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Salinity effects on the coexistence of cryptic species: a case study on marine nematodes

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Abstract The coexistence of four cryptic species of *Rhabditis (Pellioditis) marina* (Nematoda: Rhabditidae) at small geographical scale challenges ecological competition theory and was therefore studied in the laboratory at two different salinities, where their performance in combined cultures was compared with that in monospecies cultures. We found that three of the four cryptic species were able to coexist, but that interspecific interactions (competition and facilitation) were common. Salinity had an effect on these interactions, with a shift from contest to scramble competition. This shift may result from an increased population development of two of the four species at the lower salinity in the monospecific cultures. This experiment demonstrates that abiotic conditions may play an important role in achieving coexistence between cryptic species and can alter the interspecific interactions between them.

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Introduction

Behind the morphological similarity of many species hides considerable genetic diversity (e.g. Vrijenhoek et al. 1994; Williams et al. 2006; Fouquet et al. 2007). This cryptic diversity implies that biodiversity in ecosystems is significantly higher than previously thought (Bickford et al. 2007), and the consequences on ecosystem functioning are poorly understood. The coexistence of cryptic species at local scales (e.g. Trewick 1998; Ortells et al. 2003; Zhang 2004; Derycke et al. 2007; Wellborn and Cothran 2007) challenges traditional ecological competition theory, which implicitly expects competition to be most severe between closely related species (Darwin 1859; Webb et al. 2006; Violle et al. 2011), leading to competitive exclusion under constant environmental conditions (Crombie 1947; Webb et al. 2002). Neutral dynamics, where species can persist together through non-equilibrium dynamics (Hubbell 2005), and niche partitioning, where species coexistence is explained by differences in phenotype (Hutchinson and MacArthur 1959; Hughes et al. 2008), are two possible mechanisms that may explain the coexistence of closely related species and together influence community structure (Leibold and McPeek 2006). Whereas neutral dynamics—although it can potentially explain coexistence at short timescales—is usually considered important with respect to long-term coexistence (Chesson 1991), niche partitioning remains the most plausible explanation for short-term coexistence. The absence of obvious phenotypic and/or ecological differences between cryptic species at first glance renders niche differentiation unlikely. Nevertheless, despite the fact that ecological and functional differences between cryptic species are largely unknown, closely related sympatric species can display different environmental preferences (Knowlton 1993), and hence, ecological heterogeneity may

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facilitate the coexistence of the cryptic species (Leibold and McPeek 2006). In environments with fluctuating dynamics, competitively inferior species may persist because they are temporarily favoured by specific conditions (Begon et al. 1996). Under these circumstances, the results of interspecific interactions will depend on fluctuations in the abiotic (Crombie 1947; Dunson and Travis 1991) or biotic environment (e.g. food availability, predation, intraspecific competition Jensen et al. 2001).

Cryptic speciation has recently been discovered in marine nematodes (Derycke et al. 2005, 2007, 2008b; Bhadury et al. 2008), the most abundant metazoan phylum in marine sediments (Coomans 2000) with densities between 10^5 and 10^8 individuals m^{-2} (Lambshead and Boucher 2003). The high diversity of species at both global (estimates ranging from 10^5 to 10^8 nematode species worldwide Lambshead and Boucher 2003) and local (usually several tens of species m^{-2}) scales, their roles in decomposition processes (De Mesel et al. 2006), and the different functional ecology of the species contribute to the importance of nematodes for ecosystem functioning (Coull 1999). The coexistence of closely related species is important for the long-term stability of ecosystem functioning (Ettema 1998), as ecologically similar species may compensate for each other when a species goes extinct (i.e. the redundancy hypothesis Walker 1992). Hence, coexistence of cryptic species may be important for ecosystem functioning. The marine nematode, *Rhabditis (Pellioditis) marina* (Andrassy 1983) (henceforth referred to as *R. marina*), is a common bacterivore associated with decomposing macro-algae in the littoral zone of coastal and estuarine environments, a typically heterogeneous habitat, both temporally and spatially (Moens and Vincx 2000). Within the morphospecies *R. marina,* at least 10 cryptic lineages can be found (Derycke et al. 2008b). A detailed morphological study of four of these cryptic species (Pm I, Pm II, Pm III and Pm IV, not yet formally described) revealed significant morphological divergences which, however, only become apparent through a multivariate character analysis (Derycke et al. 2008a; Fonseca et al. 2008). Hence, there are no single distinguishing characters which could be used in a dichotomous identification key. These morphological divergences correspond well with the molecular divergences found at three independent loci (COI, ITS, D2D3) (Derycke et al. 2008a; Fonseca et al. 2008). No crossbreeding was observed between the two most closely related of these cryptic species (Pm I and Pm IV); hence, it is unlikely that more distant species could hybridize (Fonseca et al. 2008). Information about differences in ecology between these cryptic species, however, remains scarce. Preliminary studies indicate some differences in the food preferences of these cryptic species, Pm I and Pm IV exhibiting very similar preferences for a number of bacterial strains, but differing in these preferences from Pm II and Pm III (Derycke, unpubl data). More detailed information on their feeding ecology, as well as information about their fitness under varying environmental conditions, including salinity, hitherto remains unknown.

These four cryptic species frequently occur along the south western coast and estuaries of The Netherlands, and sympatric occurrence of two or more of these species in point samples is rule rather than exception (Derycke et al. 2005). Moreover, these four cryptic species display fluctuating abundances which may be linked to seasonal dynamics in the environment (Derycke et al. 2006). These fluctuations may for instance be linked to differential tolerances for salinity and/or temperature conditions, two of the most conspicuous environmental variables in tidal environments. If these temporal dynamics can be explained by differential tolerances of the cryptic species to abiotic conditions, coexistence of these cryptic species would be feasible (Gómez et al. 1995). Even if the cryptic species show considerable overlap in their environmental tolerances, abiotic variation can still be one of the factors shaping communities by influencing interspecific interactions between the species (Lowe et al. 2006).

In this research, effects of salinity on the coexistence of four cryptic species of *R. marina* have been studied (Pm I, Pm II, Pm III and Pm IV). Salinity is one of the determinant factors in nematode diversity and community structure (Heip et al. 1985), but its effects are mostly considered on a broader geographical scale. At a local scale, daily tidal fluctuations occur, with the highest salinity variations between low and high tide in the mid-estuary (Kaiser et al. 2005). There are also seasonal variations, which in the North Sea may range between 19 and 36, with lower salinities during early spring and higher salinities during summer (Tietjen and Lee 1972). Understanding the effects of changes in salinity may also be important in view of climate change and water level rise which may affect salinity (Dailidiene and Davuliene 2006) and its effect on community structure. Evidence for differential salinity tolerances in the cryptic species of *R. marina* can be derived from natural populations (Derycke et al. 2006, 2008b), but is ambiguous. To study the effect of salinity tolerances on interspecific interactions, we need to investigate (a) differential salinity tolerances of the different cryptic species in the absence of other species, (b) the effect of interspecific interactions on the dynamics of the different cryptic species, and (c) the interaction of the abiotic (salinity) and biotic (interspecific interactions) factors. If differential salinity tolerances exist in the cryptic lineages of *R. marina*, we may expect that (a) cryptic species show different population abundances at different salinities, (b) these differences in abundances lead to differences in interspecific interactions, and (c) the outcome of these interactions is at least partly dependent on salinity.

Materials and methods

Nematode cultures

Cultures of the different cryptic species were initially raised from single gravid females to ensure monospecificity and maintained on sloppy agar media (Moens and Vincx 1998) under standardised conditions (temperature of 20°C; salinity of 25) with unidentified bacteria from their habitat as food. Cholesterol (100 μL L^{-1}) was added as a source of sterols, because nematodes on a purely bacterial diet appear incapable of de novo synthesis of specific sterols (Vanfleteren 1980). Nematodes for the experiments were harvested from cultures in exponential growth phase.

Monospecific experiments

To study the effect of salinity on the population dynamics of the different cryptic species, monospecific cultures were reared in Petri dishes (5 cm i.d.) with 4 mL of 1% bacto agar medium prepared with artificial seawater (Dietrich and Kalle 1957) with a salinity of, respectively, 25 and 15. The pH of the medium was buffered at 7.5–8 with Tris–HCl in a final concentration of 5 mM. The addition of the buffer and the salt concentration of the agar increase the initial salinity by ca 1.2 units. Frozen-and-thawed *Escherichia coli* (strain K12) were used as food source and added every tenth day (50 μL of a suspension with a density of 3×10^{10} cells mL^{-1}).

The monospecific cultures were inoculated with five adult males and five adult females of a single cryptic species. Nematodes were manually picked up from the stock cultures, bathed in clean artificial seawater (salinity of 25) for 1 h and placed randomly on the Petri dishes. Every treatment (i.e. lower and higher salinity) was replicated four times for every cryptic species. During the first 8 days (representing at least one and at most two generations in all the treatments and species), the total numbers of adults, juveniles and eggs were counted daily. Afterwards, population dynamics were examined every fourth or fifth day until day 35 of the experiment, when a decrease in abundances of adult nematodes in all replicates—except one occurred. This decrease probably resulted from a combination of crowding and food depletion (dos Santos et al. 2008). On the 15th and the 25th day, the entire population was transferred to a larger Petri dish (resp. 8 cm i.d. and 15 cm i.d.) with new agar medium and proportional food availability.

Combined experiments

Interspecific interactions were tested by the use of combined cultures, in which all four cryptic species were simultaneously inoculated at equal numbers (five adult males and five adult females of each cryptic species). These combined cultures were prepared as described above, with the exception of food provision, agar medium and size of the microcosms: food (bacteria) was not added separately at a fixed concentration but allowed to grow on the agar medium. For this purpose, a mixture of bacto and nutrient agar in a 4:1 ratio was used. This medium ensures sufficient bacterial growth throughout (most of) the experimental test period and also eliminates the need for addition of cholesterol (Moens and Vincx 1998). Final concentration and other properties of the agar medium were the same as in the monospecific cultures and in the stock cultures of the four cryptic species. Food was sufficiently present and populations declined at the same time (around 35 days) in all treatments. This observation combined with the results of previous experiments (Moens et al. 1996; Moens and Vincx 2000) in which monospecific populations showed similar adult abundances and generation times when cultivated on bacto/nutrient agar or on bacto agar with addition of a fixed amount of food ensures that the differences in culture conditions had a negligible effect on the food availability and on nematode population growth.

Population/assemblage dynamics were studied every fifth day of the experiment by counting adults and juveniles. On every sampling occasion, ca. one-third of the adult population of each replicate was removed by handpicking and stored in acetone (70–95%) for later genetic identification. The repeated removal of adults was expected to have only a moderate impact on the population dynamics, as the removal was done randomly and only a few gravid females could already produce a substantial population due to the short generation time and the high reproductive success of the species.

Identifications of the cryptic species were initially performed by the use of restriction enzyme analyses (Fonseca et al. 2008) on the samples of 5, 10 and 25 days. Meanwhile, we developed a novel and faster qPCR-based method (Derycke et al. in preparation) and used this for the identification of nematodes from the samples of the 15th and the 35th day of the experiment. First, a DNA extraction was performed, which was similar for both identification methods. Individual nematodes were handpicked from the experimental cultures, transferred to sterile distilled water to remove traces of agar and then transferred to a 0.5 ml Eppendorf tube containing 20 μL lysis buffer (50 mM KCl, 10 mM Tris pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP 40, 0.45% Tween20). Tubes were frozen for 10 min at −20°C, after which 1 μL of proteinase K (10 mg mL^{-1}) was added. Lysis took place in an Eppendorf Mastercycler gradient PCR machine at 65°C for 1 h followed by 10 min at 95°C. Finally, the DNA samples were centrifuged for 1 min at maximum speed (14,000 rpm). Subsequently, 1 μL was used as template for qPCR. Identification of the four

species with qPCR was done using the Lightcycler 480 System and the Lightcycler 480 SYBR Green I master kit (Roche Diagnostics). Species-specific primers (Table 1) were developed in the ribosomal internal transcribed spacer region (ITS). Following optimisation of primer concentrations and cycling conditions, the qPCR mixture was prepared for a 20 µL reaction volume on 96-well plates using 10 µL LightCycler 480 SYBR Green I Master 2X solution, 3 µL PCR-grade water, 6 µL of each primer (final concentration of 1 µM for Pm I and Pm III, 500 nM for Pm II and 200 nM for Pm IV) and 1 µL of template DNA. The thermal cycling protocol comprised an initial denaturation for 10 min at 95°C followed by 40 cycles of denaturation for 10 s at 95°C, annealing for 20 s at 60°C and extension for 20 s at 72°C.

Data analyses

Only data from 0, 5, 10, 15, 25 and 35 days of the monospecific cultures were retained for the statistical analysis in order to create a balanced design with the combined culture experiment. Furthermore, data of the monospecific cultures were randomly summed to create four fictitious assemblages for every treatment, where the four cryptic species are simultaneously present without affecting each other's population development. The total and individual nematode species' abundances at the start of the experiments were thus the same in the fictitious assemblages and in the combined cultures. This enables direct comparison of assemblage structure and abundance with the combined cultures and hence assessment of the interspecific interaction effects in our experiment. For this purpose, a Permutational Based Multivariate Analysis of Variance (PERMANOVA (Anderson 2001)) on the basis of Euclidean distance with 999 permutations was used with the exact counts of adults of the assemblages (fictitious for the monospecific treatments vs. real for the combined treatment) as dependent variable and three fixed factors: time, salinity and presence/absence of interspecific interactions (i.e. monospecific vs. combined cultures). Significant terms and interactions were investigated using posterior pairwise comparisons within PERMANOVA. A SIMPER analysis was used to identify which species primarily accounted for the observed differences. PERMDISP (Anderson 2004) was executed to test the homogeneity of multivariate dispersions in order to discriminate between real location effects (output of PERMANOVA) and effects explained by differences in the multivariate dispersion for the significant factors. One-way ANOVAs were executed in R (R Development Core Team 2008) on the time-averaged numbers of adults to compare abundances within one species or one treatment.

Results

Salinity and interspecific interaction effects on assemblage structure

Interspecific interactions, salinity and the interaction between them were important factors determining the structure of the assemblages (Table 2). This significant interaction was not caused by differences in multivariate dispersion since there is homogeneity of variances ($F_{3,93} = 4.1952$, $P = 0.102$). Pm III explained most of the variation (>54% in each group) in assemblage structure between the different groups (salinity × interspecific interactions), followed by Pm I for combined cultures (>29%) and Pm IV for monospecific cultures (>15%). Figure 1 shows the effect of interspecific interactions and salinity on the time-averaged numbers of adults. In the absence of interspecific interactions (monospecific cultures), Pm III and Pm IV had higher time-averaged abundances of adults at the lower salinity compared with the higher salinity (borderline significant results of one-way ANOVA: Pm III: $F_{1,7} = 5.97$, $P = 0.050$ (log-transformed data) and Pm IV: $F_{1,7} = 5.76$, $P = 0.053$). Interspecific interactions had no negative effect on the number of adults of Pm I at both salinities and of Pm III at the higher salinity. At the higher salinity, it even had a positive effect on the numbers of adults of Pm I and Pm III (resp. increase of 110 and 119%

Table 1 Primer sequences for the four cryptic species (Pm I, Pm II, Pm III and Pm IV) of *R. marina* used in the qPCR protocol

Target	Primer sequence (5′ → 3′)	Amplicon length	Tm (°C)	GC (%)	Concentration (nM)
Pm I	F:CGCTGACCTTCACTGGAATTTT	135	53	45.45	1,000
	R:CCGACTCCGGTTCAACTCA		53	57.89	
Pm II	F:GATCATCGCTGACCTTGG	294	50	55.56	500
	R:CGCACCATGTTGCCATGA		50	55.56	
Pm III	F:AGCGGGGTGAAAGCCCA	410	52	64.71	1,000
	R:CTGAACTAGAATGGGTACATTCA		52	39.13	
Pm IV	F:CGATGGATGGTTTTCGCG	134	50	55.56	200
	R:GTGTATTGACGCTGTCCGTT		52	50.00	

Table 2 PERMANOVA results from the analyses of counts of adults of the 4 cryptic species of *R. marina* as a function of time, interspecific interactions and salinity (*$P < 0.05$)

Source	*df*	SS	MS	F	*P*
Time	5	33,142.0	6,628.4	1.716	0.001*
Interspec. interactions	1	9,148.3	9,184.3	6.535	0.001*
Salinity	1	4,048.0	4,048.0	2.880	0.048*
Time × interspec. interact.	5	30,665.0	6,133.1	4.364	0.002*
Time × salinity	5	8,821.9	1,764.4	1.255	0.231
Interspec. interact. × salinity	1	17,995.0	17,995.0	12.804	0.001*
Time × Interspec. interact. × salinity	5	10,834.0	2,166.9	1.542	0.125

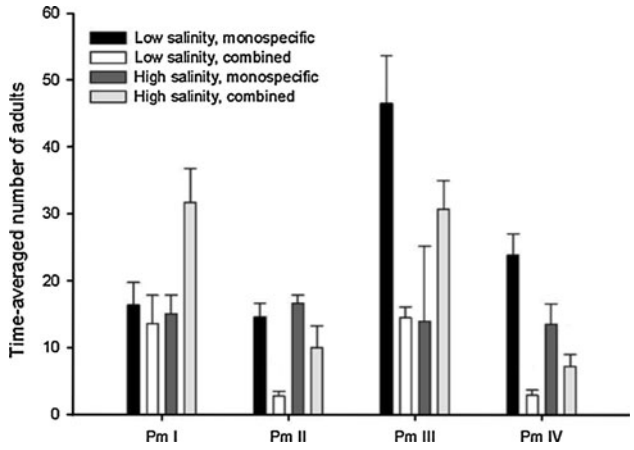

Fig. 1 Time-averaged abundances of adults of the four cryptic species of *Rhabditis (Pellioditis) marina* (mean ± SE) in monospecific and combined populations (four cryptic species together) at the two different salinities (low salinity: 15 vs. high salinity: 25) ($n = 4$ per treatment)

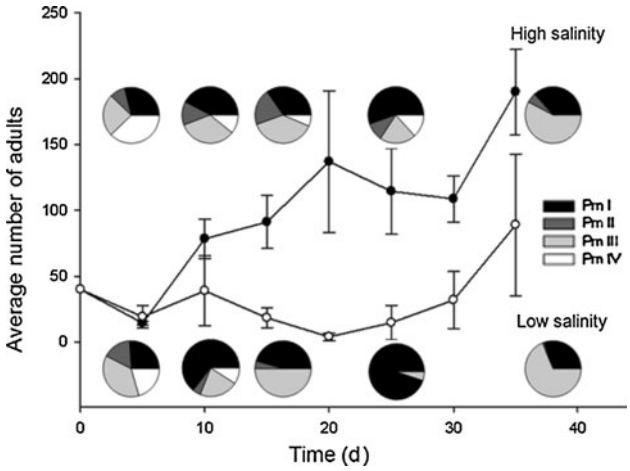

Fig. 2 Assemblage dynamics for the combined cultures (*lines*: total number of adults of all cryptic species together) at high salinity (*filled symbols*) and low salinity (*open symbols*), together with the proportional abundances (*pies*) of the four cryptic species of *R. marina* after 5, 10, 15, 25 and 35 days. Note: at 35 days, Pm II was still present, but in very low abundances ($n = 4$ per treatment)

in comparison with monospecific cultures). Pm II and Pm IV clearly suffered from the interspecific interactions with decreased average numbers of adults at the higher salinity (resp. decrease of 40 and 47% compared with monospecific cultures) and even more so at the lower salinity (resp. decrease of 82 and 86% compared with monospecific cultures). Figure 2 illustrates that Pm IV was completely absent from the assemblages at both salinities after 35 days and that at a lower salinity a bottleneck occurred after 10 days.

Time effects on assemblage structure

Assemblages changed significantly over time (see Table 2). PERMDISP displayed lack of homogeneity of variances ($F_{5,91} = 13.217$, $P = 0.001$), with higher variability as time progressed. An MDS plot revealed that differences in dispersion occurred between the different time moments and explained part of the temporal variation in assemblage structure, combined with an explicit time effect (Fig. 3).

Therefore, interactions of time with other factors must be interpreted cautiously, to discriminate between dispersion and location effects. No interaction between time and salinity was found, indicating that within the time frame of the experiment no changes in the effect of salinity on assemblage

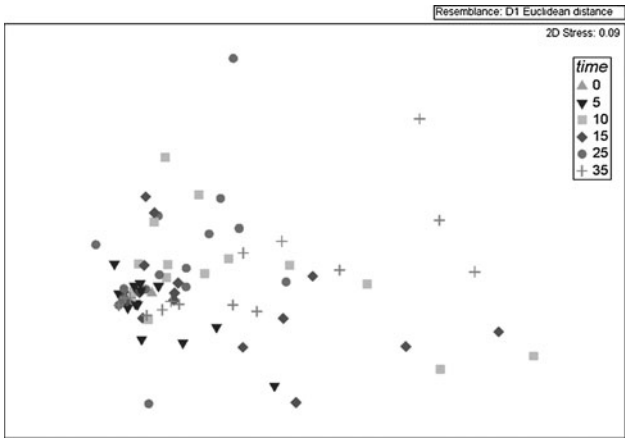

Fig. 3 MDS plot showing the effect of time (in days) on the assemblage structure of the four cryptic species of *R. marina*. An explicit time effect combined with a dispersion effect can be found with higher variability between the different assemblages at later times ($n = 96$)

Table 3 Results for the pairwise tests for PERMANOVA and homogeneity of variances between the different time moments in monospecific and combined treatments

Groups	Monospecific				Combined			
	Permanova		Permdisp		Permanova		Permdisp	
	t	*P*	*t*	*P*	*t*	*P*	*t*	*P*
0,5	2.17	0.001	5.90	1E−3	3.43	0.001	5.24	1E−3
5,10	2.17	0.024	3.34	1E−3	2.57	0.002	3.64	5E−3
10,15	0.66	0.588	0.76	0.474	0.73	0.692	0.38	0.781
15,25	1.70	0.067	1.80	0.133	1.20	0.245	1.18	0.386
25,35	1.68	0.054	1.70	0.156	2.30	0.021	1.21	0.242

structure occurred. On the other hand, an interaction between time and interspecific interactions was found (Table 2), and pairwise tests for the homogeneity of variances also revealed that dispersion effects were present (Table 3). At the start of the experiment, the differences in assemblage structure were mostly explained by the high variability in dispersion between the populations. Later on (between 25 and 35 days), time as location effect clearly explained the differences in assemblage structure in the combined treatments. Assemblage structure changed differently over time for combined and monospecific cultures (Fig. 4). Pm III and Pm IV explained most of the variation in time for cultures without interspecific interactions (resp. >73 and >14% for each time moment). In the combined populations, Pm III and Pm I explained most of the variation in time (resp. >49 and >42%), due to the high fluctuations in abundances between different time moments.

Discussion

In this experiment, coexistence between three of the four cryptic species of *R. marina* (Pm I, Pm II and Pm III) was found. The effect of salinity on this coexistence was studied, as salinity has proven to play an important role in structuring populations and communities of different marine and estuarine species (e.g. Capstick 1959; Heip et al. 1985; Williams 1998; Westerbom et al. 2002; Ortells et al. 2003; Lowe et al. 2006) and has an important impact on the life cycle of many species (e.g. Tietjen and Lee 1972; Diaz and Bevilacqua 1986; Anger 1991; Højgaard 1998; Moens and Vincx 2000). In the case of *R. marina*, all cryptic species were able to cope well with the two different salinities in our monospecific experiments. Despite this overlap in salinity tolerances, salinity can still play an important role in achieving coexistence in two different ways: (a) if species have different salinity tolerances that only partially overlap, coexistence can be achieved in the zones of overlap or (b) if the species have broad salinity tolerances, with only minor differences, even these minor differences can lead to changes in interspecific interactions between the species and thus result in coexistence (Lowe et al. 2006).

In the monospecific cultures, some differences in relative fitness between the cryptic species at the two different salinities were found. Two of the four cryptic species (Pm III and Pm IV) showed higher population abundance at the lower salinity than at higher salinity (Fig. 1). If these differences in salinity tolerances between the cryptic species are an important mechanism for coexistence, we would expect that Pm III and Pm IV would dominate the combined cultures at the lower salinity. Figure 4 shows that this is not completely true, and contrary to the expectations, Pm IV showed very low average abundances and was completely excluded after 25 days. This shows that besides the differences in salinity tolerances, other mechanisms play a role in shaping the coexistence between the cryptic species. For Pm IV, higher population abundances in monospecific cultures only occurred at the beginning of the experiment and were followed by a sudden decrease after 10 days (Fig. 5). This acceleration of development (first generation matured 1 day earlier than in the other species) may be the result of a short-term response to a sudden change in environmental conditions (Grainger 1958) and can be important to achieve coexistence with the other cryptic species. If Pm IV is capable of reaching high abundances before other species become dominant and environmental fluctuations occur on a regular base, Pm IV can coexist with the other species. The higher abundance of Pm III at the lower salinity in monospecific cultures was evident over the whole time frame of the experiment and is in line with the high abundance of Pm III in the combined cultures.

Another possible explanation is that different tolerances between the cryptic species can influence the interactions between species and that coexistence is achieved in this way (Gómez et al. 1997; Lowe et al. 2006). The differences in population development in monospecific treatments at low salinity indeed suggest that the four cryptic species have differential salinity tolerances. To further assess this hypothesis, we first have to reveal the interspecific interactions between the cryptic species. The most common interaction between species is competition. However, facilitative—i.e., the presence of one species improves the occurrence of another (Egler 1954)—and inhibitory interactions may be equally important (e.g. Ilieva-Makulec 2001; Cardinale et al. 2002; Jonsson and Malmqvist 2003; De Mesel et al. 2006; dos Santos et al. 2009). In our experiment, interactions between organisms appeared between 5 and 10 days in both salinity treatments; hence, the first generation was not affected by the presence of other species. Later on, the presence of other species had a major effect on the survival of Pm II and Pm IV, with complete exclusion

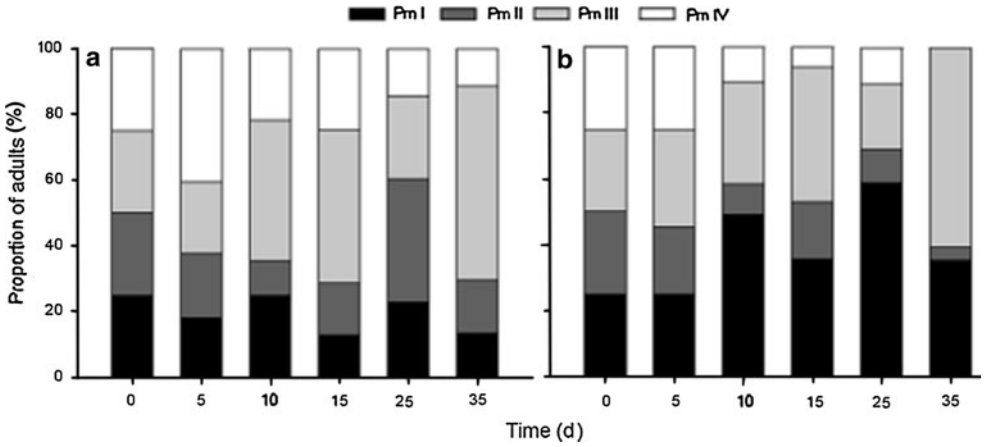

Fig. 4 Average proportional adult abundances of the four cryptic species of *R. marina* in **a** monospecific cultures and **b** combined cultures as a function of time ($n = 4$)

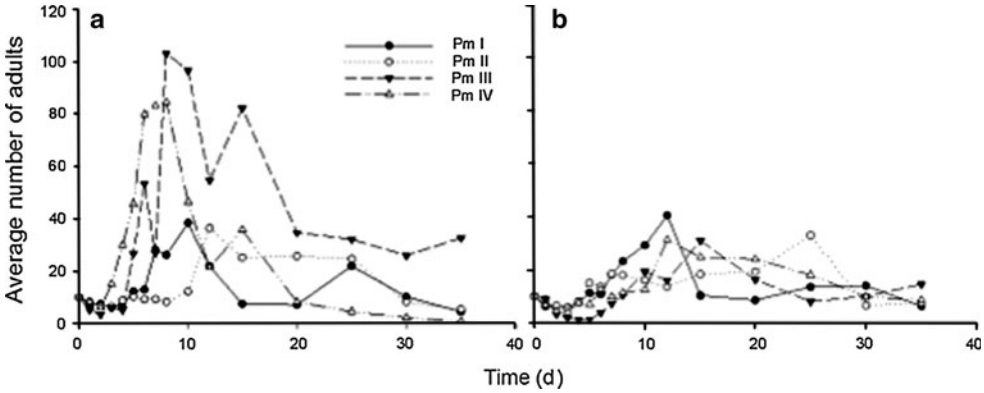

Fig. 5 Average adult abundances of the four cryptic species of *R. marina* in monospecific cultures for the different salinities over time ($n = 4$)

of Pm IV from all populations at both salinities. In natural populations in the area from which both species were isolated, Pm I and Pm IV have hitherto never been found in sympatry (Derycke et al. 2006, 2007), and in an additional experiment with only Pm I and Pm IV, inoculated at equal abundances at a salinity of 25, Pm I attained a dominance of 80 ± 8% after 15 days and completely excluded Pm IV after 35 days (De Meester, unpubl data). Pm I and Pm IV are phylogenetically the two most closely related species in the *R. marina* cryptic species complex (Derycke et al. 2005), and preliminary experiments show that Pm I and Pm IV have very similar food preferences (Derycke, unpubl data), so the absence of coexistence between Pm I and Pm IV agrees with traditional ecological competition theory. Chemical repulsion by glandular secretions produced by the nematodes is also a possible mechanism that could explain the extinction of Pm IV in the combined cultures. Although chemical repulsion was already shown between marine copepods (Chandler and Fleeger 1987), and nematodes are known to be sensitive to chemical cues (Huettel 1986), almost no information on allelochemicals in free-living nematodes is present, making it impossible to confirm this hypothesis. In contrast to Pm IV, Pm II was still present at the end of the experiment in the combined cultures at both salinities, although in very low abundances. Pm II thus clearly suffered from the interaction with the other species, but the interactions were not strong enough to completely exclude Pm II from the assemblages.

In both salinity treatments, Pm I and Pm III were highly abundant and positive interactions occurred at the higher salinity. The presence of other species possibly causes habitat amelioration—an important process in intertidal communities (Bertness and Leonard 1997)—for instance as a result of the higher densities of nematodes influencing bacterial growth. In combination with the increased secretion of mucus by nematodes, which may transport bacteria to different spots, this may make food more available (Moens et al. 2005).

But did salinity alter the outcome of these interactions? Interactions between an abiotic factor (i.e. salinity) and a biotic factor (i.e. interspecific interactions) have already been demonstrated in laboratory experiments on crustaceans (Foran 1986; Bengtsson 1987; Barata et al. 1996) and were also present in this experiment. At lower salinity the interspecific interactions between the species became stronger, leading to a population crash in all four replicates (Fig. 2); only two of the four replicates recovered from this bottleneck. The assemblage structure of these two replicates at the end of the experiment was very similar to the assemblage structure at higher salinity (Fig. 6). These

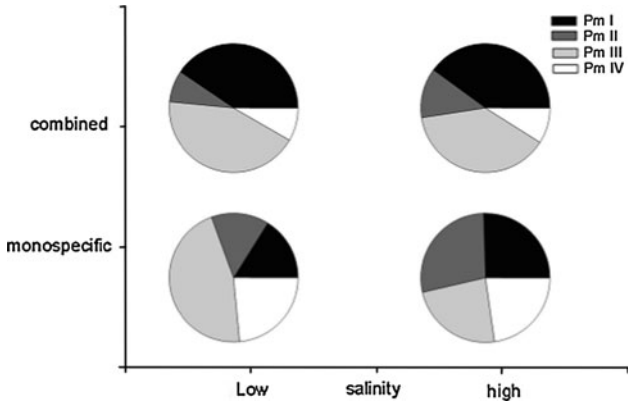

Fig. 6 Time-averaged assemblage structure of the four cryptic species of *R. marina* for the different treatments (interspecific interactions × salinity, $n = 4$ per treatment)

results indicate that salinity had no effect on the species composition of the assemblages, but rather had an effect on the type and strength of interspecific interactions. At the higher salinity, two species became dominant, while the other two suffered from the interactions. We can expect that at this salinity, resources (food, space, etc.) were unequally partitioned between the cryptic species and that some species survived (here: Pm I and Pm III) at the expense of the others (here: Pm II and Pm IV). This is an example of contest competition. In contrast, at the lower salinity, all species initially suffered from the interactions, followed by either complete extinction of all species or survival of some of the species. This suggests that (most of) the species competed equally for the resources and none was initially able to fully meet its needs. If by chance some species die off earlier than the others, the remaining species may be able to recover. This is known as scramble competition (Nicholson 1954; Lomnicki 1988; Coulson and Godfray 2007). Hence, our results strongly suggest that a shift occurred from contest competition at higher salinity to scramble competition at lower salinity. The initially higher population abundance of Pm IV at lower salinity (as seen in the monospecific cultures) may have increased interspecific interactions and may thus have contributed to this shift in the type of competition.

In this experiment, salinity did not only affect the population dynamics of the cryptic species, but also the interspecific interactions between them and the interaction between salinity and the interspecific interactions. Caution is due when linking these results to the results from natural populations as in estuarine habitats, which are characterised by large abiotic fluctuations, adaptations to both longer-term, larger-scale and short-term, local-scale (tidal) salinity fluctuations are important. These adaptations to short-term salinity fluctuations were not studied in the present experiment, which renders comparison of our results with the species composition of natural populations difficult. Over all seasons, in the area from which all species for this study were isolated, Pm I, Pm II and Pm III co-occurred at locations with an average salinity around 25 and only Pm I and Pm III co-occurred at locations with an average salinity around 15 (Derycke et al. 2005, 2006). These results are very comparable with the data from our experiment, but besides differences in salinity tolerances, also differences in tolerances to daily fluctuations or differences in tolerances to other environmental variables (temperature, food preference, etc.) between the species likely play an important role in the structuring of natural assemblages.

Differences in salinity did lead to differences in the population dynamics of some species (Pm III and Pm IV) and to differences in the interspecific interactions, suggesting that larger-scale, long-term salinity fluctuations may influence natural populations and communities. Furthermore, we can expect that seasonal dynamics in salinity can alter the species composition of the natural assemblages since shifts in cryptic species composition of *R. marina* occurred through the different seasons (Derycke et al. 2006). To prove this hypothesis, additional data from the field are necessary, but this experiment already shows that differences in salinity can have different results on the outcome of interspecific interactions and that solely focusing on the effect of salinity on monospecific cultures is highly unsatisfactory.

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