most dominant one: relative abundance of 54%), calanoid and cyclopoid copepods, and ostracods (Table 1). Both disturbance regime and salinity had a significant effect on the structure of the active

Table 1. Taxon list of the crustaceans encountered in this study, with indication of the taxa abbreviations.

Taxon	Abbreviation
Anostraca	
Tanymastix stagnalis (Linnaeus 1758)	*Tan sta*
Branchipus schaefferi Fischer 1834	*Cra sch*
Chirocephalus diaphanus Prévost 1803	*Chi dia*
Notostraca	
Triops cancriformis cancriformis (Bosc 1801)	*Tri can*
Spinicaudata	
Imnadia yeyetta Hertzog 1935	*Imn yey*
Nonchydorid Cladocera	
Daphnia magna Strauss 1820	*Dap mag*
Daphnia atkinsoni Baird 1859	*Dap atk*
Daphnia curvirostris Eylman 1887	*Dap cur*
Simocephalus sp.	*Sim* sp.
Ceriodaphnia sp.	*Cer* sp.
Moina brachiata (Jurine 1820)	*Moi bra*
Macrothrix hirsuticornis Norman and Brady 1867	*Mac hir*
Chydorid Cladocera	
Chydorus sphaericus (O.F. Müller 1776)	*Chy sph*
Alona rectangula Sars 1861	*Alo rec*
Alona elegans Kurz 1874	*Alo ele*
Alona azorica Frenzel and Alonso 1988	*Alo azo*
Pleuroxus aduncus (Jurine 1820)	*Ple adu*
Pleuroxus letourneuxi (Richard 1888)	*Ple let*
Dunhevedia crassa King 1853	*Dun cra*
Copepoda	
Calanoida	Cal cop
Cyclopoida	Cyc cop
Ostracoda	
Unidentified	Ostracod

crustacean community in each year as well as on the structure of the dormant egg banks (Table 2). In the 3 yr both factors together explained between 42.5% and 78.5% of the variation in the active and 30% of the variation in the dormant community structure.

The community trajectories over three consecutive flooded phases in the different salinity and disturbance regime treatments are visualized in Fig. 2. In the first year, overall community structure in intermediate-salinity medium (2.5) was in between the structure realized at the two lowest (0.5 and 1.0) and the highest (5.0) salinity levels. From the second year onward, two clear trajectories appeared with different end points, grouping the communities in the two lowest salinities on the one hand and the intermediate and high salinities on the other (Fig. 2A). This shift in community structure in the intermediate salinity levels toward the structure in high-salinity treatments is mainly caused by a pronounced large branchiopod density drop in the intermediate- and high-salinity treatment starting from the second year, whereas the densities of most nonchydorids (mainly *D. magna* and *Simocephalus* sp.), all chydorids, and ostracods gradually increased. Copepods, on the other hand, became gradually more abundant in the two lowest salinities. In the first year no effect of disturbance regime could be measured because at the moment of sampling (1 month after inundation) there was not yet a difference in disturbance regime (Fig. 2B). In the third year, the community trajectory in the intermediate disturbance regime differed strongly from those in the other disturbance regime treatments. The densities of many zooplankton taxa (most nonchydorids, all chydorids, and ostracods) strongly increased in the intermediate disturbance regime in the third year, whereas for copepods and large branchiopods no clear temporal disturbance regime-related trend was found.

Long-term effect on the active community—Salinity and disturbance regime together explained almost 80% of the variability in active community structure in the third year (AC_3), with disturbance regime explaining only half of the variation explained by salinity (22.7% vs. 45.8%) (Table 2). The interaction between both factors also had a significant effect on community structure, but it was weaker than both main effects. Measured environmental characteristics in the mesocosms were also significantly influenced by the applied treatments (salinity: explained variation = 22.3%, *F*-ratio = 5.738, $p = 0.001$; disturbance regime: explained variation = 19.1%, *F*-ratio = 4.720, $p = 0.001$; interaction: explained variation = 24.4%, *F*-ratio = 3.794, $p = 0.001$). Both oxygen concentration and pH were highest in the unpredictable high-disturbance regime, especially in the 1.0 salinity level (Table 3). Transparency was lowest at the two highest salinities, especially in the low-disturbance regime. Chlorophyll *a* concentration was highest in the two lowest salinity treatments, especially in the low-disturbance regime. Macrophyte cover was highest in the long (but only at the two highest salinity treatments) and intermediate disturbance regime (at all salinity treatments).

After being exposed for 2 yr to the different treatments, all large branchiopods were negatively affected by salinity, with no hatching in the highest salinity (5.0) and rather low densities in the 2.5 salinity level (Table 3, Fig. 3). Disturbance regime also had a significant effect on all species, except on *Chirocephalus diaphanus*. The two other anostracan species and *Imnadia yeyetta* performed better in the high-disturbance regime, whereas *T. cancriformis* did better in the low-disturbance regime. Overall, large branchiopod species richness was highest in the lowest salinities, but experienced no overall effect of disturbance regime.

The four zooplankton groups showed different responses. Nonchydorid cladocerans (consisting for ca. 91% of *Daphnia*), chydorids, and ostracods were significantly positively associated with salinity, reaching highest densities in the two highest salinities (Table 3, Fig. 4). They were also most abundant in the intermediate-disturbance regime. Copepods, on the other hand, were most abundant in the lowest salinity (0.5), except in the low-disturbance regimes where 1.0 salinity level appeared optimal.

Long-term effects on the dormant community—The applied treatments had a significant effect on the dormant egg bank composition after being exposed for 2 yr (DC_2) (Table 2; Fig. 5). We found significant changes in dormant egg bank sizes for several taxa in relation to salinity or

Table 2. Results of redundancy analyses (RDA) on the active (AC) and dormant (DC) crustacean community structure for each sampling.

Year	Explanatory variables	Explained variance (%)	F-ratio	p-value
AC_1	Salinity	50.2	20.16	0.001
AC_2	Salinity	24.5	6.50	0.001
	Disturbance regime	18.0	4.38	0.001
	Salinity × disturbance regime	21.2	3.12	0.001
DC_2	Salinity	12.4	2.071	0.010
	Disturbance regime	17.2	3.047	0.001
	Salinity × disturbance regime	22.0	1.614	0.015
AC_3	Salinity	45.8	16.89	0.001
	Hydroregime	22.7	5.88	0.001
	Salinity × disturbance regime	15.2	4.97	0.001

disturbance regime (Table 4), often coinciding with patterns in the active community (AC_3). Still, we found fewer significant effects than for the active communities. *Tanymastix stagnalis* dormant egg bank size significantly decreased in all treatments, except in the predictable high-disturbance regime and the highest salinity level (Fig. 6). Because of the low numbers and the large variability in data, no significant effects could be found for *Branchipus schaefferi* and *C. diaphanus*. The dormant egg bank densities of *Triops cancriformis* increased significantly in the two lowest salinities, especially in the low-disturbance regime. There was a nonsignificant trend of increased *I. yeyetta* dormant egg banks in the lowest salinity. Non-chydorid dormant egg banks increased significantly in the intermediate-disturbance regime and under the highest salinities. Chydorid dormant egg bank size significantly increased in the intermediate-disturbance regime (only at the 2.5 salinity level) and in the low-disturbance regime (only at the two highest salinity levels).

Indirect effects of large branchiopods on zooplankton communities—When corrected for the different treatments, large branchiopod density significantly influenced zooplankton community structure (F-ratio = 3.433; p = 0.024), with most taxa being negatively affected. Nevertheless, the amount of explained variance was very low (1.1%) due to the strong correlation between large branchiopod density and the applied treatments. However, a clear pattern of a negative relationship (except for the copepods) emerged when the density of different zooplankton groups was plotted against large branchiopod density (Fig. 7). Not only the biota, but also the measured environmental characteristics were significantly affected by large branchiopod density in the mesocosms (F-ratio = 2.840; p = 0.040; explained variation = 2.0%), with water transparency and macrophyte cover being negatively affected, contrary to chlorophyll *a* concentration.

Discussion

In less than 3 yr, both disturbance regime and salinity were able to alter the active crustacean communities and dormant egg bank dynamics of temporary wetlands via both direct and indirect effects.

Direct and indirect effects of salinity—Salinity was more important in explaining the structure of active crustacean communities than disturbance regime. The long-term response to salinity confirmed to a large extent the response to this variable documented during the first inundation (Waterkeyn et al. 2010). Large branchiopods reached their highest densities under the two lowest salinities (0.5 and 1.0) and were virtually absent from the high-salinity treatment (5.0). *T. cancriformis* and *I. yeyetta* even increased their dormant egg bank sizes in the low salinities. Although after one inundation it was concluded by Waterkeyn et al. (2010) that the intermediate (2.5) salinity level still is a fairly good condition for the hatching and survival of freshwater large branchiopods (average densities of 72 individuals per mesocosm), extending the exposure for two more years revealed that densities dropped to around five individuals per mesocosm. A possible explanation for this pattern is a sublethal effect of this salinity treatment allowing survival for some time but reducing reproductive output. As such, the hatched individuals observed during the first year might not have succeeded in replenishing the dormant egg bank, with negative consequences for the next hatching cohorts. Nonetheless, we did not find evidence of significantly decreased dormant egg densities in the 2.5 salinity level, possibly due to high variability among replicates or reduced hatching under unfavorable salinity conditions. Although Camargue large branchiopods are probably locally adapted to higher salinities than published tolerance limits (Waterkeyn et al. 2009), apparent tolerance reduced after long-term exposure and was therefore lower than what was concluded after a short-term exposure (Waterkeyn et al. 2010). Expected increased salinity in the wetlands due to rising of the saline groundwater table and increasing evaporation rates as predicted by climate change scenarios might therefore be more detrimental than earlier assumed. The interplay of salinization and reduction of successful inundations that last long enough for reproduction to take place is likely to drastically reduce the number of wetlands suitable for freshwater large branchiopods.

Copepod numbers were also negatively associated with salinity and performed better in the lowest salinity levels. The applied salinity range in our mesocosm study was, however, not limiting for most cladocerans and ostracods

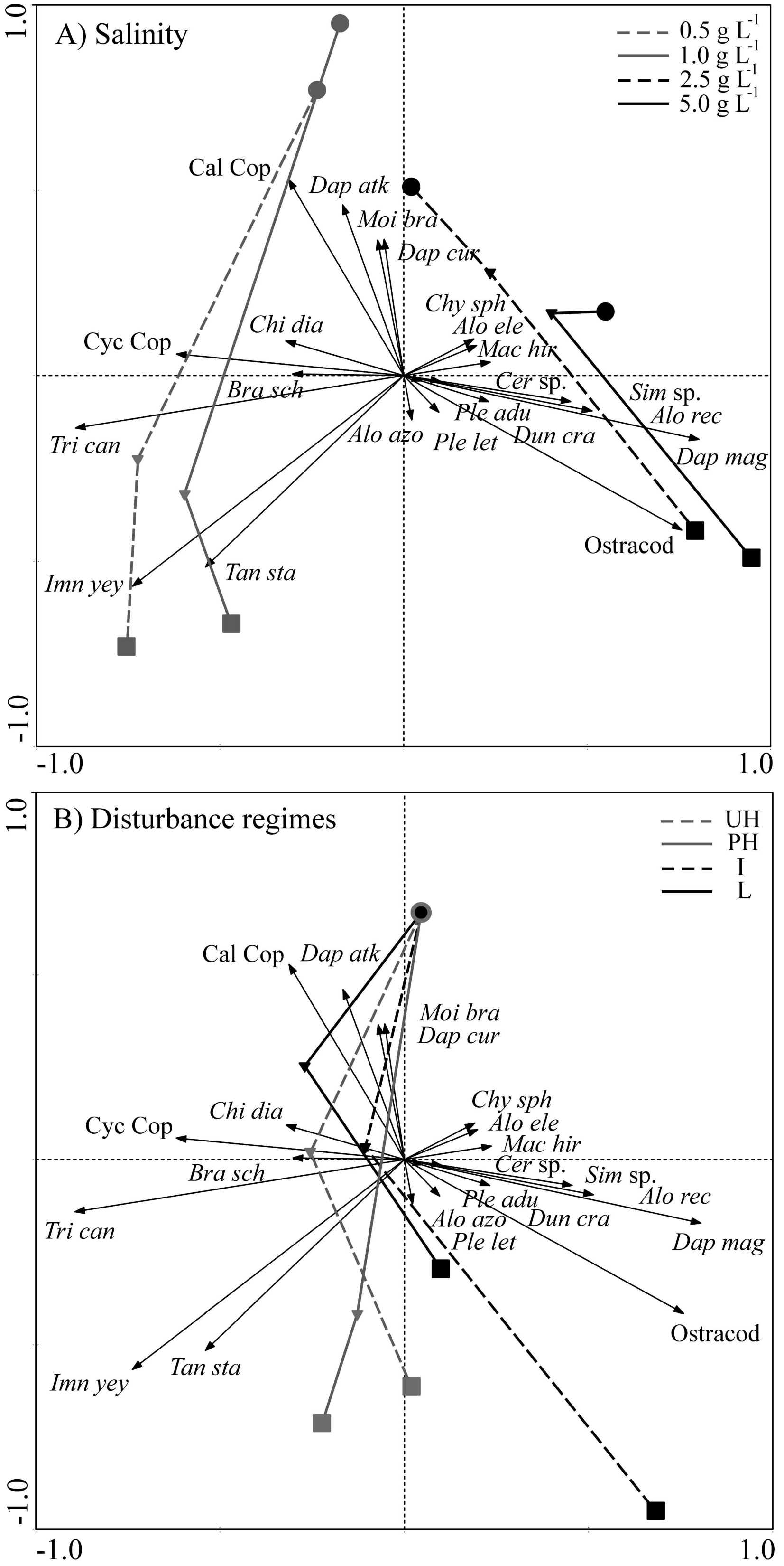

Fig. 2. Ordination diagram of PCA of the crustacean taxa (arrows, abbreviations: *see* Table 1), with time (year) as a supplementary variable for (A) each salinity or (B) disturbance regime treatment separately. Community trajectory lines connect interactions between year and treatment, starting at the circles (AC_1) and ending at the squares (AC_3).

Table 3. Results (*p*-values) of ANOVA for the effect of salinity (df = 3), disturbance regime (df = 3) treatments, and their interaction (df = 9) on the measured environmental variables and taxon richness and density of large branchiopod and zooplankton taxa.

Response variable	Salinity	Disturbance regime	Salinity × disturbance regime
Environmental			
Oxygen concentration	0.391	<0.001	0.001
pH	0.598	0.003	<0.001
Transparency	<0.001	0.298	0.002
Chlorophyll *a*	0.176	0.023	0.087
Macrophyte cover	0.020	<0.001	<0.001
Large branchiopods			
Species richness	<0.001	0.145	0.006
Density *T. stagnalis*	<0.001	<0.001	0.008
Density *B. schaefferi*	0.187	0.017	0.562
Density *C. diaphanus*	<0.001	0.175	0.705
Density *T. cancriformis*	<0.001	0.004	<0.001
Density *I. yeyetta*	<0.001	<0.001	<0.001
Zooplankton			
Taxon richness	0.256	0.095	<0.001
Density non-Chydoridae	<0.001	<0.001	<0.001
Density Chydoridae	0.002	<0.001	<0.001
Density Copepoda	<0.001	0.460	0.015
Density Ostracoda	<0.001	<0.001	<0.001

that were even positively related to salinity and reached highest densities in the two highest salinity levels. These results were confirmed in laboratory hatching experiments (A. Waterkeyn unpubl. data) but are in contrast to findings for other species and regions. Zooplankton hatching from Australian dormant egg banks, for example, was significantly reduced above 1.0 (Nielsen et al. 2003*b*, 2008; Brock et al. 2005). Several zooplankton taxa, such as some chydorid and nonchydorid cladocerans (especially *D. magna*) and ostracods are known to have broad salinity tolerances, especially in regions where a long history of natural salinity may have contributed to local adaptation (Pinder et al. 2005; Gonçalves et al. 2007; Brucet et al. 2009), as was also proposed for the Camargue (Waterkeyn et al. 2008, 2009).

Besides direct effects of a stressor under consideration, also second-order effects often play an important role when assessing the effect at community level (Scheffer et al. 2006; Pérez et al. 2007). In our experiment, community structure trajectories in the different experimental salinities evolved toward two distinct end points. In the two lowest salinities, communities were characterized by high densities of large branchiopods and copepods, but low densities of most cladoceran taxa (one of the exceptions being *Daphnia atkinsoni*). The low-salinity mesocosms were also less transparent, with fewer macrophytes and high chlorophyll *a* concentrations. Conversely, communities in the two highest salinities evolved toward communities with many cladocerans and ostracods and an almost total absence of large branchiopods in clear water with macrophytes. This apparent regime shift (Scheffer et al. 1993) may be explained by second-order effects of the salinity-mediated presence of large branchiopods. Large branchiopods indeed are competitively superior to zooplankton, whereas especially the tadpole shrimp *Triops* is a top predator that, together with clam shrimps, can cause high turbidities through bioturbation (Luzier and Summerfelt 1997; Jocqué et al. 2010; Waterkeyn et al. 2011).

In an attempt to test this hypothesis, we found a significant effect of large branchiopod density, when corrected for the different treatments, on zooplankton community structure, with most of the zooplankton taxa being negatively affected by the presence of large branchiopods. Copepods, however, coped better with the biotic pressure imposed by large branchiopods, probably because they can escape predation by swimming faster, as was also confirmed in Waterkeyn et al. (2011). Also the measured environmental variables were influenced by large branchiopod density when corrected for the treatments, with transparency and macrophyte cover being negatively affected, contrary to chlorophyll *a*. However, the amount of explained variation by these models was rather low, probably due to strong covariation between large branchiopod density and our applied treatments. Regime shifts induced by changes in salinity have already been reported in the literature (Davis et al. 2003; Strehlow et al. 2005; Sim et al. 2006), but for much broader salinity ranges. Sim et al. (2006), for example, reported shifts from submerged macrophytes toward benthic microbial communities (comprised of bacteria, cyanobacteria, and algae) starting at salinities above 45. Jeppesen et al. (2007) showed that regime shifts can even occur at lower salinities. These

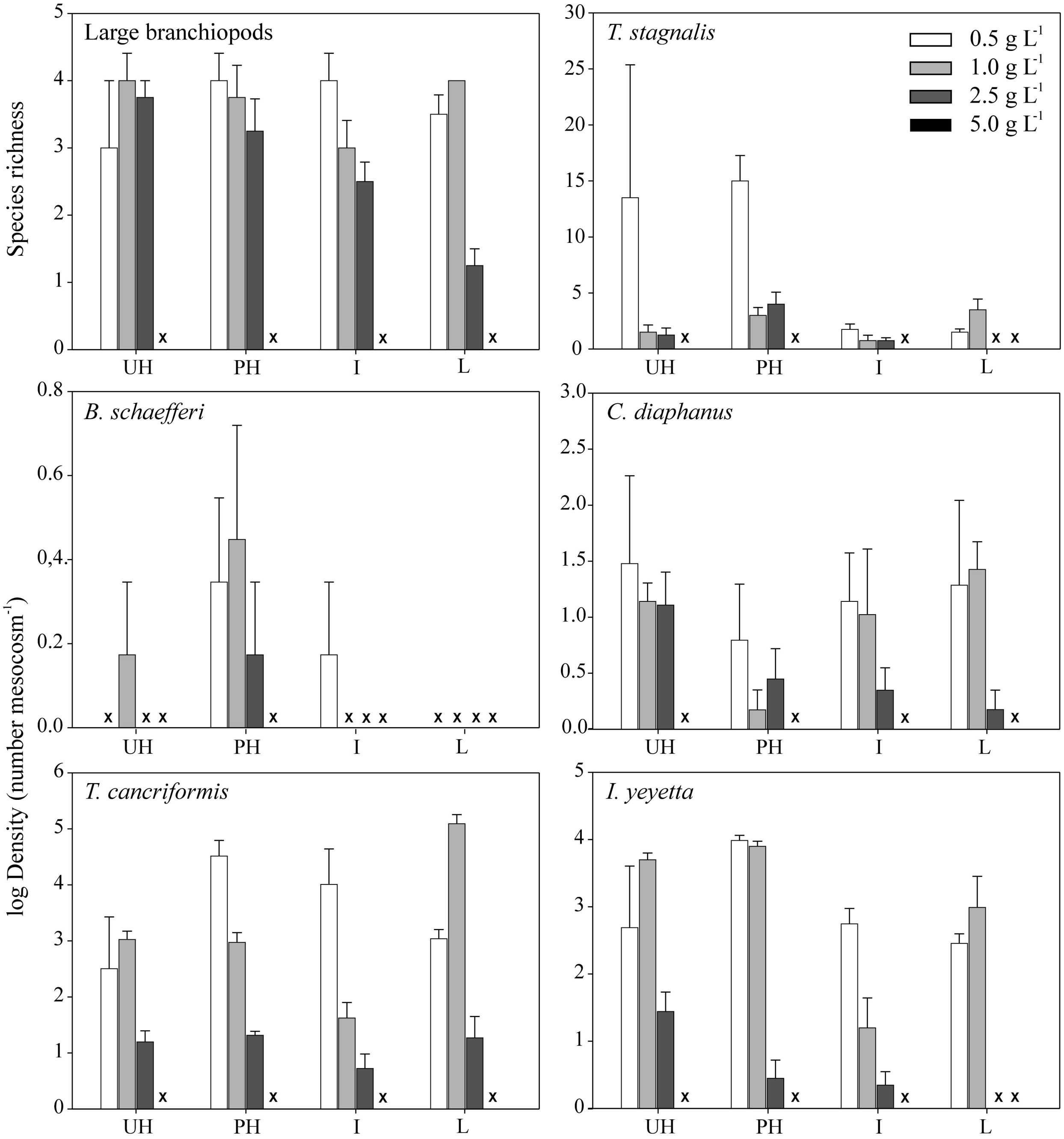

Fig. 3. Average (± SE) species richness and density of large branchiopods in AC_3 in all treatments. x indicates when none of the replicates of a treatment contained any individuals.

researchers demonstrated shifts from a clear-water *Daphnia*-dominated state toward a turbid copepod- and rotifer-dominated state at salinities above 6–8, but only at high nutrient levels. It is possible that in temporary wetlands housing large branchiopods, a first shift (from a turbid large branchiopod-dominated state to a clear-water zooplankton [*Daphnia*]-dominated state) occurs at salinities above 2.5–5.0 and that further salinity increases exceeding the tolerance of Camargue *Daphnia* (at least 16.8; Waterkeyn et al. 2008) could lead to another shift, back toward a turbid state. However, more research is needed to confirm this.

Effect of disturbance regime—Disturbance regime also had a significant effect on the active crustacean communities. After 2 yr of exposure *Tanymastix stagnalis*, *B. schaefferi*, and *I. yeyetta* reached highest densities in high-disturbance regimes, whereas *Triops cancriformis* thrived in mesocosms that held water longest. *C. diaphanus* performed equally in all disturbance regimes. These species-specific responses most likely reflect differences in their life history strategies. The anostracans *Tanymastix stagnalis* and *B. schaefferi* (Defaye et al. 1998; Waterkeyn et al. 2009) and the spinicaudatan *I. yeyetta* (Defaye et al. 1998) have a very short life cycle, sometimes maturing as soon as 7 d after

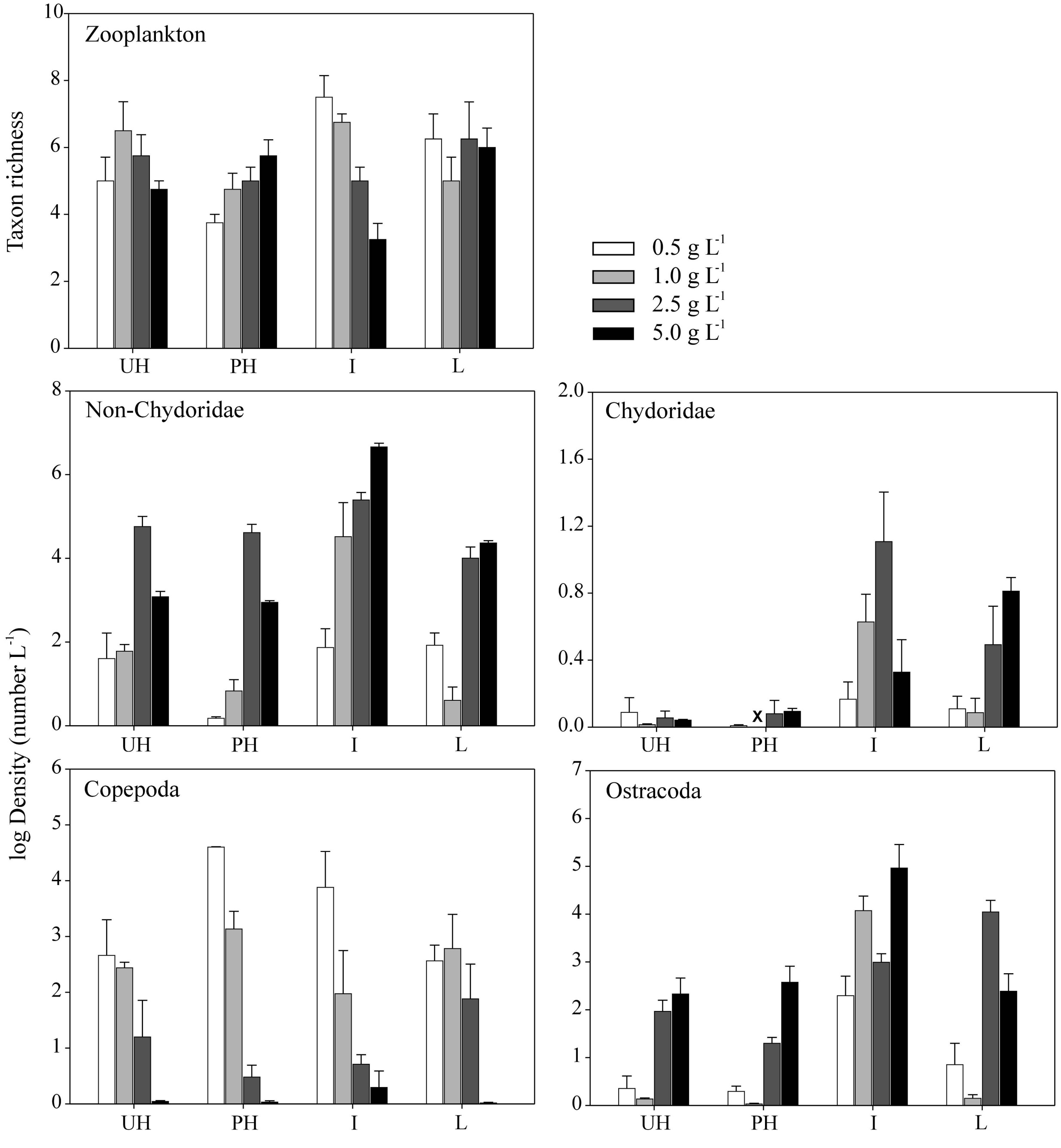

Fig. 4. Average (± SE) taxon richness and density of zooplankton in AC_3 in all treatments. x indicates when none of the replicates of a treatment contained any individuals.

inundation. For these species, disturbance regimes with several flooding and drying cycles during one season are preferable since it gives them several opportunities to hatch and replenish the dormant egg bank. For species that do not manage to reproduce (or to produce sufficient numbers of dormant eggs) in time, such high-disturbance regimes can result in abortive hatchings, eventually resulting in dormant egg bank depletion. Although *Triops cancriformis* also matures soon after inundation (around 15 d; Defaye et al. 1998), they have a much longer life span and an increasing fecundity with age (6 to 8 months; Thiéry 1988). In our experiment *T. cancriformis* indeed managed to produce massive amounts of dormant eggs in the low-disturbance regime, generating five times larger dormant egg bank densities after two long flooded phases. *C. diaphanus*, on the other hand, matured just in time to start producing dormant eggs in the 1-month hydroperiods, whereas in longer hydroperiods adults became very big (sometimes reaching up to 4 cm) and survived up to 5 months, probably with increased dormant egg production. For this species, several relatively short reproduction periods could be equally beneficial as a lower number of longer ones.

Although copepods were most abundant in the predictable high-disturbance regime (but only in the lowest

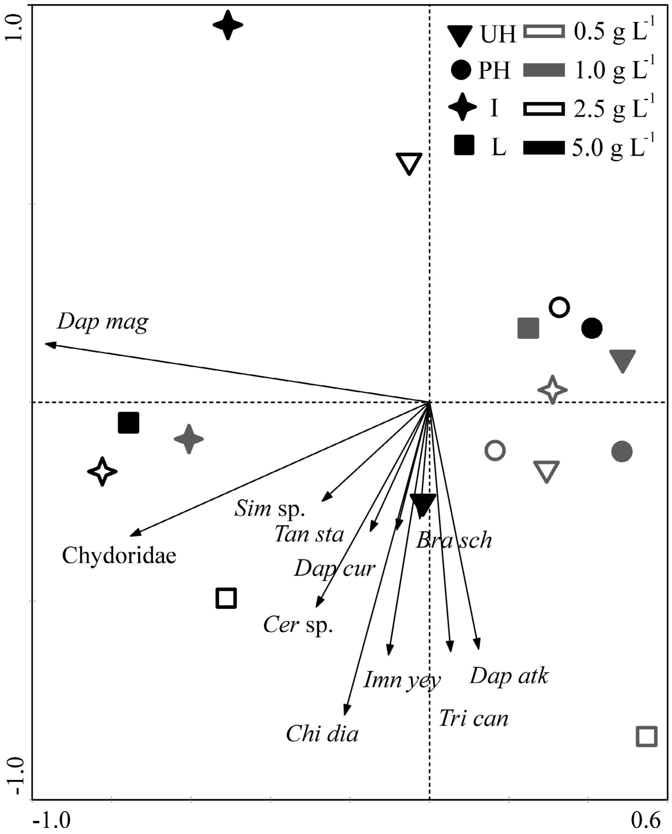

Fig. 5. Ordination diagram of PCA of the dormant large branchiopod and cladoceran taxa in DC_2 (arrows, abbreviations: *see* Table 1), with the interaction between salinity and disturbance regime plotted as supplementary variables (symbols).

salinity), all other zooplankton groups (chydorids, nonchydorids, and ostracods) reached their highest densities in the intermediate-disturbance regime during the third year. Taxon richness was also highest in this condition. Community truncation with renewed hatching twice a year (in autumn and spring) therefore seems to be more favorable than to have one long inundation period with a population crash in winter (Waterkeyn et al. 2010). This result can also be interpreted in the light of the intermediate-disturbance hypothesis (Connell 1978; Shea et al. 2004) with maximal biodiversity at intermediate levels of disturbance. When disturbance occurs frequently (i.e., frequent drought spells during the flooded phase), communities will not progress beyond the pioneer stage with low diversity (Jocqué et al. 2007; Boven and Brendonck 2009). Only the organisms with fast hatching and short life cycles, such as Anostraca and Spinicaudata, can benefit from such a high-disturbance regime. When the disturbance interval increases, more time is available for colonization by more species. Chydorids and ostracods, for example, bloom late in the succession (Waterkeyn et al. 2010) and failed to do so in the high-disturbance regimes. On the other hand, when disturbance frequency is too low, communities will reach and remain in climax with strong biotic interactions (e.g., competitive exclusion, predation), reducing diversity. For example, the high density of *T. cancriformis* in the low-disturbance regime may have reduced zooplankton abundances and taxon richness.

Multiple stressors—Besides main effects, significant interactions between the two applied experimental treatments were also revealed. Some combinations of disturbance regime and salinity turned out to be beneficial for some species but unfavorable for others. For most large branchiopods, salinization up to 2.5 was less detrimental in the high- compared with the low-disturbance regimes, suggesting an antagonistic effect. Several short-term exposures to this salinity may have been less detrimental than long-term exposure during long inundations. However, this was not the case for *T. cancriformis,* copepods, cladocerans, and ostracods where patterns were indicative of a synergistic effect. For *T. cancriformis* and copepods, the optimal salinity condition (maximum density) under low-disturbance regimes was 1.0, compared with 0.5 under the other disturbance regimes. For nonchydorid and chydorid cladocerans and ostracods, salinization up to 5.0 was also worse under the high- compared with the low-disturbance regime. For many species the combined effect of salinization and more intense disturbance regimes due to shorter hydroperiods might therefore be worse than the effect predicted by the single effects. The importance of the

Table 4. Results (p-values) of ANOVA for the effect of salinity (df = 3), disturbance regime (df = 3), and their interaction (df = 9) on change in dormant egg bank size (compared with the start of the experiment: DC_2–DC_0) of large branchiopods and zooplankton (only cladocerans).

Response variable	Salinity	Disturbance regime	Salinity × disturbance regime
Large branchiopods			
T. stagnalis	0.036	0.026	0.667
B. schaefferi	0.193	0.598	0.430
C. diaphanus	0.947	0.149	0.393
T. cancriformis	<0.001	<0.001	0.090
I. yeyetta	0.083	0.337	0.427
Zooplankton			
Non-Chydoridae	0.046	<0.001	0.385
Chydoridae	<0.001	<0.001	<0.001

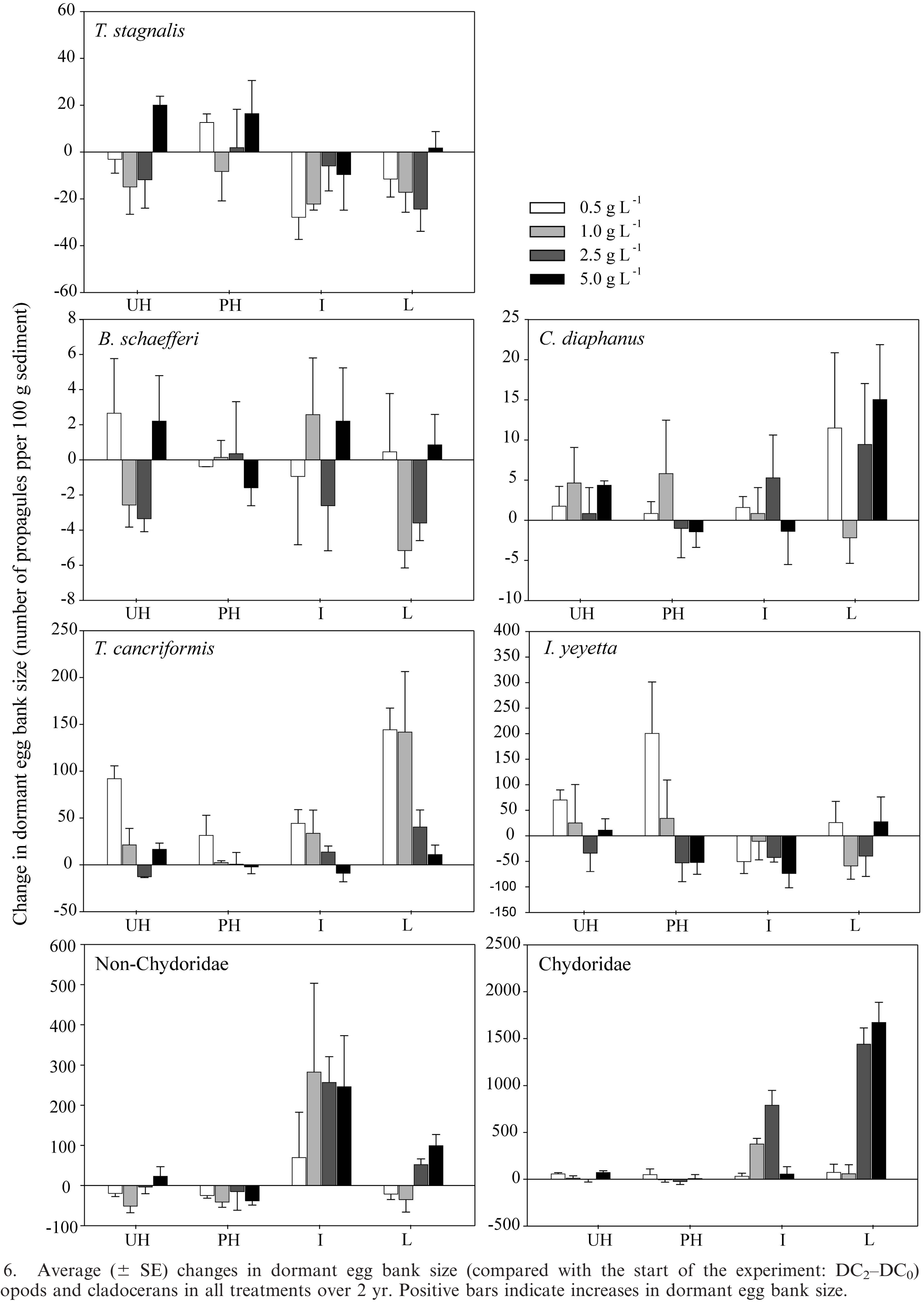

Fig. 6. Average (± SE) changes in dormant egg bank size (compared with the start of the experiment: DC_2–DC_0) of large branchiopods and cladocerans in all treatments over 2 yr. Positive bars indicate increases in dormant egg bank size.

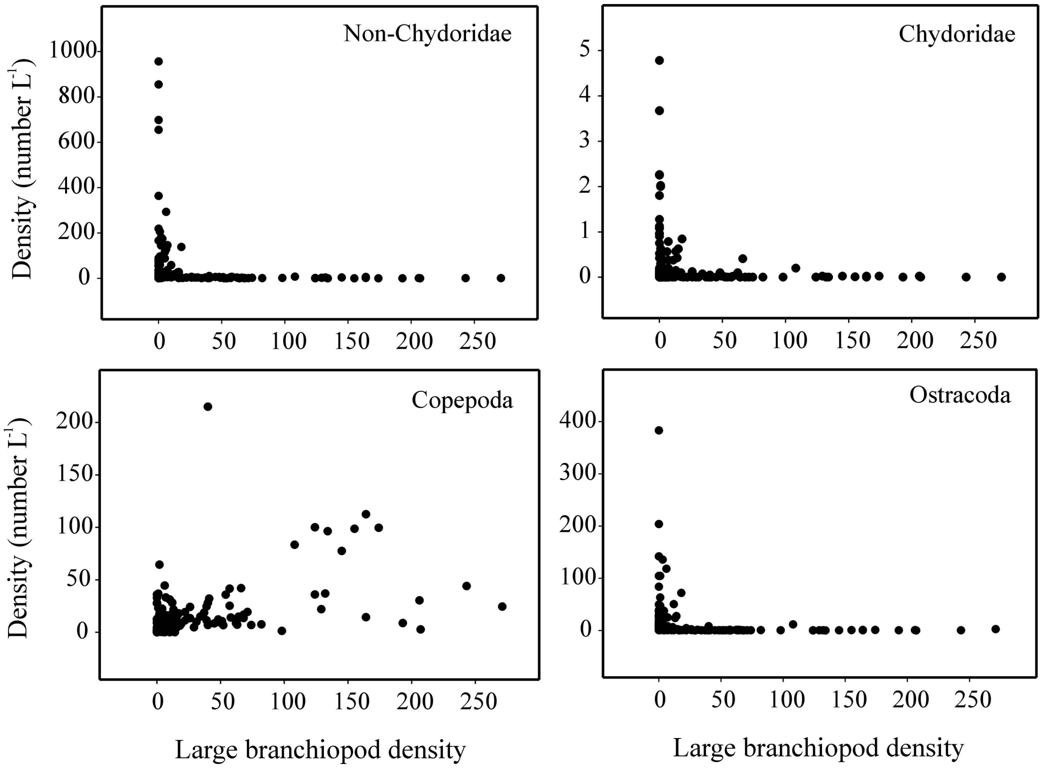

Fig. 7. Scatter plot of the density of zooplankton groups in relation to large branchiopod density.

interaction and the fact that, in the field, changes in salinity are often related to hydrological modification in a complex way (Nielsen and Brock 2009) indicate that salinity and disturbance regime should not be considered separately when modeling the effect of climate change on biodiversity and ecosystem functions and in designing conservation measures.

Perspectives—The interacting effects of salinity and disturbance regime significantly influenced diversity, density, reproduction, and overall community structure of the permanent invertebrate communities of temporary wetlands. After being exposed for 2 yr to the different treatments, considerable shifts in community composition had occurred, both in active communities and at the level of the dormant egg bank. Although dormant egg banks buffer against unfavorable conditions (Brendonck and De Meester 2003), there are limits to this capacity. Even though in some conditions dormant egg bank sizes declined, none was exhausted in 2 yr. A restoration of favorable conditions or adaptation at the level of hatching fractions (bet-hedging strategies) would therefore probably allow communities to return to a healthy state. However, in view of predicted fast changes and prolonged unfavorable conditions due to climate change, rehabilitation or adaptation is not very likely. Several treatments in our experiment caused dormant egg bank reduction, ranging from 15–20% (*C. diaphanus* and chydorids) to 45–50% (nonchydorids, *Tanymastix stagnalis*, *Triops cancriformis*, and *I. yeyetta*), even up to 70% (*B. schaefferi*). By extrapolating these results, we expect that another 2 to 8 yr of exposure to these conditions would lead to dormant egg bank exhaustion.

Predicted aridification (resulting in more intense disturbance regimes with, on average, shorter hydroperiods in temporary wetlands) may also lead to the loss of late successional species (several cladoceran and ostracod species), whereas salinization may cause the loss of sensitive freshwater species, such as large branchiopods. This may be even more important in more fragmented landscapes where suitable patches are scarce and isolated, as predicted by Nielsen and Brock (2009). Additionally, our results also demonstrated that predictions of the effects of changing environmental factors are not always straightforward, since they are often complicated by unknown sublethal effects, changed biotic interactions (due to appearance or disappearance of other species), and indirect effects (due to other changing environmental factors).

We also want to stress the merits of long-term multiple stressor community-level experiments as a tool for designing conservation measures as they integrate higher-order interactions, such as species interactions, nutrient cycling, and productivity (Angeler et al. 2006). Our understanding of ecological effects, and how to manage them, would also be incomplete without detailed knowledge of interactive effects among multiple stressors. In addition, long-term exposure is necessary to uncover sublethal effects or to eliminate delaying effects due to the presence of any buffering dormant egg banks. Such experiments therefore provide more realistic information compared with short-term single species testing with one stressor.

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