Active and species-specific dispersal behaviour in a marine nematode cryptic species complex

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ABSTRACT: Dispersal is a 3-step process, consisting of a departure, transience and settlement step. In marine nematodes, dispersal is expected to be passive because nematodes lack an active planktonic phase. The transoceanic distribution of several species suggests effective large-scale dispersal. Nevertheless, it remains unclear whether nematodes can actively influence their dispersal and whether species-specific differences exist. Such differences could contribute to the commonly observed co-existence of ecologically similar species. Three separate experiments were conducted on 4 species of the cryptic species complex of *Litoditis marina* (Pm I, Pm II, Pm III and Pm IV) to test the effect of species identity and behaviour in the dispersal steps. A flume experiment demonstrated that nematode emergence increased with increasing flow speed and that they could also actively emerge from decomposing algae (departure step). Movement analysis of nematodes in seawater highlighted behaviour which contributes to the transience step. The settlement experiment demonstrated that *L. marina* can choose on which substratum to settle in still water. Species-specific differences were found in the departure and transience steps. In the departure step, Pm IV exhibited more active behaviour than Pm I. In the transience step, Pm II and Pm IV showed the highest body bend frequency. These species-specific differences correspond with geographical distribution patterns, which are smaller in the species with weaker dispersal behaviour, and lend support to the idea that dispersal can facilitate coexistence of closely related species through a trade-off between competition and dispersal.

KEY WORDS: Active dispersal · Cryptic species · Nematodes

INTRODUCTION

Dispersal refers to every movement of individuals away from their natal habitat which may lead to gene flow (Ronce 2007). Dispersal is a 3-step process consisting of (1) a departure step, in which an organism leaves the patch where it lives, followed by (2) a transience step, in which the transport of the organism towards a new patch takes place, and finally (3) a settlement step, where organisms may succeed in establishing a new population in the patch where they have just arrived (Clobert et al. 2009). Thus, dispersal determines both the present and potential distribution of species and affects processes of adaptation and speciation (Gibbs et al. 2010).

Large-scale dispersal in the majority of marine macrobenthic invertebrate species occurs through a planktonic larval stage (Ullberg & Ólafsson 2003, Levin 2006). In contrast, meiobenthic organisms (small invertebrates with sizes from 40 µm up to a few mm; Mare 1942) lack such an active planktonic phase to facilitate dispersal (Giere 2009). The transoceanic distribution of several species nevertheless suggests effective large-scale dispersal and has been ascribed

to transport with currents, either with (rafting) or without the help of a floating substratum (Chandler & Fleeger 1983, Bhadury et al. 2008, Derycke et al. 2008b). In the absence of such a substratum, organisms eroded into the water column can be transported by currents to distant locations. Repeated patterns of resuspension, displacement and settlement after a series of tidal events can result in dispersal over small to larger scales (Thomas & Lana 2011). Alternatively, rafting on floating objects, like drifting macroalgae, is an efficient mechanism for both small-scale and larger-scale dispersal (Hicks 1988). Meiofauna have been found on coconut shells, cyanobacterial mats, sea turtle carapaces and seaweeds (Micoletzky 1922, Gerlach 1977, Gaudes et al. 2006, dos Santos et al. 2018). Some meiofauna, e.g. many nematodes, may use secretions from their caudal glands to attach themselves (Micoletzky 1922, Fonsêca-Genevois et al. 2006).

Nevertheless, it remains poorly known whether dispersal is a purely passive process or whether meiofauna may be at least partially capable of actively influencing these processes. For nematodes, which are by far the most abundant and one of the most species-rich animal phyla in marine soft sediments (Coomans 2000), it is expected that most dispersal processes are passive (Jensen 1981, Palmer 1984, Chandler & Fleeger 1987) because of their small size and poor swimming capacities. Their wide distribution and their appearance in the water column suggests that marine hydrodynamic processes must play an important role in dispersal (Hagerman & Rieger 1981, Palmer & Gust 1985, Palmer 1988). Nevertheless, nematodes may actively influence at least some steps of the dispersal process. In the departure step, water column entry not only occurs through passive erosion by water currents (both tidal currents and waves), but also by active (behavioural) migration from the bottom (Jensen 1981, Palmer 1984, Walters & Bell 1986). Although nematodes show limited swimming behaviour, this may still be an important mechanism to actively emerge from the sediment (Jensen 1981, Palmer 1984, Thomas & Lana 2011) and to stay in suspension, which is important for the second step of dispersal, the transience step. In addition, some nematode species live in or migrate to more superficial layers and become suspended more easily, particularly in intertidal sediments. In contrast, some nematode species prevent resuspension through water currents by moving deeper into the sediment (Eskin & Palmer 1985, Steyaert et al. 2001). Resuspension by water currents thus depends on behavioural activities and may not be a purely passive process (Steyaert et al. 2001). Possible short-term advantages of active meiofaunal emergence include avoidance of crowded or unfavourable conditions and finding resources and mating partners (Service & Bell 1987, Hicks 1988). The major disadvantage of emergence is the possibility of transport to unfavourable areas (Palmer & Gust 1985) and increasing susceptibility to predators (McCall & Fleeger 1995). However, this disadvantage can be limited if organisms are able to manipulate their settlement. Indeed, nematodes are able to at least partly control this third step of dispersal by actively choosing a preferred type of substrate (Ullberg & Ólafsson 2003, Schratzberger et al. 2004, Guilini et al. 2011, Lins et al. 2013, Mevenkamp et al. 2016), for instance, by actively influencing their horizontal movement when descending towards a patch with better food conditions (Lins et al. 2013).

Litoditis marina Sudhaus 2011, formerly known as *Pellioditis marina* Andrassy 1983 and *Rhabditis marina* Bastian 1865, is a sexually reproducing (up to 600 eggs female^{-1}) (Vranken & Heip 1983), oviparous to ovoviviparous bacterivore nematode (Moens & Vincx 2000, Derycke et al. 2008a). Species of the *L. marina* species complex are typical colonizers of decaying algae and show explosive population growth and rapid colonization/extinction dynamics (Derycke et al. 2007). Its close association with decomposing and living macroalgae appears in intertidal zones around the world (Sudhaus & Nimrich 1989, Derycke et al. 2008b). The wide distribution of *L. marina* is partly explained by the presence of multiple cryptic species (Derycke et al. 2005) (i.e. species that are morphologically highly similar but show consistent and pronounced genetic differences), but also by occasional long-distance dispersal (Derycke et al. 2008b). Cross-breeding between the 2 most closely related cryptic species (Pm I and Pm IV) has not been detected (Derycke et al. 2008a), rendering it unlikely that hybridization between more diverged cryptic species would occur. Four cryptic species (Pm I, Pm II, Pm III and Pm IV) frequently occur along the southwestern coasts and estuaries of The Netherlands (Derycke et al. 2006, 2008b). Sympatric occurrence of 2 or more of these species on decomposing algae is rule rather than exception (Derycke et al. 2006, 2008b). The coexistence of these cryptic species at local scales 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. Niche differentia-

tion, however, may produce stable species coexistence (Leibold & McPeek 2006). Ecological differences in population responses to temperature and salinity conditions, food resource utilization, microhabitat preferences, functional roles in decomposition processes and competitive abilities were found between the 4 cryptic species (De Meester et al. 2011, 2012, 2015a,c, 2016, Derycke et al. 2016, R. M. Guden unpubl. data). Next to ecological differences, sufficiently large dispersal differences between species would provide a mechanism to achieve coexistence (Aiken & Navarrete 2014). Active small-scale (cm) dispersal was already investigated in the cryptic species complex and proved to be species-specific (De Meester et al. 2012, 2015b). Inter- and intraspecific competition were found to be important triggers of dispersal. The investigated dispersal was, however, restricted to active short-distance movement. Little is known about species-specific differences in long-distance dispersal in this cryptic species complex or even in marine nematodes in general. Differences in short- and large-scale dispersal abilities can allow competitively inferior species to persist in a certain environment by occupying a dispersal niche that differs from that of one or more superior species (Aiken & Navarrete 2014).

Here, we investigated (1) whether dispersal is at least partially active in one or more of its 3 steps and (2) whether species-specific differences exist between the cryptic species of *Litoditis marina* in any of the 3 dispersal steps (Fig. 1). We might expect that (1) active processes will take place in the departure and settlement phase, as other nematode species can influence their emergence (Jensen 1981, Palmer 1984, Walters & Bell 1986) and settlement (Ullberg & Ólafsson 2003, Schratzberger et al. 2004, Guilini et al. 2011, Lins et al. 2013, Mevenkamp et al. 2016). By contrast, limited information on possible active behaviour of nematodes in the transience phase is available. Phylogenetic analyses showed that Pm III is most distantly related to the 3 other species (Derycke et al. 2008a) and is also morphologically more distinct when combining different morphological traits (Fonseca et al. 2008). These differences are also reflected in life-history traits (De Meester et al. 2015a) and active dispersal behaviour (De Meester et al. 2012).

We can therefore expect that Pm III also differs from the other cryptic species in the 3 steps of dispersal: active behaviour during the departure step, which may increase its chance of becoming suspended; the duration of remaining in suspension (transience step); and the choice of substrate during the settlement step. Moreover, the most closely related pair of cryptic species of the *L. marina* complex (Derycke et al. 2008a) — Pm I and Pm IV — may benefit from differences in dispersal niche, which may facilitate their coexistence.

MATERIALS AND METHODS

Nematode culture

To investigate species-specific differences in active dispersal behaviour, dispersal in 4 cryptic species (Pm I, Pm II, Pm III and Pm IV) of *Litoditis marina* was investigated in 3 different laboratory experiments. The cryptic species were collected from *Fucus* sp. stands at the Paulina mudflat-salt marsh, Westerschelde Estuary (Pm I, Pm II and Pm III), or from *Ulva* sp. stands in Lake Grevelingen (Pm IV), both in The Netherlands, and cultivated on agar medium. Stock cultures derived from a single gravid female were maintained in the lab (salinity of 25, temperature of
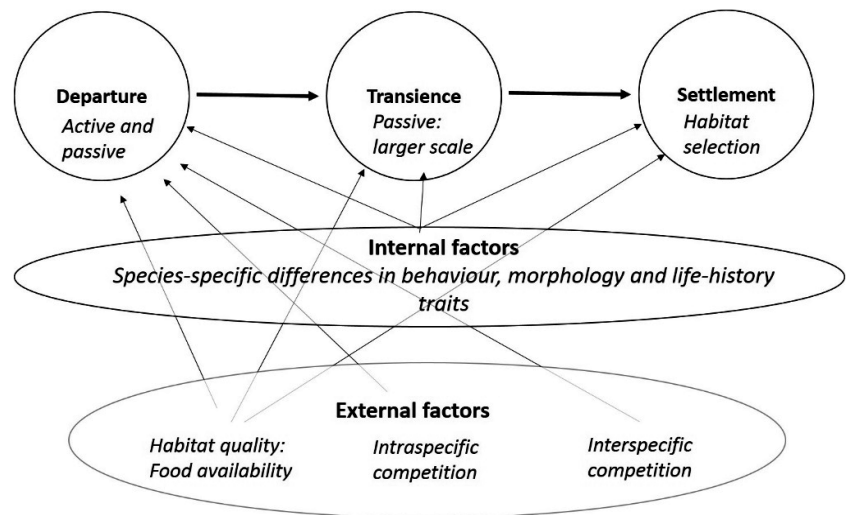

Fig. 1. Conceptual framework to investigate individual variation in dispersal (adapted from Clobert et al. 2009) applied to the *Litoditis marina* cryptic species complex. Relationships between the 3 dispersal steps (departure, transience and settlement), phenotype-dependent dispersal (i.e. dependence on internal state) and condition-dependent dispersal (i.e. dependence on external factors) are illustrated by arrows. Departure and settlement can be partly active processes; transience is mostly passive. Factors influencing internal state and external factors are *italicized*. Habitat quality may influence the transience step when rafting

20°C in the dark) on 1% bacto and nutrient agar (ratio 4:1, Difco) (Moens & Vincx 1998), made up with sterile artificial seawater (ASW; Dietrich & Kalle 1957). TRIS-HCl buffer was added in a final concentration of 5 mM to maintain a pH of 7.5 to 8. Nematodes for the experiments were harvested from stock cultures by picking them out manually (transience experiment) or by rinsing the surface of the agar layers with 3 ml of ASW (salinity 25) (departure and settlement experiments). The nematode density obtained in the suspension was determined by taking the average of 4 successive counts of 1 ml of suspension under a stereomicroscope (Wild Heerbrugg; magnification 15× 10×).

Departure step: flume experiment

Experimental design

To test if current velocities affect the emergence of nematodes and if nematodes are capable of actively emerging from decomposing algal thalli, a flume experiment was conducted. Nematodes were bred on dead thalli of *Fucus* sp. These algae were collected from rocks at the eastern margin of the Paulina salt marsh during low tide. In order to completely defaunate the algae, they were rinsed with tap water and heated in an oven at 60°C for 48 h, followed by 2 d of rehydration in ASW with a salinity of 25. During the rehydration, 50 µl of bacterial suspension was added in order to allow bacteria to colonize the algae. This bacterial suspension consisted of bacteria collected from *Fucus* sp. from the Paulina salt marsh by shaking the algae in ASW. Subsequently, this seawater was filtered 3 times over a Whatman GF/C filter with 1.2 µm pores in order to remove all meiofauna and microalgae. Monospecific cultures of Pm I, Pm II, Pm III or Pm IV were added to 4 to 6 g dry weight of algae by spreading up to 100 nematodes from the stock cultures directly onto the defaunated algae. The colonization by nematodes was regularly inspected under a stereomicroscope (Wild Heerbrugg; magnification 15× 10×) until nematode populations were sufficiently dense (i.e. until several 100s of nematodes were visible on the thalli of the alga and every part of the thalli was colonized) for the algae to be used in the flume experiment.

Current velocities were created in the laboratory with an annular flume (Van Colen et al. 2009) based on the design of Widdows et al. (1998) (Fig. 2) and made of polystyrene with a 10 cm wide and 40 cm deep circular channel. The inner and outer diameter measured 44 and 64 cm, respectively. The total volume of the flume comprised 68 l. The water current in the flume was created by 4 paddles (9 × 10 cm) that entered the water only in the top layer. These paddles were attached to a support system driven by a single DC motor. The speed of the paddles was adjustable and an outflow of the water was made possible through stopcocks. Secondary flows, i.e. inherent flows in the boundary layers due to the viscosity of the fluid, were minimal (~3% tangential flow) and were unlikely to have an effect on the course of this experiment (Widdows et al. 1998). A volume of 60 l of natural seawater was used in the flume. This seawater was filtered 3 times over a GF/C filter (diameter 1.2 µm) and diluted to a salinity of 25.

The algae were attached to a metal weight and slowly lowered to the bottom of the flume. After 30 min, the algae were carefully removed with a magnet connected to a rope and rinsed over a 32 µm sieve to obtain the remaining nematodes. The water from the flume was also sieved over a 32 µm sieve to obtain the suspended nematodes. In the Paulina tidal flat, current velocities are moderate (generally not exceeding 20 cm s^{-1}) (Bouma et al. 2005), so for this reason, 3 flow rates (5, 10 and 20 cm s^{-1}) were tested for every cryptic species of *L. marina*. A treatment without flow was added as a treatment for active dispersal. For every treatment, 4 replicate runs were conducted.

Data analysis

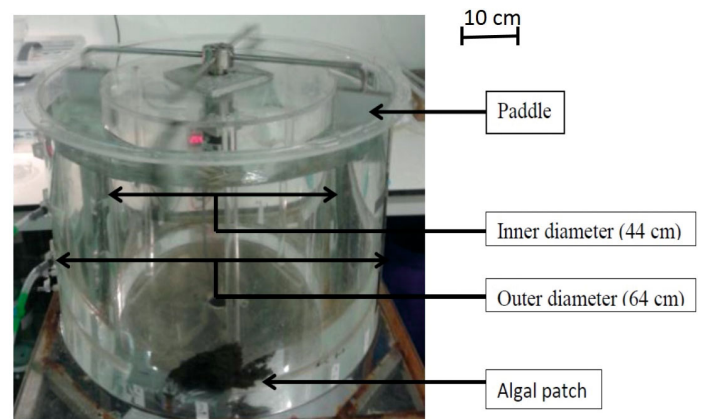A 'resuspension rate' was calculated by dividing the number of nematodes found in the water column

Fig. 2. Side view of the annular flume; total volume comprised 68 l. The water current in the flume is created by 4 paddles (9 × 10 cm) that enter the water only in the top layer. An algal patch with *Litoditis marina* was attached to the bottom to test the emergence from the nematodes into the water at different flow rates

by the total number of nematodes (present in the water column + on the algae). The effects of species identity and flow rate on the resuspension rate were tested with permutational based multivariate analysis of variance (PERMANOVA; Anderson et al. 2008) in R (R Development Core Team 2008), because the data did not meet the assumptions for parametric tests, even after transformation. A PERMANOVA on the basis of Euclidian distance with 999 permutations was performed with the percentage of the suspended nematodes as the dependent variable and species and flow rate as the 2 independent variables. Posterior pairwise comparisons within PERMANOVA were conducted when interaction factors were significant. Furthermore, a PERMDISP was used to check if the multivariate dispersions were homogeneous and the observed effect could not be the result of variation among the replicates

Transience step: body bending

Experimental design

To investigate differences in the transience step between the cryptic species, movement analysis of the behaviour of the cryptic species in seawater was performed. *L. marina* does not show undulating swimming behaviour, but performs a series of frequent body bends when no 'solid' substrate is present (Sudhaus 1974). To elucidate the effect of active behaviour during the transience step, number of body bends per second was used as an indication of duration of suspension in the water column. We chose body bending behaviour because we have often observed that in still water, nematodes which actively bend their bodies remain in suspension (much) longer than nematodes which are not really moving (authors' pers. obs.). Admittedly, however, other mechanisms of remaining in suspension exist, and several nematode species can remain afloat for hours in still water whilst not or only sparsely moving. *Viscosia viscosa*, for instance, probably uses its mucus secretions to remain afloat (T. Moens pers. obs.). However, in *L. marina*, active bending movements appear to be the main mechanism to remain in suspension (authors' pers. obs.). A total of 20 well-fed individuals of each cryptic species were washed in ASW and placed in a well of 2.5 cm in diameter filled with 1 ml of ASW (salinity 25). Nematodes were left in the well for 10 min before the start of the experiment to acclimate to their new environment. In total, 4 replicates species^{-1} were conducted. Each replicate

was recorded for 2 min with a Canon 600D camera connected to a Leica stereomicroscope (10447157) with a magnification of 4× 10×. The recordings were analyzed with the WRMTRCK plug-in for ImageJ (Pedersen 2008), and the average number of body bends per second per individual was calculated.

Data analysis

To test if the average number of body bends per second differed between the different species, a Kruskal-Wallis rank sum test was conducted in R, as the assumptions for a parametric test were not met. Pairwise tests with Bonferroni correction were conducted to find out which species differed from each other in frequency of body bends.

Settlement step: substrate preference

Experimental design

In a third experiment, we tested if cryptic species of *L. marina* preferentially settle on macroalgae compared with bare sediment, and if this settlement is species-specific. Cylindrical non-transparent containers (30 cm diameter), in which the bottom layer was divided into 4 quadrants completely isolated by 4 cm high metal separation plates (Fig. 3), were used. These 4 quadrants were filled with a 2 cm layer of defaunated sand from the intertidal zone of
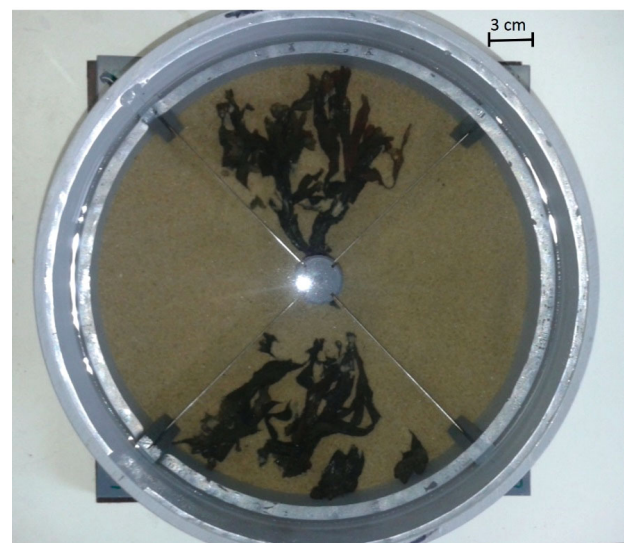

Fig. 3. Top view of the settling containers with 2 sediment quadrants (left and right) and 2 algae quadrants (top and bottom), separated by 4 metal separation plates (14 × 4 cm)

a sandy beach at De Haan at the Belgian coast. In order to remove fine sediment particles, detritus and meiofauna, the sand was shaken 5 times in demineralized water and rapidly decanted. To remove the remaining meiofauna and bacteria, the sand was dried in an oven at 120°C for 48 h. Subsequently, ASW was gently added until the sediment was water-saturated. A total of 15.5 ± 1.5 g of defaunated algae (*Fucus* sp.) with a bacterial suspension (same as in the departure experiment) was placed in 2 opposing quadrants; in the other 2 quadrants, the sediment surface remained bare. Approximately one-third of the algae was buried under the sand to prevent them from floating. The containers were filled with a water column with a height of 8 cm. At the start of the experiment, monospecific cultures of each cryptic species containing ca. 1000 nematodes were added to the water in a circular motion using a 1 ml micro-pipette. The water surface was superficially and carefully stirred for 5 s in a circular motion to spread the nematodes as evenly as possible over the water surface. In total, 4 replicates species^{-1} were incubated. An extra set of 4 containers received no nematodes and served as a control to check if the algae, the sediment and the used seawater were properly defaunated.

After 48 h, the overlaying water was gently removed with a 20 ml pipette from each container and sieved over a 32 μm sieve to retain the nematodes. Nematodes were collected in DESS (a versatile solution for preserving morphology and extractable DNA of nematodes) (Yoder et al. 2006). The sediment from the 2 opposing quadrants without algae was also collected and pooled. The nematodes were collected from the sediment by repeated decantation (5 times) and sieving over a 32 μm sieve. The algae from the other 2 opposing quadrants were collected, and algae from both quadrants were combined and thoroughly rinsed over a 32 μm sieve. The sediment from these quadrants was treated as described above. Hence, 3 nematode samples were collected from each container: the nematodes present in the water column, the nematodes present in the 2 opposing sediment quadrants and the nematodes present in the 2 opposing quadrants with sediment and algae. The samples were left overnight in order to let the nematodes precipitate. DESS was then gently removed with a pipette from top to bottom until only 10 ml was left. All the nematodes in this 10 ml residue were counted in a counting dish under a stereomicroscope (Wild Heerbrugg; magnification 15× 10×).

Data analysis

Relative abundances per substrate were calculated by dividing the number of nematodes found at one substrate by the total number of nematodes in one experimental microcosm. Because these abundances are not independent, no statistical test was performed on the relative abundances. However, proportions of nematodes remaining in the water column until the end of the settlement experiment were compared between the species with a 1-way ANOVA. In addition, the preference of a species for the algae was calculated using an adapted version of Ivlev's electivity index (Strauss 1979): $E = (o − e) / (o + e)$, where E is the electivity, o is the observed number of nematodes, and e is the expected number of nematodes (half of the total number of nematodes retrieved from the sediment and/or algae). Nematodes that stayed in the water column were not included in the analysis. The index ranges from −1 to 1; negative values indicate avoidance of, and positive values indicate positive selection for the sectors with algae. A single sample *t*-test was conducted in R for every electivity value to check if the values were significantly different from 0, followed by 1-way ANOVA (after testing the assumptions) to test if differences in electivity scores existed among the species. Moreover, a replicated *G*-test (Sokal & Rohlf 1995) was also applied to confirm the results of the electivity test.

RESULTS

Departure experiment

The proportion of suspended nematodes was influenced by both flow rate ($F_{3,64} = 29.2$, p = 0.001) and species identity ($F_{3,64} = 5.6$, p = 0.004); there was no significant interaction between both factors ($F_{9,64} = 1.5$, p = 0.18). PERMDISP analysis showed that multivariate dispersions were homogeneous for both flow rate and species (flow rate: $F_{3,64} = 2.0131$, p = 0.145; species: $F_{3,64} = 0.42459$, p = 0.748). The proportion of suspended nematodes was significantly different for all flow rates and zero flow, with increasing resuspension at increasing flow rates (Table 1). However, even under a zero flow, an important portion of the nematodes (mean ± SE of 38.9 ± 0.025 % regardless species identity) was found suspended in the water column after 30 min (Fig. 4). Although the differences were rather small, species identity did have a significant effect on the resuspension rate, with

Table 1. Pairwise comparisons in PERMANOVA between the 4 different species of the cryptic species complex of *Litoditis marina* (Pm I, Pm II, Pm III and Pm IV) and different flow rates. **Bold** text indicates significant differences (p < 0.05)

Pairwise tests	*t*-value	p-value
Pm I, Pm II	1.594	0.121
Pm I, Pm III	4.188	**0.001**
Pm I, Pm IV	2.341	**0.041**
Pm II, Pm III	2.122	**0.04**
Pm II, Pm IV	0.206	0.841
Pm III, Pm IV	2.271	**0.033**
0 cm s^{-1}, 5 cm s^{-1}	3.706	**0.002**
0 cm s^{-1}, 10 cm s^{-1}	5.912	**0.001**
0 cm s^{-1}, 20 cm s^{-1}	9.811	**0.001**
5 cm s^{-1}, 10 cm s^{-1}	2.128	**0.042**
5 cm s^{-1}, 20 cm s^{-1}	5.552	**0.001**
10 cm s^{-1}, 20 cm s^{-1}	3.245	**0.002**

higher numbers of Pm III (57.4 ± 4.3% SE) compared with Pm IV (51.3 ± 2.8%), Pm II (50.7 ± 4.4%) and Pm I (46.7 ± 2.2%) over all flow treatments (including zero flow). Pm I also had a significantly lower resuspension rate compared with Pm IV (Table 1, Fig. 4).

Transience experiment

Average number of body bends per second differed significantly between the cryptic species ($H_{3,109}$ = 15 641; p = 0.001), with Pm I having a lower average number of body bends per second compared with Pm II (p = 0.003) and Pm IV (p = 0.01) (Fig. 5).

Settlement experiment

The relative abundance of nematodes in the algae quadrants was higher compared with the abundances in the sediment quadrants (43.3 ± 2.9% compared with 23.3 ± 1.9%, regardless of species identity). Nevertheless, an important fraction of the nematodes remained present in the water column, and species-specific differences were present (ANOVA: $F_{3,16}$ = 19.01, p < 0.001; Fig. 6), with Pm IV showing the highest proportion of nematodes remaining in the water (55.1 ± 2.4%) compared with the other species (Pm II: 34.4 ± 5.8%; Pm I: 22.7 ± 1.8%; Pm III: 21.1 ± 2.8%). The electivity values (preference towards algae) were all positive and significantly different from 0 in all species (all p < 0.05) except Pm I. No species-specific differences in electivity were found ($F_{3,12}$ = 0.60, p = 0.63) (Fig. 7). The replicated *G*-test confirmed these results: the proportion of nematodes found on the algae was higher than expected for all 4 species (for all individual *G*-tests: p < 0.021). Species-specific differences were not present (*G*-test for heterogeneity: G = 0.648, p = 0.89).

DISCUSSION

Our results showed that *Litoditis marina* has behavioural characteristics that support successful dispersal through emergence, transport and settlement. Moreover, differences in dispersal behaviour exist between the cryptic species, which may facilitate coexistence of closely related species.
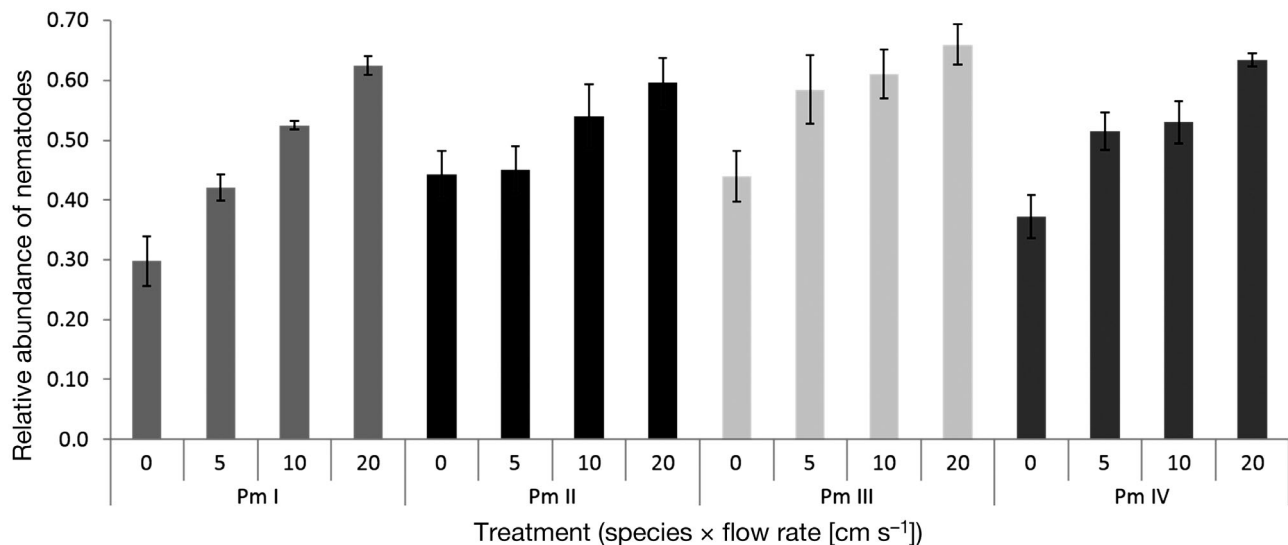

Fig. 4. Mean (±SE) abundance of *Litoditis marina* resuspended during a 30 min run for every treatment: species (Pm I, Pm II, Pm III and Pm IV) × flow rate (0, 5, 10 and 20 cm s^{-1})
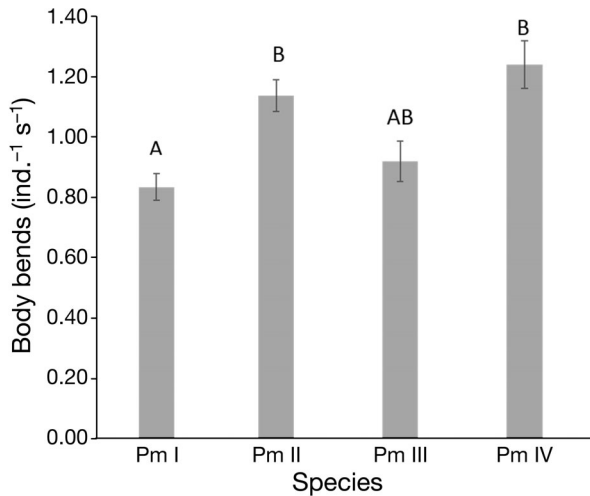
Fig. 5. Mean (±SE) number of *Litoditis marina* body bends. Letters indicate pairwise significant differences (p < 0.05)
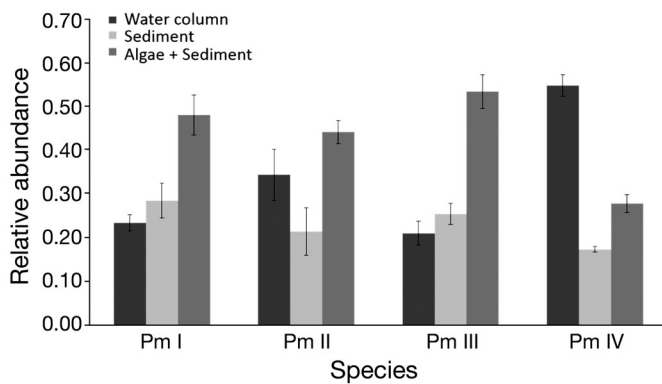

Fig. 6. Mean (±SE) relative abundances of the 4 cryptic species of *Litoditis marina* on the 2 substrates (sediment and sediment + algae) and in the water column
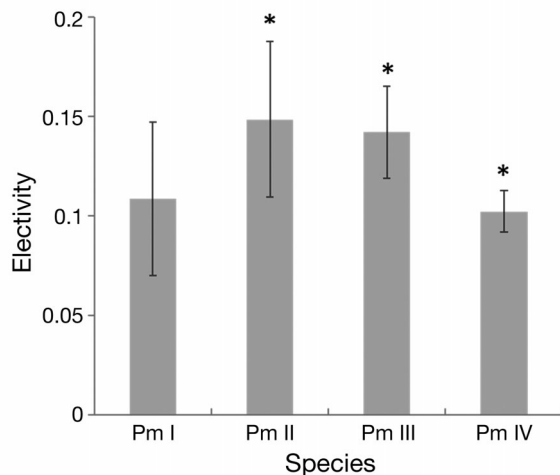

Fig. 7. Electivity values for the algae by the 4 cryptic species of *Litoditis marina*. Positive values indicate attraction to algae. (*) shows when electivity is significantly different from 0

L. marina shows active behaviour in the transience and settlement steps of dispersal

L. marina was able to at least partly control its dispersal behaviour. Active behaviour was found in the transience and settlement step. Some individuals (1 ind. of Pm II and several of Pm IV; data not shown) not only showed typical body bending, but also an undulating swimming behaviour, which may indicate that at least some of the cryptic species are able to partly actively influence their dispersal behaviour. Undulating swimming (even at small scales) and body bending behaviour can increase the duration of their stay in the water column (Thomas & Lana 2011, authors' pers. obs.).

L. marina also influenced its settling by choosing macroalgae over sediment. Attraction to the algae probably results from the presence of bacteria as food (Moens et al. 1999, Ullberg & Ólafsson 2003). Ullberg & Olafsson (2003) made similar observations: 7 times more nematodes settled on algae compared with other food patches after 2 h in a 1 m water column. Chemotaxis to food (here, bacteria) is the most plausible mechanism for the selective attraction of bacterivorous nematodes (Moens et al. 1999, Höckelmann et al. 2004), but other chemical signals, e.g. pheromone signaling between nematodes once the first nematodes have arrived in a patch can also play a role (Huettel 1986). Importantly, the settlement experiment was conducted in still water and is therefore not including the possible effect of flow on the settlement step. Consequently, we can not exclude the possibility that organisms move within the benthic boundary layer to select better habitats or may be passively eroded again in non-favourable areas in the presence of a flow (Butman 1987, Fleeger et al. 1995).

Our results do not provide convincing evidence that active processes are involved in the departure step. In the absence of current velocities, a considerable proportion (38.9 ± 2.4%) of *L. marina* nematodes were found in suspension. This might be a bias due to experimental handling: the algae were slowly and gently lowered to the bottom of the flume, which may nevertheless have caused a substantial part of the nematodes to become released into the water column. Alternatively, it could also indicate that *L. marina* actively emerges into the water column. Such behaviour is common in some other meiofauna (e.g. copepods: Hicks & Coull 1986, Walters & Bell 1986), but was hitherto considered rather rare in nematodes (e.g. *Chromadorita tenuis*; Jensen 1981). In addition, the complex structure of macroalgae can lead to a

reduced flow around the surface of the algae. As a consequence, nematodes have to move away from the surface during emergence to overcome this reduced flow, which may also indicate some active behaviour (Atilla et al. 2005).

Active dispersal behaviour may be important to avoid unfavourable conditions, such as inter- and intraspecific competition or food depletion, because species may be able to choose when to leave or where to settle. The typical habitat of *L. marina* consists of ephemeral patches of macroalgal wrack washed ashore, and local populations are hence subject to pronounced colonization–extinction dynamics. If active dispersal is common, patches where species go extinct can easily become recolonized (Derycke et al. 2007), which can contribute to the resilience of populations (Harrison 1979). In this way, populations have a metapopulation structure in which coexistence on a spatial and temporal scale can be achieved (Hanski 1999) by mechanisms such as patch dynamics, spatial storage, mass-effect or neutral dynamics (Leibold et al. 2004). Metapopulation structure has also been described for populations in other meiofauna taxa (Denis et al. 2009, Yamasaki et al. 2014).

Long-distance dispersal of *L. marina*

Passive emergence was prominent in the *L. marina* complex: when flow rate increased, more nematodes became suspended, in agreement with observations on *Diplolaimelloides bruciei* living on *Spartina* sp. leaves (Alkemade et al. 1994). Around 60% of the nematodes were found in suspension at the highest flow rate used here (see Fig. 4). Current velocities are moderate in the Paulina tidal mudflat (up to 20 cm s^{-1}, the maximum speed in our experiment), although they can be higher at the edges (up to 60 cm s^{-1}; Bouma et al. 2005). The results of our study cannot predict how a higher flow rate would affect nematode resuspension, but demonstrate that hydrodynamic processes, even at low to moderate current speeds, can cause nematodes to become increasingly suspended and passively transported.

Nevertheless, successful transport by currents over longer distances is questionable (Boeckner et al. 2009), because there is limited food on which nematodes can feed during transport in water. However, rafting on macroalgae may offer both a substratum and a food source as well as possible protection from predation (Heck & Thoman 1981, Holmquist 1994). Drifting macroalgae often carry a rafting population

of nematodes (Thiel & Gutow 2005); a large number of recruits enhances the chances of successful colonization and thus contributes to successful dispersal. In our experiment, despite resuspension of a large portion of the nematodes at low to moderate flow rates, a significant portion (>30%) of the nematodes remained on the macroalgae even at the highest flow rate, implying that *L. marina* can, to some extent, withstand the shear from water currents. Attachment through secretions from caudal glands is a known mechanism for some nematodes (Micoletzky 1922), but in *L. marina* it is more likely that the oesophageal glands secrete mucus (Jensen 1987, T. Moens, S. Derycke and N. De Meester pers. obs.) that could help with attachment to macroalgae. Another possibility is that they may hide inside specific structures of the algae, such as receptacula or bladders, where they may be at least partly sheltered from currents (R. M. Guden unpubl. data), or that the flow is modified around the algae and nematodes do not have to overcome shear by using mucus or hiding into algal structures, but can just remain on the algae (see above, Atilla et al. 2005). In the natural environment, macroalgae often detach from the substrate and drift in the water column according to the prevailing water currents. Consequently, macroalgae could transport nematodes over considerable distances. The speed of drifting macroalgae is more or less linearly proportional to current velocities (Biber 2007). Flow velocities of 10 to 20 cm s^{-1} correspond to drift speeds of 8 to 16 km d^{-1} (Qiao et al. 2011). Hence, rafting on floating algae may be an efficient mechanism for long-distance dispersal.

The transoceanic distribution of Pm III (Derycke et al. 2008b) suggests that effective long-distance dispersal has occurred in this species. Rafting on macroalgae may be one plausible mechanism here, next to transport in ship ballast water or with migrating birds (Derycke et al. 2008b). Long-distance dispersal could have also played an important role for the sympatric distribution of the cryptic species. Derycke et al. (2008b) suggested that allopatric speciation is the most plausible mechanism for speciation in this nematode species complex, followed by long-distance dispersal events leading to sympatry.

Species-specific differences in dispersal as a mechanism of coexistence

Species-specific differences were found in at least 2 dispersal steps: the transience step and the departure step. In the departure step, Pm IV showed signif-

icantly higher resuspension rates compared with Pm I over all flow rates. This experiment did not study possible triggers of dispersal, but a main reason why nematodes emerge may be the presence of density-dependent dispersal as a result of intraspecific competition (De Meester et al. 2012, 2015b). Pm I and Pm IV had similar start densities in the experiment (pairwise Wilcoxon rank sum test with Bonferroni correction, p = 0.80), which indicates that other triggers must have caused the differences between the 2 cryptic species. Pm IV may exhibit more active behaviour to facilitate dispersal than Pm I, or Pm I may attach more to the macroalgae to avoid becoming eroded. Pm IV is mostly found on *Ulva* sp., rather than *Fucus* sp., which could also explain why Pm IV dispersed faster from the *Fucus* sp., as this may not be the favourite substrate of the species. Density-dependent dispersal may have played a role for Pm III. A higher proportion of Pm III became suspended in the water column compared with the other species, but density on the macroalgae at the start of the experiment was 10 times higher for Pm III (average of 100771 ± 22458 nematodes compared with 12070 ± 1802 nematodes for Pm I, 13977 ± 1925 for Pm II and 8477 ± 1353 for Pm IV) than for the other cryptic species (pairwise Wilcoxon rank sum test with Bonferroni correction, all p < 0.002). The higher reproduction rate of Pm III (De Meester et al. 2015a) led to these high densities of Pm III on the macroalgae and may have triggered this species to emerge. Other physical and chemical cues, such as differences in tolerances to low oxygen levels or sulphide concentrations (Vopel et al. 1998) may also induce species-specific dispersal behaviour. Small differences in tolerances to a variety of abiotic factors between cryptic species exist (Rocha-Olivares et al. 2004, De Meester et al. 2015a, Monteiro et al. 2018) and can result in differential, species-specific timing of emergence from the sediment. Another possible trigger are other biotic factors, such as predators (Heck & Thoman 1981, McCall & Fleeger 1995).

In the transience step, Pm II and Pm IV showed the highest number of body bends in the water column while Pm I showed the lowest. Species showing more body bends are expected to remain in the water column longer (Eskin & Palmer 1985, Thomas & Lana 2011, authors' pers. obs.). This might partly explain why Pm IV had the highest proportions of nematodes remaining in the water column until the end of the settlement experiment compared with the other cryptic species. Some individuals of Pm II and Pm IV also showed undulating swimming behaviour (see above), which may also cause a longer duration of suspension. Thus, the behaviour of Pm IV, and to a lesser extent Pm II, consistently favoured dispersal through the water column more than in the other 2 cryptic species. Next to active behaviour, body morphology (Eskin & Palmer 1985) is also an important characteristic contributing to suspension. Small differences in body size (Pm IV is larger than Pm I and Pm II; Fonseca et al. 2008) could also contribute to the differences in duration of suspension, with bigger species being likely to sink more rapidly. As a consequence, the larger Pm IV should be more active to overcome this problem.

In the settlement step, a preference for algae (*Fucus* sp.) was found for Pm II, Pm III and Pm IV, which indicates their ability to actively influence their settlement (in agreement with Ullberg & Ólafsson 2003, Schratzberger et al. 2004, Guilini et al. 2011, Lins et al. 2013, Mevenkamp et al. 2016). Significant species-specific differences in the settlement step were, however, not found, indicating that all species studied here actively choose algae over sediment. For Pm IV it means that even if the algae are not the preferred species (*Fucus* sp. and not *Ulva* sp.; see above), they prefer algae as a substrate over bare sediment.

Pm I generally appeared the least mobile of these 4 cryptic species, regardless of substrate: it showed the lowest frequency of body bends when suspended in water, had the lowest resuspension rate and was the slowest active disperser on agar plates (De Meester et al. 2012). The slower movements of Pm I may shorten its time in suspension, and hence restrict its transport through the water column to smaller spatial scales. Pm I is a competitively strong species (De Meester et al. 2011); hence, our results may well indicate a trade-off between competition and dispersal, where good dispersers will disperse before competition becomes too strong, whereas good competitors, such as Pm I, will disperse later and/or over shorter distances (McPeek & Holt 1992). Pm IV was a weak competitor in competition culture experiments (De Meester et al. 2011), but may be a better disperser judging from its higher number of body bends and higher resuspension rate. This also holds for Pm II, which showed a higher frequency of body bends and is a poor competitor (De Meester et al. 2011). Dispersal of Pm III is highly density-dependent (De Meester et al. 2015b, our departure experiment) and its higher reproduction rate (De Meester et al. 2015a) may cause faster dispersal compared with the other species. These results are also in agreement with the geographical distribution of the species (Derycke et al. 2008b). Pm III has been found in a transoceanic distribution, which relates to the good dispersal

capacities in this experiment. Pm I has a more limited distribution, which could be explained by the more limited dispersal capacities found in this experiment, but is mostly dominant. Pm II is widely distributed in Europe, but highly absent if Pm I or Pm III are present, which also nicely correlates with their high dispersal and low competitive abilities (De Meester et al. 2011). Pm IV is not very abundant when other species are present, which also nicely relates to its lower competitive abilities (De Meester et al. 2011). Nevertheless, Pm IV is not a widely distributed species (Derycke et al. 2008b), which is in contrast with their dispersal behaviour as found in this experiment. One of the possible explanations of the absence of Pm IV in several geographical locations is that their favourite substrate (*Ulva* sp.) is absent there. In a heterogeneous habitat, where patches vary spatially and temporally, differential dispersal strategies are likely to evolve among closely related species (McPeek & Holt 1992). These species-specific differences in dispersal behaviour may be one of the important mechanisms for coexistence of closely related species. Despite the fact that most coexistence can occur without differences in dispersal strategies, sufficiently large dispersal differences between species may produce stable coexistence (Aiken & Navarrete 2014). Differences in dispersal abilities (for instance, time until dispersal) can lead to a competitively inferior species being able to persist in a certain environment by occupying a dispersal niche that differs from that of a superior competitor (Aiken & Navarrete 2014). Further studies including competition might be interesting to further reveal the possible differences in dispersal niche.

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