

Tidal elevation and parasitism: patterns of infection by the rhizocephalan parasite *Sacculina carcini* in shore crabs *Carcinus maenas*

Andreas M. Waser^{1,2,*}, M. Anouk Goedknecht^{1,2}, Rob Dekker¹, Niamh McSweeney¹, Johannes IJ. Witte¹, Jaap van der Meer^{1,2}, David W. Thieltges^{1,3}

¹Department of Coastal Systems, NIOZ Royal Netherlands Institute for Sea Research and Utrecht University, PO Box 59, 1790 AB Den Burg, Texel, The Netherlands

²Department of Animal Ecology, Faculty of Earth & Life Sciences, VU University Amsterdam, de Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

³Department of Marine Benthic Ecology and Evolution, GELIFES, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

ABSTRACT: While the distinct zonation patterns of benthic organisms along intertidal elevation gradients have been extensively documented, relatively little is known about the impact that tidal elevation has on the distribution and abundance of marine parasites that are common in intertidal ecosystems. In this study, we investigated the distribution of shore crabs *Carcinus maenas* infected with the rhizocephalan parasite *Sacculina carcini* at 12 locations and in 3 adjacent habitats (intertidal mussel beds, intertidal bare sand flats and subtidal gullies) along a tidal elevation gradient in the Dutch Wadden Sea. Our sampling revealed that of the 27 629 crabs investigated, most infected crabs were found in the subtidal gullies and almost none on intertidal bare sand flats or mussel beds at all of the 12 locations. This probably resulted from a parasite-induced manipulation of infected crabs to behave like egg-bearing females which migrate towards deeper waters, as the same pattern was observed in the distribution of non-infected ovigerous females. The prevalence of both infected crabs and ovigerous females in the gullies was significantly correlated with water depth, and both tended to increase (albeit not significantly) with increasing salinity. As water depth and salinity are expected to affect larval survival of both parasites and crabs, this suggests that the migration into subtidal habitats may result in favourable conditions for reproduction and dispersal. By using a replicated and nested sampling design as well as a large sample size, our study significantly increases the limited understanding of parasite distributions along tidal elevation gradients.

KEY WORDS: Parasitism · Tidal zonation patterns · Rhizocephalan barnacles · Parasite manipulation of host behaviour · Brood mimicry · Wadden Sea · Feminisation

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

The distinct zonation patterns of benthic organisms along intertidal elevation gradients have fascinated marine biologists for a long time, and the effect of tidal elevation on the distribution and abundance of marine organisms has been extensively documented (e.g. Lewis 1964, Benson 2002, Bertness et al. 2014).

In contrast, little is known about the relationship between tidal elevation gradients and the distribution and abundance of marine parasites, which are common in intertidal ecosystems (Mouritsen & Poulin 2002, Torchin & Høeg 2008). Parasites may be affected by tidal elevation gradients in several ways. First, as parasites depend on the presence of their hosts, they will inevitably follow the distribution of

their hosts along elevation gradients and thus only occur where their host is present. Second, parasites may show distinct distribution patterns within the tidal range occupied by a host species if the exposure of hosts to parasites varies within this range. This could result from changing abiotic and biotic factors known to affect parasite transmission along tidal elevation gradients (for reviews of potential factors see Pietrock & Marcogliese 2003, Thieltges et al. 2008). In addition, in the case of parasites with complex life cycles, it could result from distributional patterns of intermediate hosts up-stream in a parasite's life cycle, as those are known to be strong drivers of infection patterns (Hechinger & Lafferty 2005, Thieltges & Reise 2007). Finally, parasites may show a distinct distribution along tidal elevation gradients due to parasite-induced behavioural changes of their hosts. For example, snails serving as first intermediate hosts for trematodes have been shown to move higher up the shore when infected, presumably to increase transmission to the down-stream hosts in their life cycles (Curtis 1987, McCarthy et al. 2000). Apart from trematodes, however, distributional patterns along tidal elevation gradients have been rarely studied for most intertidal parasite-host systems.

This is also the case for common shore crabs *Carcinus maenas* (L., 1758) infected with the rhizocephalan barnacle *Sacculina carcini* Thompson, 1836. Shore crabs are native to European and North African shores and have been introduced to many coastal areas worldwide (Carlton & Cohen 2003). They occur in 2 colour morphs, which are associated with the crab's moulting stage; green crabs are those which have recently moulted, and red crabs have undergone a prolonged duration of intermoult (Crothers 1968, McGaw & Naylor 1992, Reid et al. 1997, Styriehave et al. 2004). In intertidal sedimentary ecosystems like the European Wadden Sea, shore crabs occur in both subtidal and intertidal habitats, with particularly high densities reported from biogenic structures like mussel beds and seagrass meadows (Klein Breteler 1976, Reise 1985, Thiel & Dornedde 1994).

Shore crabs are infected by a range of parasites (Torchin et al. 2001), with the rhizocephalan *S. carcini* being the most conspicuous one (Høeg 1995, Høeg & Lützen 1995). Shore crabs and many other portunid crab species (see Øksnebjerg 2000 for host range of *S. carcini*) become infected with this parasite when cyprid larvae of *S. carcini* settle on the crab cuticle, penetrate into the hemocoel and develop an internal root-like network, the interna, throughout the tissue of the crab. After a duration of 1 to 3 yr, *S.*

carcini matures, and part of the interna ruptures the crab's abdominal exoskeleton and forms a distinct sac-like structure underneath the pleon of infected crabs (Lützen 1984). This structure is called the externa and contains the reproductive organs of the parasite (Høeg & Lützen 1995). The parasite infects and castrates both sexes of *C. maenas* (Høeg 1995). Moreover, male crabs become to a certain extent morphologically feminized, induced by effects of the parasite on the hormonal system of the host, involving an enlargement of the pleon and a reduction in cheliped size (Rubilianiet al. 1980, Høeg 1995, Kristensen et al. 2012). The morphological feminisation occurs stepwise with every moulting event of the infected host, while *S. carcini* remains internal (Høeg 1995). Once the externa emerges and crabs become externally infected, moulting ceases (parasitic anecdydys; O'Brien & Van Wyk 1985). These morphological changes are also accompanied by behavioural changes. Infected individuals exhibit brood mimicry by carrying the externa in the same place where egg-bearing females keep their eggs (underneath the pleon) and by behaving like ovigerous females, for instance grooming the parasite externa (Høeg & Lützen 1995). In addition, it has been proposed that infected crabs migrate towards deeper waters, thus copying the behaviour of ovigerous females (Rasmussen 1959, Rainbow et al. 1979, Lützen 1984). However, replicated studies at several locations along a tidal elevation gradient or a direct comparison of distributional patterns of parasitized crabs and ovigerous females along such a gradient are lacking.

In this study, we investigated the distribution of shore crabs infected with *S. carcini* along a tidal elevation gradient and compared it with the distribution of ovigerous females at identical locations. To do so, we sampled crabs at 12 locations in the Dutch Wadden Sea from 3 adjacent habitats: intertidal mussel beds, intertidal bare sand and subtidal gullies. This sampling design together with data on water depth and salinity allowed us to investigate 3 main research questions: (1) Do the levels of infection with *S. carcini* differ between the 3 habitats and does this co-vary with patterns observed for ovigerous females? (2) To what extent are water depth and salinity drivers of infection levels with *S. carcini* and the occurrence of ovigerous females? (3) Do infection levels differ among sexes or colour morphs? By using a replicated and nested sampling design, as well as a large sample size, our study significantly increases the limited understanding of parasite distributions along tidal elevation gradients.

Table 1. Locations and location codes (W: west; E: east) used in Fig. 1 as well as coordinates and dates at which shore crabs *Carcinus maenas* were collected in the Dutch Wadden Sea in 2012 (west) and 2013 (east). For each location, the number of traps set on mussel beds as well as the number of hauls on intertidal bare sand flats and in subtidal gullies are given. Numbers in parentheses show the total number of crabs investigated per habitat. For details of sampling design see 'Materials and methods: Sampling of crabs'

Code	Location	Coordinates	Date	Mussel bed Trap (n)	Intertidal flat Haul (n)	Subtid. gully Haul (n)
W001	De Cocksdorp	53° 9' 31.97" N, 4° 53' 20.8" E	7 & 8 Jun	30 (593)	13 (659)	5 (613)
W007b	Vlieland	53° 16' 14.45" N, 5° 1' 19.64" E	6 & 7 Jun	19 (379)	7 (248)	6 (401)
W012	Krassekeet	53° 6' 51.77" N, 4° 55' 11.85" E	5 Jun	18 (427)	10 (190)	6 (371)
W013	Kuitje	52° 56' 2.22" N, 4° 48' 56.14" E	29 May	18 (398)	10 (1383)	5 (3346)
W015	Amsteldiep	52° 55' 48.07" N, 4° 54' 8.57" E	4 Jun	17 (319)	10 (858)	6 (690)
W017	Napoleonendam	52° 56' 42.94" N, 4° 51' 8.56" E	30 May	18 (467)	9 (700)	6 (4824)
E010	Brakzand	53° 26' 31.67" N, 6° 12' 37.91" E	17 Jun	17 (571)	10 (504)	8 (1292)
E013	Roode Hoofd	53° 26' 9.10" N, 6° 9' 59.02" E	18 Jun	8 (110)	5 (263)	8 (756)
E015	Schiermonnikoog	53° 27' 51.16" N, 6° 10' 53.07" E	19 Jun	16 (611)	10 (570)	8 (1675)
E022	Ternaard south	53° 23' 56.33" N, 5° 56' 14.33" E	11 Jun	10 (55)	10 (844)	8 (1622) ^a
E032	Ternaard north	53° 24' 38.45" N, 5° 57' 34.47" E	11 Jun	10 (51)	7 (556)	8 (1622) ^a
E031	Kromme Balg	53° 22' 37.16" N, 5° 39' 33.29" E	12 Jun	16 (583)	5 (514)	7 (186)

^aSame adjacent gully

MATERIALS AND METHODS

Sampling of crabs

Carcinus maenas were sampled in early summer 2012 and 2013. In 2012, 6 locations were sampled in the western part of the Dutch Wadden Sea (Weeks 22

and 23) and in 2013, 6 locations in the eastern part (Weeks 24 and 25; Fig. 1 and Table 1). At each location, 3 adjacent habitat types were sampled: intertidal mussel bed, bare intertidal sand flat and subtidal gully. On mussel beds, crabs were caught with baited, funnel-shaped, plastic crayfish traps (61 cm long × 31.5 cm wide × 25 cm high) with inverted

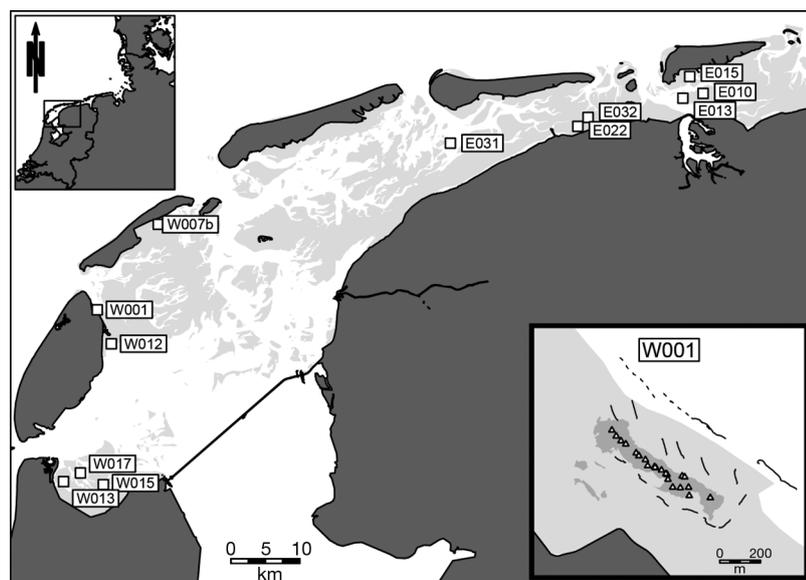


Fig. 1. Sampling locations in the Dutch Wadden Sea. At each location (white squares), intertidal mussel beds and adjacent intertidal bare sand flats and subtidal gullies were sampled. For location names, geographic coordinates and numbers of crabs sampled see Table 1. White areas: subtidal; light grey areas: intertidal flats exposed during low tide; intermediate grey (in inset): mussel beds; dark grey: land. Inset: Specific sampling design of 1 site. White triangles: positions of traps; lines: hauls taken by beam trawl (dashed lines: hauls at low tide; solid lines: hauls at high tide)

entry cones at both ends. The traps were baited with several (4–7) frozen juvenile (<7 cm) herring *Clupea harengus* and were set at low tide and emptied after high tide periods. Intertidal mudflats adjacent to mussel beds were investigated at high tide (water depth 0.5–1.5 m) using a 2 m beam trawl (mesh size 5.5 mm) towed by a small motorboat. With the same gear, the gully closest to each mussel bed was sampled during high and low tide. However, in a few cases (9 out of 73 hauls) fishing in the deep subtidal (>5 m water depth) was carried out with a 3 m beam trawl (mesh size 10 mm) towed by a larger research vessel (RV 'Navicula' of NIOZ Royal Netherlands Institute for Sea Research). For 2 mussel beds (E022 and E032), the closest gully to the respective mussel bed was the same for both beds (Table 1). The location and exact distance of each haul was determined using a Global Positioning System (GPS) receiver.

For all crabs, carapace width (CW), which is the maximum distance between the 2 prominent lateral spines, was measured with digital callipers to the nearest 0.01 mm. Their colour morph (white, yellow or green-coloured abdomen was recorded as green and a predominantly orange or red abdomen as red) and sex were determined by visual inspection. Although parasitized males resemble females in appearance (feminisation), both sexes, even if parasitized, can be clearly distinguished from each other (for photographs see Kristensen et al. 2012). In addition, the ventral abdomen was inspected to identify ovigerous females as well as specimens infected with *Sacculina carcini* (clearly visible externa).

Crab density, water depth and salinity in subtidal gullies

Density of crabs was calculated using the known haul length and net width of the beam trawls. Data on the water depth of subtidal gullies (m below mean tide level, MTL) were generated based on the tracks of the hauls and a bathymetric grid (20 × 20 m) of the Dutch Wadden Sea provided by Rijkswaterstaat (Dutch Ministry of Infrastructure and Environment; 'vaklodgingen'; <http://opendap.deltares.nl>). Salinities (psu) in the Wadden Sea are substantially influenced by high amounts of freshwater discharges of several outlet sluices and thus subject to seasonal and tidal fluctuations of up to 6 psu (van Aken 2008), making point measurements during sampling unreliable. To obtain more accurate measures of local salinities, we used model output from a salinity model with a grid of 250 × 250 m, simulating salinities over a period of 2 years (2009–2010; Duran-Matute et al. 2014). For each gully, the mean water depth and salinity of all grid-points overlapping with the tracks of the hauls was calculated.

Data analysis

The total number of crabs caught was used to plot the size frequency distributions of infected and uninfected crabs (both for males and females separately) as well as that of ovigerous females. Following this, the proportions of infected crabs and ovigerous females of the total sample of crabs per habitat and location were calculated (hereafter called prevalence). As the prevalences in the subtidal gullies did not differ between the high tide and low tide samples (Student's *t*-test, *S. carcini*: $t = -0.96$, $p = 0.35$; oviger-

ous females: $t = -0.21$, $p = 0.84$), data from both tidal periods were summed for further analysis. Statistical differences in prevalence of infections and ovigerous females among the 3 habitats were tested using generalized linear mixed models (GLMM) with a binomial distribution (preliminary exploration did not show overdispersion). The model included habitat as a fixed factor and location as a random factor. As the main interest was the comparison among the 3 habitats, locations from both years were included in the analyses, despite the possibility that the difference in sampling year could confound location effects. For the 2 locations that shared the same adjacent subtidal gully (E022 and E032; Table 1, Fig. 1), the value of this gully was used for both locations, considering this mild pseudoreplication to be unproblematic for the question at hand.

The effect of water depth, salinity and crab density on the prevalence of infected crabs and ovigerous females was tested using generalised linear models (GLM) with a quasi-binomial error distribution (to correct for overdispersion observed in preliminary explorations). For these analyses only crabs from the 11 subtidal gullies were used (locations E022 and E032 shared the same gully).

Finally, statistical differences between the different colour morphs and sexes in parasite infection status of the crabs caught in the subtidal gullies was tested using likelihood ratio *G*-tests. Comparisons were based on the totals of subtidal crabs ($N = 15\,086$, $n = 10$ locations: same gully for E022 and E032; no parasitized crabs at W015).

All statistical analyses were performed using R v3.2.1 (R Development Core Team 2015) supplemented by the package lme4 (Bates et al. 2015). For spatial data handling and production of the map, we used the R packages sp (Pebesma & Bivand 2015), rgeos (Bivand & Rundel 2015), rgdal (Bivand et al. 2015), maptools (Bivand & Lewin-Koh 2015) and raster (Hijmans 2015). For plotting, the package ggplot2 (Wickham 2009) was used.

RESULTS

Of the 27 629 crabs investigated at the 12 locations, 217 carried an externa of the parasite *Sacculina carcini*. The size of these infected crabs ranged from 12.7 to 62.8 mm CW (Fig. 2A,B), and the size frequency distribution of infected crabs reflected more or less the one of uninfected crabs (Fig. 2A,B). In contrast, ovigerous females were generally larger (20.5–57.8 mm CW; Fig. 2C). In general, the preva-

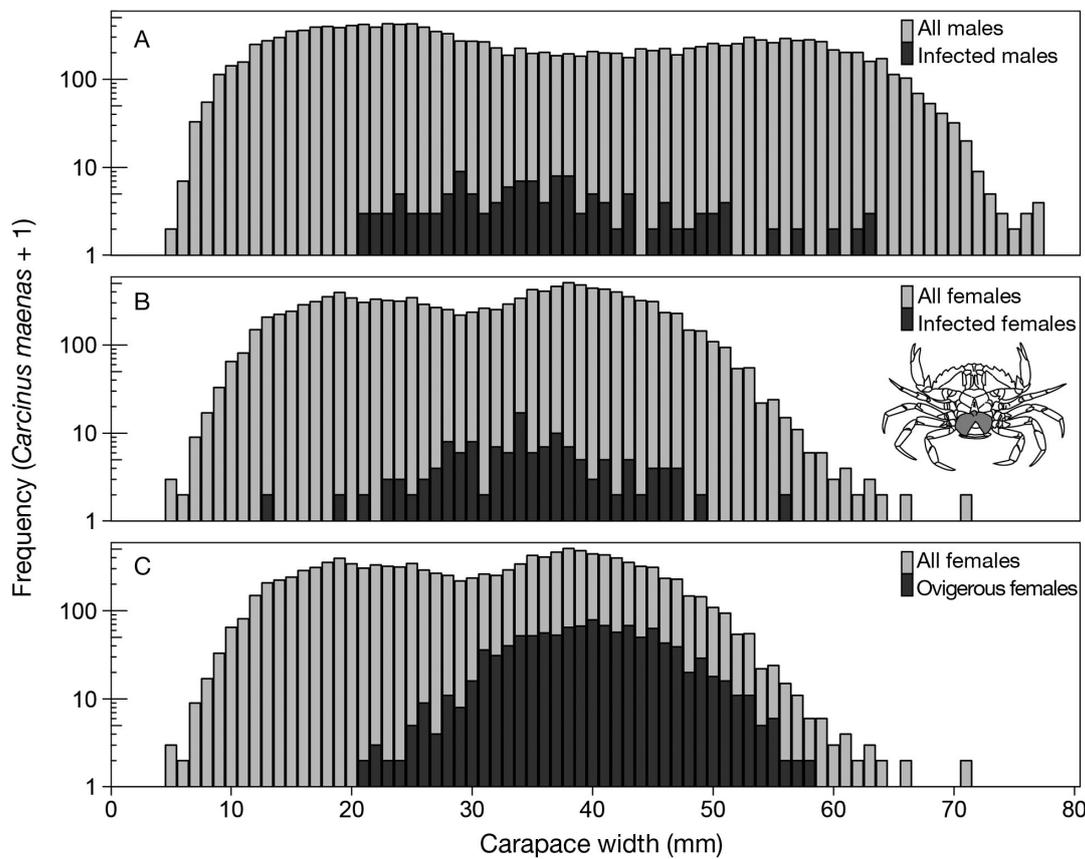


Fig. 2. Size frequency distributions of *Carcinus maenas* (on a log-scale) summed for all locations and habitats: (A) male crabs infected with *Sacculina carcini* in comparison to all male crabs caught; (B) female crabs infected with *S. carcini* and the total of female crabs caught; and (C) ovigerous female crabs compared to all female crabs caught. All *Carcinus maenas* frequency values were increased by 1 in order to make frequencies of 1 visible

lence of infected crabs differed among the 3 habitats (GLMM, likelihood ratio test, $\chi^2 = 119.3$, $p < 0.001$; Table 2, Fig. 3A): these crabs showed highest prevalence (up to 2.8%) and occurred at all locations but one (W015) in the subtidal gullies, where in total 206 infected crabs were found. Infections at the 2 intertidal habitats only occurred at a few locations with low prevalence (below 0.5%; Fig. 3A). The distribution of ovigerous females also significantly differed among habitats (GLMM, likelihood ratio test, $\chi^2 = 619.94$, $p < 0.001$; Table 2, Fig. 3B). Egg-bearing females occurred with higher prevalence and at all but one (W015) location in the subtidal gullies, while they only occurred at a few locations and with very low prevalence on intertidal sand flats and mussel beds (Fig. 3B). In general, the size frequency distribution of crabs was similar in the intertidal and the subtidal, and similar sizes of crabs were infected in both habitats (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m545p215_supp.pdf).

Table 2. Results of general mixed effects model (binomial error structure) analysing the prevalences of infected crabs and ovigerous females in different habitats. Table shows predictor estimates, standard errors (SE), Wald statistic (z) and p-values. Model included habitat as a fixed factor and location as a random factor. The habitat intertidal mussel bed is included in the intercept. Alpha was set at 0.05. There was an overall significance of habitat for prevalences of infected crabs (likelihood ratio test statistic = 119.3, $df = 2$, $p < 0.001$) and ovigerous females between habitats (likelihood ratio test statistic = 619.94, $df = 2$, $p < 0.001$)

Predictors	Estimate	SE	z	p
Infected crabs				
Intercept	-7.37	0.537	-13.714	<0.001
Bare sand flat	0.01	0.609	0.025	0.98
Subtidal gully	2.50	0.463	5.403	<0.001
Ovigerous females				
Intercept	-5.73	0.527	-10.87	<0.001
Bare sand flat	-1.88	0.545	-3.46	<0.001
Subtidal gully	2.52	0.219	11.53	<0.001

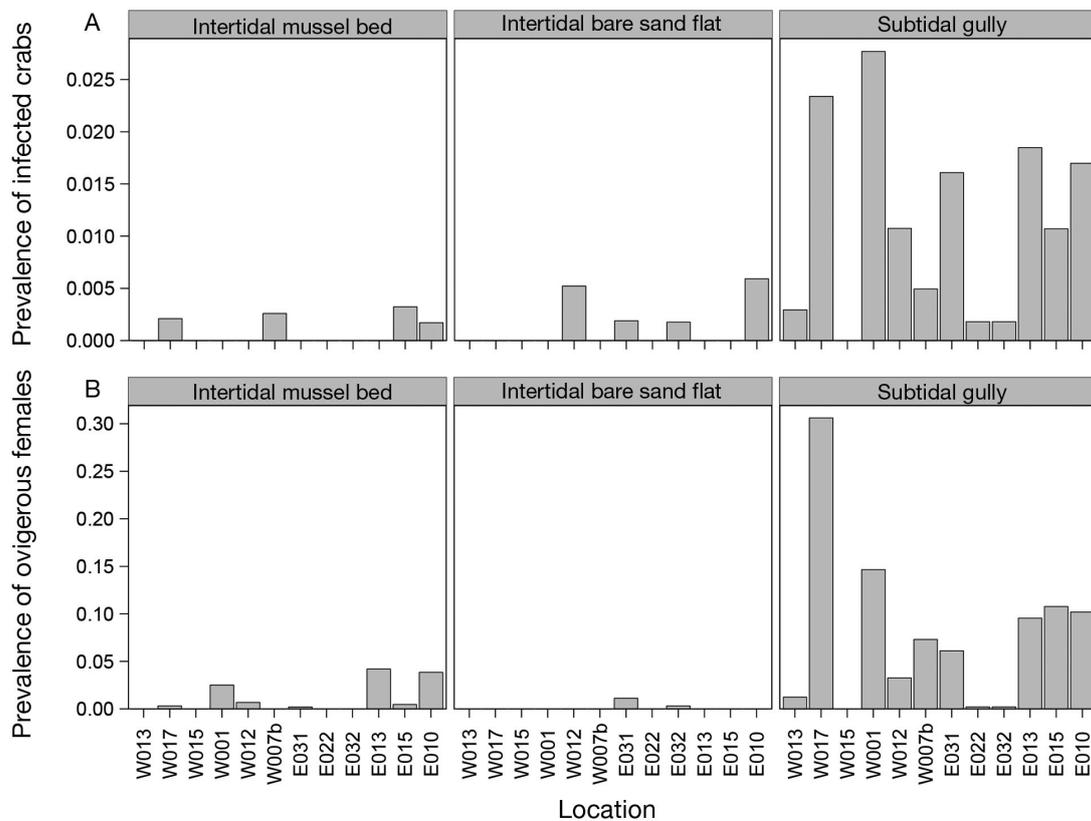


Fig. 3. Prevalence of (A) shore crabs infected with *Sacculina carcini* and (B) ovigerous female crabs in 3 habitats at 12 locations in the Dutch Wadden Sea. For location codes and sample sizes see Table 1. Note that mussel beds E022 and E032 shared the same subtidal gully and hence the same value

Both prevalence of infected crabs and prevalence of ovigerous females differed among the subtidal gullies (Table 2, Fig. 3), and both were positively correlated with each other (Pearson correlation; $r = 0.77$, $p < 0.01$; see Fig. S2 in the Supplement; www.int-res.com/articles/suppl/m545p215_supp.pdf). This covariance was likely the result of a similar effect of the same abiotic factors on parasite and ovigerous female prevalence. Water depth explained prevalence of infected crabs and ovigerous females the best; prevalence of both generally increased with water depth (GLM with binomial distribution and quasibinomial error fix, because of overdispersion; Table 3, Fig. 4). Moreover, both infected crabs and ovigerous females tended to occur more frequently at locations with higher salinity, al-

Table 3. General linear models (quasibinomial error structure) of prevalences of infected crabs and ovigerous females. Predictors in the full models are depth (m below mean tide level), density of crabs (ind. m^{-2}) and salinity (psu). The final model was obtained by model selection: all predictors with the highest p-values were deleted in a stepwise, backward selection procedure. Alpha was set at 0.05. Predictor estimates, standard errors (SE), t , p and R-squared values and dispersion parameters (ϕ) are shown

Model	Predictors	Estimate	SE	t	p	R^2	ϕ
Infected crabs							
Full	Intercept	-8.77	3.993	-2.197	0.064	0.64	6.47
	Depth	0.17	0.107	1.586	0.157		
	Density	-0.19	0.59	-0.315	0.762		
	Salinity	0.16	0.137	1.157	0.285		
Final	Intercept	-5.39	0.482	-11.187	<0.001	0.45	7.11
	Depth	0.26	0.096	2.704	<0.05		
Ovigerous females							
Full	Intercept	-5.98	5.588	-1.07	0.32	0.7	37.11
	Depth	0.36	0.155	2.309	0.054		
	Density	-0.31	0.806	-0.389	0.709		
	Salinity	0.12	0.193	0.608	0.562		
Final	Intercept	-3.82	0.684	-5.579	<0.001	0.63	34.96
	Depth	0.46	0.126	3.652	<0.01		

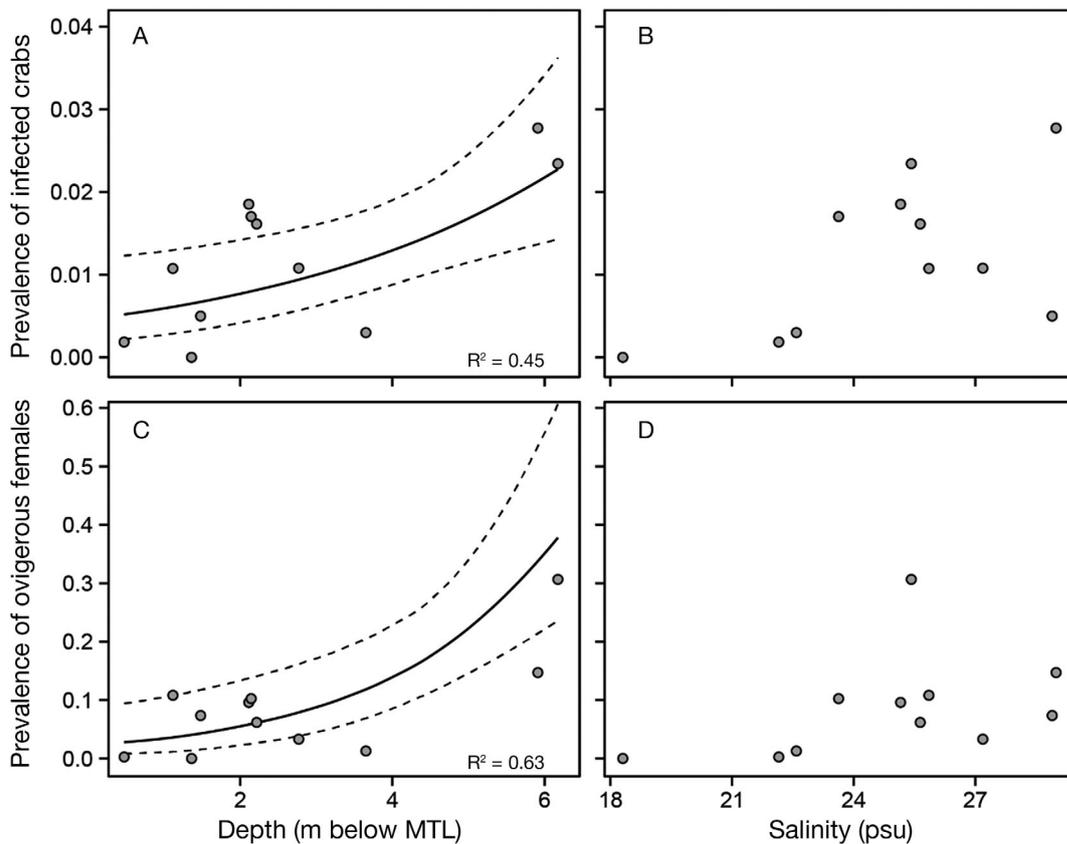


Fig. 4. Prevalence of (A,B) shore crabs infected with *Sacculina carcini*, and (C,D) ovigerous female crabs in subtidal gullies depending on (A,C) water depth (m below mean tide level, MTL) and (B,D) salinity (psu). Plots show observed values (dots) and fitted values of the final generalised linear models (GLMs) (black line) with 95 % confidence intervals (dashed lines)

though this was not or only marginally significant (Fig. 4; Pearson correlations, infected crabs: $r = 0.57$, $p = 0.07$; ovigerous females: $r = 0.42$, $p = 0.2$, see Fig. S2), although salinity was dropped in the model selection procedures and was not included in the final model (Table 3).

Finally, infection status of the crabs caught in subtidal gullies significantly differed between the different colour morphs and sexes (G -test: $G = 440.35$, $df = 3$, $p < 0.001$; Table 4). In males only 1.15% (0.11% green and 1.04% red) of the population was found to be externally infected. The non-infected males accounted for 98.85% (90.66% green and 8.19% red) (G -test: $G = 374.96$, $df = 1$, $p < 0.001$; Table 4). In contrast, in the females the proportion of individuals of the green morph was much less. Only 44.42% of the females were found to be green-coloured and non-infected, while 0.18% were externally infected green females. Most of the red females, 53.93% of the total female population, carried no externa of *S. carcini* and 1.47% of the females were red and externally infected (G -test: $G = 58.253$, $df = 1$, $p < 0.001$; Table 4).

Table 4. Percentage of non-infected and infected shore crabs (visible externa) belonging to either the green- or the red-coloured morph for male and female *Carcinus maenas* in the subtidal gullies. Data are from 10 locations (same gully for E022 and E032; no parasitized crabs at W015)

Colour morph	Males (N = 8566)		Females (N = 6520)	
	Non-infected	Infected	Non-infected	Infected
Green	90.66	0.11	44.42	0.18
Red	8.19	1.04	53.93	1.47

DISCUSSION

Our study reveals that most infected crabs were found in the subtidal gullies and almost none on intertidal bare sand flats or mussel beds at all of the 12 locations. This pattern was also observed in rhizocephalans infecting hermit crabs along the shore of Okinawa Island, Ryukyu Archipelago, southwestern Japan (R. Yoshida pers. comm.) and corroborates earlier notes in the literature based on very limited

observations that infected *Carcinus maenas* migrate towards deeper waters (Rasmussen 1959, Rainbow et al. 1979, Lützen 1984). We observed a similar pattern in the distribution of ovigerous females, which also predominantly occurred in the subtidal habitat. The migration towards the subtidal is thought to result from parasite-mediated behavioural changes in infected crabs which lead to typical behaviour patterns of ovigerous females, even in the case of infected males, ultimately resulting in the observed distribution pattern (brood mimicry; Høeg & Lützen 1995). However, an alternative explanation for the dominant occurrence in deeper waters may be a higher exposure of crabs to infective propagules in subtidal compared to intertidal habitats. As infected crabs accumulate in the subtidal gullies, the release of nauplius larvae will also predominately take place here, suggesting a higher infection risk in the subtidal habitat that could lead to a positive feedback loop. However, the development from the nauplius to the infective cypris larval stage takes about 4–5 d (at 12–18°C; Høeg & Lützen 1995); thus, the site of larval release and the site of actual infection are most likely disconnected due to some dispersal of the infective stages, especially in turbulent and current rich tidal ecosystems like the Wadden Sea. This suggests that crabs may become infected either in the intertidal and then migrate towards deeper waters or, alternatively, they become infected in the subtidal and never migrate into the intertidal. The actual causality will only be ascertained experimentally, for example by releasing marked infected crabs in the intertidal and recapturing them over a tidal elevation gradient.

Both the prevalence of infected crabs and of ovigerous females significantly increased with water depth. Furthermore, there was an indication (marginally significant or non-significant) of an increase in prevalence of infected crabs with salinity. Hence, both abiotic factors are probably relevant for reproduction, and the migration towards the subtidal gullies may ultimately be related to the conditions faced by the larval stages of the crab and the parasite. Deep and saline water may provide favourable physiological conditions for the larval stages of both the host and the parasite. Indeed, mortality of larval stages of crabs and rhizocephalan barnacles is known to increase with decreasing salinity (Thresher 1996, Anger et al. 1998, Kashenko & Korn 2002, Anger 2003). In addition, larval release and subsequent dispersal may be more successful in deeper waters than in the intertidal, where larval stages most likely will face higher risks of heat stress in the warmer months (Thresher 1996). Regardless of the

exact mechanisms, water depth and salinity seem to explain at least in part the observed differences in prevalence among the 12 locations investigated in the Wadden Sea. They may also underlie the differences in prevalence of *Sacculina carcini* infection compared to other locations where the parasite has been studied. In general, prevalences of *S. carcini* infection are known to vary widely with prevalence of over 50% observed at some localities along the native range of the shore crabs (Bourdon 1960, Minchin 1997, Torchin et al. 2001, J. T. Høeg pers. comm.). However, more detailed studies will be necessary to unravel the underlying mechanisms of the large-scale distribution of the parasite.

In general, mature *S. carcini* were mainly observed in crabs between 20 and 45 mm CW in both sexes. In male crabs, however, some infected individuals reached 60 mm in CW. Ovigerous females were also absent in the very small-sized females, with 20 mm CW as the minimum size for egg-bearing females. In contrast to parasitized female crabs, ovigerous females were also numerous in sizes beyond 45 mm CW, reaching a maximum of about 60 mm CW. Similar patterns have been observed in previous studies (e.g. Lützen 1984, Dittmann & Villbrandt 1999, Costa et al. 2013) and result from the biology of crabs and parasites. Infection with *S. carcini* preferentially occurs on recently moulted crabs compared to crabs in intermoult stages (Glennier & Werner 1998). As smaller crabs have higher moulting rates than larger crabs, they face a higher infection risk. Moreover, as moulting ceases in infected crabs with an externa (O'Brien & Van Wyk 1985), they do not increase in size, causing the observed size distribution. Crabs below 20 mm CW, i.e. crabs of 1 yr or younger (Klein Breteler 1975), barely featured a mature externa of *S. carcini*, which can be attributed to the maturation period of the parasite which at minimum takes about 1 yr (Lützen 1984). Even in the very small infected crabs, measuring only a few mm in CW, an externa generally does not appear before crabs reach a CW of around 20 mm (e.g. Lützen 1984, Costa et al. 2013, J. T. Høeg pers. comm.).

Hence, as barely any crabs below 20 mm CW were infected with *S. carcini*, the calculated prevalence values for the different habitats depend to a certain extent on the size range used for calculations. On intertidal bare sand flats, for example, the inclusion of the very abundant juvenile ~1 yr old crabs (<20 mm CW) in the prevalence estimates leads to an underestimation in prevalence of infected crabs. If only the potentially infected size range was used, prevalences would be slightly higher, and compar-

isons with other studies need to take the actual size range of the respective samples into consideration. The actual size range sampled in our study also differed slightly between habitats, as baited traps are known to catch preferentially larger crabs (Williams & Hill 1982, Smith et al. 2004) due to larger individuals being more aggressive, thereby restricting the likelihood of smaller animals entering the trap. Hence, our prevalence values calculated for mussel beds may be relatively higher compared to intertidal sand and subtidal gullies. However, as there were only very few crabs infected with *S. carcini* on intertidal mussel beds and at intertidal sand flats (11 out of 217 infected crabs), some imprecision in the actual prevalence values among habitats does not affect the overall distributional pattern observed. Another potential problem of using baited crab traps may be a difference in catch efficiency between infected and non-infected crabs, for example caused by lower feeding activity of infected crabs. However, experiments with *S. carcini* infected and non-infected shore crabs did not find any differences in prey consumption rates (Larsen et al. 2013), so that this is unlikely to confound the method. Baited crab traps may also catch less ovigerous females due to the bias towards catching preferentially bigger and male individuals, suggesting the absence of ovigerous females on mussel beds to be an sampling artefact. However, our trawling (where this potential sampling bias is absent) in the intertidal revealed that ovigerous females are generally scarce on sandy flats. In addition, our long-term experience in the field and previous qualitative literature reports (Rasmussen 1959, Rainbow et al. 1979, Lützen 1984) also indicate that ovigerous females and infected crabs are rarely found on mussel beds and in the intertidal in general. This suggests that the pattern of a predominantly subtidal occurrence of ovigerous females and infected crabs is unlikely to be an artefact of our sampling design.

Finally, while the prevalence of *S. carcini* did not differ between sexes, the majority of the infected crabs were of the red colour morph. This dominance of the red colour morph in infected crabs probably results from the fact that moulting ceases after the emergence of the externa in infected crabs (parasitic anecydysis; O'Brien & Van Wyk 1985), with infected crabs remaining at this intermoult stage as long as they carry the externa (Andrieux 1968). A prolonged duration of intermoult stages has generally been associated with a red carapace colour, while recently moulted crabs show a green carapace (Crothers 1968, McGaw & Naylor 1992, Reid et al. 1997, Sty-

ishave et al. 2004). The exact mechanism for this colour change is not known, but it has been suggested that it is related to a denaturation of pigments in the carapace (Reid et al. 1997). The lack of a difference in infection levels between males and females contrasts with previous findings. For example, Costa et al. 2013 found significantly higher prevalence of *S. carcini* in females than in males at some but not all sampling events at the Mondego estuary in Portugal. However, Lützen (1984) found generally lower infection levels in female than in male crabs in the Isefjord in Denmark, and Werner (2001) found similar infection levels of both sexes on the west coast of Sweden. Whether these differences are related to some underlying mechanism or just result from spurious correlations remains to be investigated.

In conclusion, *S. carcini* shows a distinct distributional pattern in the intertidal ecosystem of the Wadden Sea, with most infected crabs occurring in the subtidal and probably driven by water depth and salinity. This indicates that other parasite groups besides the well-documented effects of trematodes are able to affect the distributional patterns of their hosts along tidal elevation gradients. Hence, parasite-mediated distributional patterns may be much more common in marine systems than currently known. More well-replicated studies at multiple locations and use of a nested sampling design including tidal elevation would be valuable to further assess the effect of tidal elevation gradients on the distribution and abundance of other marine parasites.

Acknowledgements. We thank B. Fey, K.-J. Daalder, W.-J. Boon and H. de Vries, crew of the Royal NIOZ-research vessel RV 'Navicula', for their help. We also thank A. Asjes, A. Dairain, N. de Jong, E. Koelemij, L. Meeuwissen, F. Ribas, A. Rippen, R. Twijnstra, M. van Regteren and K. Wätjen, who volunteered and contributed to the collection and processing of the samples. We further appreciate the valuable feedback on an earlier draft by J. T. Høeg (Department of Biology, University of Copenhagen) and 3 anonymous reviewers. Financial support for this study came from the Dutch Waddenfonds, the Ministry of Infrastructure and Environment (Rijkswaterstaat) and the provinces of Fryslân and Noord Holland via the project Mosselwad. M.A.G. and D.W.T. acknowledge support from NWO and BMBF (NWO-ZKO project 839.11.002).

LITERATURE CITED

- Andrieux N (1968) Etude de la cuticule chez *Carcinus mediterraneus* (Czerniavsky) indenne et parasité par *Sacculina carcini* (Thompson). Bull Soc Zool Fr 93:611–627
- Anger K (2003) Salinity as a key parameter in the larval biology of decapod crustaceans. Invertebr Reprod Dev 43: 29–45

- Anger K, Spivak E, Luppi T (1998) Effects of reduced salinities on development and bioenergetics of early larval shore crab, *Carcinus maenas*. *J Exp Mar Biol Ecol* 220: 287–304
- Bates D, Maechler M, Bolker B, Walker S (2015) lme4: linear mixed-effects models using 'Eigen' and S4. R package version 1.1-8. <https://cran.rproject.org/web/packages/lme4/lme4.pdf>
- Benson KR (2002) The study of vertical zonation on rocky intertidal shores—a historical perspective. *Integr Comp Biol* 42:776–779
- Bertness MD, Bruno JF, Silliman BR, Stachowicz JJ (2014) Marine community ecology and conservation. Sinauer Associates, Sunderland, MA
- Bivand R, Lewin-Koh N (2015) maptools: tools for reading and handling spatial objects. R package version 0.8-36. <https://cran.r-project.org/web/packages/maptools/maptools.pdf>
- Bivand R, Rundel C (2015) rgeos: interface to geometry engine –open source (GEOS). R package version 0.3-11. <https://cran.r-project.org/web/packages/rgeos/rgeos.pdf>
- Bivand R, Keitt TH, Rowlingson B (2015) rgdal: bindings for the Geospatial Data Abstraction Library. R package version 1.0-4. <https://cran.r-project.org/web/packages/rgdal/rgdal.pdf>
- Bourdon R (1960) Rhizocéphales et isopodes parasites des décapodes marcheurs de la Baie de Quiberon. *Bull Soc Sci Nancy* 19:134–153
- Carlton JT, Cohen AN (2003) Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. *J Biogeogr* 30:1809–1820
- Costa S, Bessa F, Pardal MA (2013) The parasite *Sacculina carcini* Thompson, 1836 (Cirripedia, Rhizocephala) in the crab *Carcinus maenas* (Linnaeus, 1758) (Decapoda, Portunidae): influence of environmental conditions, colour morphotype and sex. *Crustaceana* 86:34–47
- Crothers JH (1968) The biology of the shore crab *Carcinus maenas* (L.) 2. The life of the adult crab. *Field Studies* 2: 579–614
- Curtis LA (1987) Vertical distribution of an estuarine snail altered by a parasite. *Science* 235:1509–1511
- Dittmann S, Villbrandt M (1999) Size frequency, distribution and colour variation of *Carcinus maenas* in the Spiekeroog Backbarrier System. In: Dittmann S (ed) *The Wadden Sea ecosystem: stability properties and mechanisms*. Springer-Verlag, Berlin, p 163–173
- Duran-Matute M, Gerkema T, De Boer GJ, Nauw JJ, Gräwe U (2014) Residual circulation and freshwater transport in the Dutch Wadden Sea: a numerical modelling study. *Ocean Sci* 10:611–632
- Glenner H, Werner M (1998) Increased susceptibility of recently moulted *Carcinus maenas* (L.) to attack by the parasitic barnacle *Sacculina carcini* Thompson 1836. *J Exp Mar Biol Ecol* 228:29–33
- Hechinger RF, Lafferty KD (2005) Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. *Proc R Soc B* 272:1059–1066
- Hijmans RJ (2015) raster: geographic data analysis and modeling. R package version 2.4-15. <https://cran.r-project.org/web/packages/raster/raster.pdf>
- Høeg JT (1995) The biology and life cycle of the Rhizocephala (Cirripedia). *J Mar Biol Assoc UK* 75:517–550
- Høeg JT, Lützen J (1995) Life cycle and reproduction in the Cirripedia Rhizocephala. *Oceanogr Mar Biol Annu Rev* 33:427–485
- Kashenko SD, Korn OM (2002) Effects of temperature and salinity on the larvae of two species of rhizocephalan (Crustacea: Cirripedia). *Invertebr Reprod Dev* 41: 179–184
- Klein Breteler WCM (1975) Growth and moulting of juvenile shore crabs, *Carcinus maenas*, in a natural population. *Neth J Sea Res* 9:86–99
- Klein Breteler WCM (1976) Settlement, growth and production of the shore crab, *Carcinus maenas*, on tidal flats in the Dutch Wadden Sea. *Neth J Sea Res* 10:354–376
- Kristensen T, Nielsen AI, Jørgensen AI, Mouritsen KN and others (2012) The selective advantage of host feminization: a case study of the green crab *Carcinus maenas* and the parasitic barnacle *Sacculina carcini*. *Mar Biol* 159: 2015–2023
- Larsen MH, Høeg JT, Mouritsen KN (2013) Influence of infection by *Sacculina carcini* (Cirripedia, Rhizocephala) on consumption rate and prey size selection in the shore crab *Carcinus maenas*. *J Exp Mar Biol Ecol* 446:209–215
- Lewis JR (1964) *The ecology of rocky shores*. English Universities Press, London
- Lützen J (1984) Growth, reproduction, and life span in *Sacculina carcini* Thompson (Cirripedia: Rhizocephala) in the Isefjord, Denmark. *Sarsia* 69:91–105
- McCarthy HO, Fitzpatrick S, Irwin S (2000) A transmissible trematode affects the direction and rhythm of movement in a marine gastropod. *Anim Behav* 59:1161–1166
- McGaw IJ, Naylor E (1992) Distribution and rhythmic locomotor patterns of estuarine and open-shore populations of *Carcinus maenas*. *J Mar Biol Assoc UK* 72:599–609
- Minchin D (1997) The influence of the parasitic cirripede *Sacculina carcini* on its brachyuran host *Carcinus maenas* within its home range. In: Thresher RE (ed) *Proc 1st Int Workshop on the Demography, Impacts and Management of the Introduced Populations of the European Crab, Carcinus maenas*, Mar 20–21, 1997, Hobart. Centre for Research on Introduced Marine Pests. Technical Report Number 11. CSIRO Marine Laboratories, Hobart, p 76–79
- Mouritsen KN, Poulin R (2002) Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology* 124(Suppl):S101–S117
- O'Brien J, Van Wyk P (1985) Effects of crustacean parasitic castrators (epicaridean isopods and rhizocephalan barnacles) on growth of crustacean hosts. In: Wenner AM (ed) *Crustacean issues: factors in adult growth*. A.A. Balkema, Rotterdam, p 191–218
- Øksnebjerg B (2000) The Rhizocephala (Crustacea: Cirripedia) of the Mediterranean and Black seas: taxonomy, biogeography, and ecology. *Isr J Zool* 46:1–102
- Pebesma E, Bivand R (2015) sp: classes and methods for spatial data. R package version 1.1-1. <https://cran.r-project.org/web/packages/sp/sp.pdf>
- Pietroock M, Marcogliese DJ (2003) Free-living endo-helminth stages: at the mercy of environmental conditions. *Trends Parasitol* 19:293–299
- R Development Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Rainbow PS, Ford MP, Hepplewhite I (1979) Absence of gregarious settling behaviour by female cypris larvae of British parasitic rhizocephalan barnacles. *J Mar Biol Assoc UK* 59:591–596
- Rasmussen E (1959) Behaviour of sacculinized shore crabs

- (*Carcinus maenas* Pennant). Nature 183:479–480
- Reid DG, Abelló P, Kaiser MJ, Warman CG (1997) Carapace colour, inter-moult duration and the behavioural and physiological ecology of the shore crab *Carcinus maenas*. Estuar Coast Shelf Sci 44:203–211
 - Reise K (1985) Tidal flat ecology. Springer-Verlag, Berlin
 - Rubiliani C, Rubiliani Durozoi M, Payen GG (1980) Effets de la sacculine sur les gonades, les glandes androgenes et le systeme nerveux central des crabes *Carcinus maenas* (L.) et *C. mediterraneus* Czerniavsky. Bull Soc Zool Fr 105: 95–100
 - Smith KD, Hall NG, De Lestang S, Potter IC (2004) Potential bias in estimates of the size of maturity of crabs derived from trap samples. ICES J Mar Sci 61:906–912
 - Styriehave B, Rewitz K, Andersen O (2004) Frequency of moulting by shore crabs *Carcinus maenas* (L.) changes their colour and their success in mating and physiological performance. J Exp Mar Biol Ecol 313:317–336
 - Thiel M, Dornedde T (1994) Recruitment of shore crabs *Carcinus maenas* on tidal flats: mussel clumps as an important refuge for juveniles. Helgol Mar Res 48:321–332
 - Thieltgies DW, Reise K (2007) Spatial heterogeneity in parasite infections at different spatial scales in an intertidal bivalve. Oecologia 150:569–581
 - Thieltgies DW, Jensen KT, Poulin R (2008) The role of biotic factors in the transmission of free-living endohelminth stages. Parasitology 135:407–426
 - Thresher RE (1996) Environmental tolerances of larvae of the European parasitic barnacle, *Sacculina carcini* (Rhizocephala: Sacculinidae): implications for use as a biological control agent against the introduced European crab, *Carcinus maenas*. Centre for Research on Introduced Marine Pests. Technical Report Number 7. CSIRO Marine Laboratories, Hobart, p 1–23
 - Torchin ME, Høeg JT (2008) Rhizocephalan barnacles. In: Denny MW, Gaines SD (eds) Encyclopedia of tidepools and rocky shores. University of California Press, Berkeley, CA, p 465–468
 - Torchin ME, Lafferty KD, Kuris AM (2001) Release from parasites as natural enemies: increased performance of a globally introduced marine crab. Biol Invasions 3: 333–345
 - van Aken HM (2008) Variability of the salinity in the western Wadden Sea on tidal to centennial time scales. J Sea Res 59:121–132
 - Werner M (2001) Prevalence of the parasite *Sacculina carcini* Thompson 1836 (crustacea, rhizocephala) on its host crab *Carcinus maenas* (L.) on the west coast of Sweden. Ophelia 55:101–110
 - Wickham H (2009) ggplot2: elegant graphics for data analysis. Springer, New York, NY
 - Williams MJ, Hill BJ (1982) Factors influencing pot catches and population estimates of the portunid crab *Scylla serrata*. Mar Biol 71:187–192

Editorial responsibility: Romuald Lipcius,
Gloucester Point, Virginia, USA

Submitted: August 24, 2015; Accepted: December 21, 2015
Proofs received from author(s): February 23, 2016