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 Goedknegt[1)2)], Rob Dekker[1)], Niamh McSweeney[1)], Johannes IJ. Witte[1)], Jaap van der Meer[1)2)], David W. Thieltges[1)3)]

1) NIOZ Royal Netherlands Institute for Sea Research, PO Box 59, 1790 AB Den Burg/Texel, The Netherlands

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

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

Corresponding author:

* andreas.waser@nioz.nl

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 three adjacent habitats along a tidal elevation gradient in the Dutch Wadden Sea: intertidal mussel beds, intertidal bare sand flats and subtidal gullies. Our sampling revealed that of the 27629 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. Both the prevalence of infected crabs and of ovigerous females in the gullies were 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.

Keywords

Parasitism, tidal zonation patterns, rhizocephalan barnacles, parasite manipulation of host behaviour, brood mimicry, Wadden Sea, feminisation

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.

66 This is also the case for common shore crabs (*Carcinus maenas* L., 1758) infected with the
67 rhizocephalan barnacle *Sacculina carcini* Thompson, 1836. Shore crabs are native to
68 European and North African shores and have been introduced to many coastal areas
69 worldwide (Carlton & Cohen 2003). They occur in two colour morphs, which are associated
70 with the crab's moulting stage; green crabs have recently moulted and red crabs have
71 undergone a prolonged duration of intermoult (Crothers 1968, McGaw & Naylor 1992, Reid
72 et al. 1997, Styrishave et al. 2004). In intertidal sedimentary ecosystems like the European
73 Wadden Sea, shore crabs occur in both subtidal and intertidal habitats, with particularly high
74 densities reported from biogenic structures like mussel beds and seagrass meadows (Klein
75 Breteler 1976, Reise 1985, Thiel & Dernedde 1994).
76 Shore crabs are infected by a range of parasites (Torchin et al. 2001), with the
77 rhizocephalan *S. carcini* being the most conspicuous one (Høeg 1995, Høeg & Lützen 1995).
78 Shore crabs and many other portunid crab species (see Øksnebjerg 2000 for host range of *S.*
79 *carcini*) become infected with this parasite when cyprid larvae of *S. carcini* settle on the crab
80 cuticle, penetrate into the hemocoel and develop an internal root-like network, the interna,
81 throughout the tissue of the crab. After a duration of 1-3 y, *S. carcini* matures and part of the
82 interna ruptures the crab's abdominal exoskeleton and forms a distinct sac-like structure
83 underneath the pleon of infected crabs (Lützen 1984). This structure is called the externa and
84 contains the reproductive organs of the parasite (Høeg & Lützen 1995). The parasite infects
85 and castrates both sexes of *C. maenas* (Høeg, 1995). Moreover, male crabs become to a
86 certain extent morphologically feminized, induced by effects of the parasite on the hormonal
87 system of the host, involving an enlargement of the pleon and a reduction in cheliped size
88 (Rubilianiet al. 1980, Høeg 1995, Kristensen et al., 2012). The morphological feminisation
89 occurs stepwise with every moulting event of the infected host, while *S. carcini* remains
90 internal (Høeg 1995). Once the externa emerges and crabs become externally infected,

91 moulting ceases (parasitic anecdysis; O'Brien & van Wyk 1985). These morphological
92 changes are also accompanied by behavioural changes. Infected individuals exhibit brood
93 mimicry by carrying the externa in the same place where egg-bearing females keep their eggs
94 (underneath the pleon) and by behaving like ovigerous females, for instance grooming the
95 parasite externa (Høeg & Lützen 1995). In addition, it has been proposed that infected crabs
96 migrate towards deeper waters, thus copying the behaviour of ovigerous females (Rasmussen
97 1959, Rainbow et al. 1979, Lützen 1984). However, replicated studies at several locations
98 along a tidal elevation gradient or a direct comparison of distributional patterns of parasitized
99 crabs and ovigerous females along such a gradient are lacking.
100 In this study, we investigated the distribution of shore crabs infected with *S. carcini* along a
101 tidal elevation gradient and compared it with the distribution of ovigerous females at identical
102 locations. To do so, we sampled crabs at 12 locations in the Dutch Wadden Sea from three
103 adjacent habitats: intertidal mussel beds, intertidal bare sand and subtidal gullies. This
104 sampling design together with data on water depth and salinity allowed us to investigate three
105 main research questions: 1) Do the levels of infection with *S. carcini* differ between the three
106 habitats and does this co-vary with patterns observed for ovigerous females?; 2) To what
107 extend are water depth and salinity drivers of infection levels with *S. carcini* and the
108 occurrence of ovigerous females?; and 3) Do infection levels differ among sexes or colour
109 morphs? By using a replicated and nested sampling design as well as a large sample size, our
110 study significantly increases the limited understanding of parasite distributions along tidal
111 elevation gradients.

Material and Methods

Sampling of crabs

C. maenas were sampled in early summer 2012 and 2013. In 2012, six locations were sampled in the western part of the Dutch Wadden Sea (week 22-23) and in 2013 six locations in the eastern part (week 24-25; Fig. 1 and Table 1). At each location, three 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 entry cones at both ends. The traps were baited with several (4-7) frozen juvenile (< 7cm) herring (*Clupea harengus)*, and were set at low tide and emptied after high tide periods. Intertidal mudflats adjacent to mussel beds were investigated at hide tide (water depth 0.5 – 1.5 m) using a 2 m beam trawl (mesh size 5.5 mm) towed by a small motor boat. 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). For two 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 two 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 *S. 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 (metre 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; "vaklodingen", 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 0.6 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 two 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*: $t = -0.96$, $p = 0.35$; ovigerous 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 three 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 three 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 two 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 fix 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 G-tests. Comparisons were based on the totals of subtidal crabs (N = 15086, 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 producing 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.

184 **Results**

185 Of the 27629 crabs investigated at the 12 locations, 217 carried an externa of the parasite *S.*
186 *carcini*. The size of these infected crabs ranged from 12.7 to 62.8 mm CW (Fig. 2 A, B) and
187 the size frequency distribution of infected crabs reflected more or less the one of uninfected
188 crabs (Fig. 2 A, B). In contrast, ovigerous females were generally larger (20.5 - 57.8 mm CW;
189 Fig. 2 C). In general, the prevalence of infected crabs differed among the three habitats
190 (GLMM, likelihood ratio test, $\chi^2 = 119.3$, $p < 0.001$; Table 2, Fig. 3 A): crabs showed highest
191 prevalence (up to 2.8 %) and occurred at all locations but one (W015) in the subtidal gullies,
192 where in total 206 infected crabs were found, while infections at the two intertidal habitats
193 only occurred at a few locations with low prevalence (below 0.5 %; Fig. 3 A**)**. The distribution
194 of ovigerous females also significantly differed among habitats (GLMM, likelihood ratio test,
195 $\chi^2 = 619.94$, $p < 0.001$; Table 2, Fig. 3 B). Egg-bearing females occurred with higher
196 prevalence and at all but one (W015) location in the subtidal gullies, while they only occurred
197 at a few locations and with very low prevalence on intertidal sand flats and mussel beds (Fig.
198 3 B). In general, the size frequency distribution of crabs was similar in the intertidal and the
199 subtidal and similar sizes of crabs were infected in both habitats (Fig. S1).

200 Both prevalence of infected crabs and prevalence of ovigerous females differed among the
201 subtidal gullies (Table 2, Fig. 3 A & B) and both were positively correlated with each other
202 (Pearson correlation; $r = 0.77$, $p < 0.01$; see Fig. S2). This covariance was likely the result of a
203 similar effect of the same abiotic factors on parasite and ovigerous female prevalence. Water
204 depth explained prevalence of infected crabs and ovigerous females the best; prevalence of
205 both generally increased with water depth (GLM with binomial distribution and quasi-
206 binomial error fix (because of overdispersion), Table 3, Fig. 4). Moreover, both infected crabs
207 and ovigerous females tended to occur more frequently at locations with higher salinity,
208 although this was not or only marginally significant (Fig. 4; Pearson correlations, infected

209 crabs: r = 0.57, p = 0.07; ovigerous females: r = 0.42, p = 0.2, see Fig. S2), although salinity
210 was dropped in the model selection procedures and was not included in the final model (Table
211 3).
212 Finally, infection status of the crabs caught in subtidal gullies significantly differed
213 between the different colour morphs and sexes (G-test: G = 440.35, df = 3, p < 0.001; Table
214 4). In males only 1.1 % (0.1 % green and 1 % red) of the population was found to be
215 externally infected. The non-infected males accounted for 98.9 % (90.7 % green and 8.2 %
216 red) (G-test: G = 374.96, df = 1, p < 0.001; Table 4). In contrast, in the females the proportion
217 of individuals of the green morph was much less. Only 44.4 % of the females were found to
218 be of green colour and non-infected while 0.2 % were externally infected green females. Most
219 of the red females, about 53.9 % of the total female population, carried no externa of *S.*
220 *carcini* and about 1.5 % of the females were red and externally infected (G-test: G = 58.253,
221 df = 1, p < 0.001; Table 4)**.**
222
223 **Discussion**
224 Our study revealed that most infected crabs were found in the subtidal gullies and almost
225 none on intertidal bare sand flats or mussel beds at all of the 12 locations. This pattern was
226 also observed in rhizocephalans infecting hermit crabs along the shore of Okinawa Island
227 (Ryukyu Archipelago, southwestern Japan, R. Yoshida pers. comm.) and corroborates earlier
228 notes in the literature based on very limited observations that infected *C. maenas* migrate
229 towards deeper waters (Rasmussen 1959, Rainbow et al. 1979, Lützen 1984). We observed a
230 similar pattern in the distribution of ovigerous females which also predominantly occurred in
231 the subtidal habitat. The migration towards the subtidal is thought to result from parasite-
232 mediated behavioural changes in infected crabs which lead to typical behaviour patterns of
233 ovigerous females, even in the case of infected males, ultimately resulting in the observed

234 distribution pattern (brood mimicry; Høeg & Lützen 1995). However, an alternative
235 explanation for the dominant occurrence in deeper waters may be a higher exposure of crabs
236 to infective propagules in subtidal compared to intertidal habitats. As infected crabs
237 accumulate in the subtidal gullies, the release of nauplius larvae will also predominately take
238 place here, suggesting a higher infection risk in the subtidal habitat that could lead to a
239 positive feedback loop. However, the development from the nauplius to the infective cypris
240 larval stage takes about 4-5 days (at 12-18 °C; Høeg & Lützen 1995), thus the site of larval
241 release and the site of actual infection are most likely disconnected due to some dispersal of
242 the infective stages, especially in turbulent and current rich tidal ecosystems like the Wadden
243 Sea. This suggests that crabs may become infected either in the intertidal and then migrate
244 towards deeper waters or, alternatively, they become infected in the subtidal and never
245 migrate into the intertidal. The actual causality will only be ascertained experimentally, for
246 example by releasing marked infected crabs in the intertidal and recapturing them over a tidal
247 elevation gradient.
248 Both the prevalence of infected crabs and of ovigerous females significantly increased with
249 water depth. Furthermore, there was an indication (marginally significant or non-significant)
250 of an increase in prevalence of infected crabs with salinity. Hence, both abiotic factors are
251 probably relevant for reproduction and the migration towards the subtidal gullies may
252 ultimately be related to the conditions faced by the larval stages of the crab and the parasite.
253 Deep and saline water may provide favourable physiological conditions for the larval stages
254 of both the host and the parasite. Indeed, mortality of larval stages of crabs and rhizocephalan
255 barnacles is known to increase with decreasing salinity (Anger et al. 1998, Anger 2003,
256 Thresher 1996, Kashenko & Korn 2002). In addition, larval release and subsequent dispersal
257 may be more successful in deeper waters than in the intertidal where larval stages most likely
258 will face higher risks of suffering from heat stress in the warmer months (Thresher 1996).

259 Regardless of the exact mechanisms, water depth and salinity seem to explain at least in part
260 the observed differences in prevalence among the 12 locations investigated in the Wadden
261 Sea. They may also underlie the differences in prevalence of *S. carcini* compared to other
262 locations where the parasite has been studied. In general, prevalences are known to vary
263 widely with prevalence of over 50 % observed at some localities along the native range of the
264 shore crabs (Bourdon 1960, Minchin 1997, Torchin et al. 2001, J. T. Høeg pers. comm.).
265 However, more detailed studies will be necessary to unravel the underlying mechanisms of
266 the large scale distribution of the parasite.
267 In general, mature *S. carcini* were mainly observed in crabs between 20 and 45 mm CW in
268 both sexes. In male crabs, however, some infected individuals reached 60 mm in CW.
269 Ovigerous females were also absent in the very small sized females, with 20 mm CW as the
270 minimum size for egg-bearing females. In contrast to parasitized female crabs, ovigerous
271 females were also numerous in sizes beyond 45 mm CW, reaching a maximum of about 60
272 mm CW. Similar patterns have been observed in previous studies (e.g. Lützen 1984, Dittmann
273 & Villbrandt 1999, Costa et al. 2013) and result from the biology of crabs and parasites.
274 Infection with *S. carcini* preferentially occurs on recently moulted crabs compared to crabs in
275 intermoult stages (Glenner & Werner 1998). As smaller crabs have higher moulting rates than
276 larger crabs, they face a higher infection risk. Moreover, as moulting ceases in infected crabs
277 with an externa (O'Brien & van Wyk1985), they do not increase in size, causing the observed
278 size distribution. Crabs below 20 mm CW, i.e. crabs of 1 y or younger (Klein Breteler 1975),
279 barely featured a mature externa of *S. carcini*, which can be attributed to the maturation period
280 of the parasite which at minimum takes about 1 y (Lützen 1984). Even in the very small
281 infected crabs, measuring only a few mm in CW, an externa generally does not appear before
282 crabs reach a CW of around 20 mm (e.g. Lützen 1984, Costa et al. 2013, J. T. Høeg pers.
283 comm.).

284 Hence, as barely any crabs below 20 mm CW were infected with *S. carcini*, the calculated
285 prevalence values for the different habitats depend to a certain extent on the size range used
286 for calculations. On intertidal bare sand flats for example, the inclusion of the very abundant
287 juvenile ~1 y old crabs (< 20 mm CW) in the prevalence estimates leads to an underestimation
288 in prevalence of infected crabs. If only the potentially infected size range was used,
289 prevalences would be slightly higher and comparisons with other studies need to take the
290 actual size range of the respective samples into consideration. The actual size range sampled
291 in our study also differed slightly between habitats as baited traps are known to catch
292 preferentially larger crabs (Williams and Hill 1982, Smith et al. 2004), due to larger
293 individuals being more aggressive, thereby restricting the likelihood of smaller animals
294 entering the trap. Hence, our prevalence values calculated for mussel beds may be relatively
295 higher compared to intertidal sand and subtidal gullies. However, as there were only very few
296 crabs infected with *S. carcini* on intertidal mussel beds and at intertidal sand flats (11 out of
297 217 infected crabs), some imprecision in the actual prevalence values among habitats does not
298 affect the overall distributional pattern observed. Another potential problem of using baited
299 crab traps may be a difference in catch efficiency between infected and non-infected crabs, for
300 example caused by lower feeding activity of infected crabs. However, experiments with *S.*
301 *carcini* infected and non-infected shore crabs did not find any differences in prey consumption
302 rates (Larsen et al. 2013) so that this is unlikely to confound the method. Baited crab traps
303 may also catch less ovigerous females due to the bias towards catching preferentially bigger
304 and male individuals, suggesting the absence of ovigerous females on mussel beds to be an
305 sampling artefact. However, our trawling (where this potential sampling bias is absent) in the
306 intertidal revealed that ovigerous females are generally scarce on sandy flats. In addition, our
307 long-term experience in the field and previous qualitative literature reports (Rasmussen 1959,
308 Rainbow et al. 1979, Lützen 1984) also indicate that ovigerous females and infected crabs are

309 rarely found on mussel beds and in the intertidal in general. This suggests that the pattern of a
310 predominantly subtidal occurrence of ovigerous females and infected crabs is unlikely to be
311 an artefact of our sampling design.

312 Finally, while the prevalence of *S. carcini* did not differ between sexes, the majority of the
313 infected crabs were of the red colour morph. This dominance of the red colour morph in
314 infected crabs probably results from the fact that moulting ceases after the emergence of the
315 externa in infected crabs (parasitic anecdysis; O'Brien & van Wyk 1985), with infected crabs
316 remaining at this intermoult stage as long as they carry the externa (Andrieux 1968). A
317 prolonged duration of intermoult stages has generally been associated with a red carapace
318 colour, while recently moulted crabs show a green carapace (Crothers 1968, McGaw &
319 Naylor 1992, Reid et al. 1997, Styrishave et al. 2004). The exact mechanism for this colour
320 change is not known but it has been suggested to be related to a denaturation of pigments in
321 the carapace (Reid et al. 1997). The lack of a difference in infection levels between males and
322 females contrasts with previous findings. For example, Costa et al. 2013 found significantly
323 higher prevalence of *S. carcini* in females than in males at some but not all sampling events at
324 the Mondego estuary in Portugal. However, Lützen (1984) found generally lower infection
325 levels in female than in male crabs in the Isefjord in Denmark and Werner (2001) found
326 similar infection levels of both sexes on the West coast of Sweden. Whether these differences
327 are related to some underlying mechanism or just result from spurious correlations remains to
328 be investigated.

329 In conclusion, *S. carcini* shows a distinct distributional pattern in the intertidal ecosystem
330 of the Wadden Sea, with most infected crabs occurring in the subtidal and probably driven by
331 water depth and salinity. This indicates that also other parasite groups besides the well
332 documented effects of trematodes are able to affect the distributional patterns of their hosts
333 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 using 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.

Acknowledgments

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 wish to 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. Goedknegt and D. W. Thieltges 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) indemne 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 Bio 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

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

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

Benson KR (2002) The study of vertical zonation on rocky intertidal shores-a historical perspective. Integr Comp Biol 42:776–779

Bertness, M. D., J. Bruno, B. R. Silliman JJS (2014) Marine Community Ecology and Conservation. Sinauer Associates, Sunderland, MA

Bourdon R (1960) Rhizocéphales et isopodes parasites des décapodes marcheurs de la Baie de Quiberon. Bulletin de la Soci´et´e des Sciences de Nancy 19: 134–153

374 Carlton JT, Cohen AN (2003) Episodic global dispersal in shallow water marine organisms:
375 The case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. J
376 Biogeogr 30:1809–1820

377 Costa S, Pardal M a., Bessa F (2013) The parasite *Sacculina carcini* Thompson, 1836
378 (Cirripedia, Rhizocephala) in the crab *Carcinus maenas* (Linnaeus, 1758) (Decapoda,
379 Portunidae): influence of environmental conditions, colour morphotype and sex.
380 Crustaceana 86:34–47

381 Crothers JH (1968) The biology of the shore crab *Carcinus maenas* (L.) 2. The Life of the
382 Adult Crab. F Stud 2:579–614

383 Curtis LA (1987) Vertical distribution of an estuarine snail altered by a parasite. Science
384 235:1509–1511

385 Dittmann S, Villbrandt M (1999) Size Frequency, Distribution and Colour Variation of
386 *Carcinus maenas* in the Spiekeroog Backbarrier System. In: Dittmann S (ed) The
387 Wadden Sea Ecosystem: Stability Properties and Mechanisms. Springer Berlin
388 Heidelberg, Berlin, Heidelberg, p 163–173

389 Duran-Matute M, Gerkema T, Boer GJ De, Nauw JJ, Gräwe U (2014) Residual circulation
390 and freshwater transport in the Dutch Wadden Sea: A numerical modelling study. Ocean
391 Sci 10:611–632

392 Glenner H, Werner M (1998) Increased susceptibility of recently moulted *Carcinus maenas*
393 (L.) to attack by the parasitic barnacle *Sacculina carcini* thompson 1836. J Exp Mar Bio
394 Ecol 228:29–33

395 Hechinger RF, Lafferty KD (2005) Host diversity begets parasite diversity: bird final hosts
396 and trematodes in snail intermediate hosts. Proc Biol Sci 272:1059–1066

397 Høeg JT (1995) The biology and life cycle of the Rhizocephala (Cirripedia). J Mar Biol Assoc
398 UK 75:517–550

399 Høeg JT, Lützen J (1995) Life cycle and reproduction in the Cirripedia Rhizocephala.
400 Oceanogr Mar Biol 33:427–485

401 Hijmans RJ (2015) raster: Geographic data analysis and modeling. R package version 2.4-15.
402 https://cran.r-project.org/web/packages/raster/raster.pdf

403 Kashenko SD, Korn OM (2002) Effects of temperature and salinity on the larvae of two
404 species of rhizocephalan (Crustacea: Cirripedia). Invertebr Reprod Dev 41:179–184

405 Klein Breteler WCM (1975) Growth and moulting of juvenile shore crabs, carcinus maenas,
406 in a natural population. Netherlands J Sea Res 9:86–99

407 Klein Breteler WCM (1976) Settlement, growth and production of the shore crab, *Carcinus*
408 *maenas*, on tidal flats in the Dutch Wadden Sea. Netherlands J Sea Res 10:354–376

409 Kristensen T, Nielsen AI, Jørgensen AI, Mouritsen KN, Glenner H, Christensen JT, Lützen J,
410 Høeg JT (2012) The selective advantage of host feminization: A case study of the green
411 crab *Carcinus maenas* and the parasitic barnacle *Sacculina carcini*. Mar Biol 159:2015–
412 2023

413 Larsen MH, Høeg JT, Mouritsen KN (2013) Influence of infection by *Sacculina carcini*
414 (Cirripedia, Rhizocephala) on consumption rate and prey size selection in the shore crab
415 *Carcinus maenas*. J Exp Mar Bio Ecol 446:209–215

416 Lewis JR (1964) The Ecology of Rocky Shores. English Universities Press, London

417 Lützen J (1984) Growth, reproduction, and life span in *Sacculina carcini* Thompson
418 (Cirripedia: Rhizocephala) in the Isefjord, Denmark. Sarsia 69:91–105

419 McCarthy H, Fitzpatrick S, Irwin S (2000) A transmissible trematode affects the direction and
420 rhythm of movement in a marine gastropod. Anim Behav 59:1161–1166

421 McGaw IJ, Naylor E (1992) Distribution and rhythmic locomotor patterns of estuarine and
422 open-shore populations of *Carcinus maenas*. J Mar Biol Assoc UK 72:599–609

423 Minchin D (1997) The influence of the parasitic cirripede *Sacculina carcini* on its brachyuran
424 host *Carcinus maenas* within its home range. In: Thresher RE (ed) Proc 1st Int
425 Workshop on the Demography, Impacts and Management of the Introduced Populations
426 of the European Crab, *Carcinus maenas*, Mar 20–21, 1997, Hobart. Centre for Research

427 on Introduced Marine Pests. Technical Report Number 11. CSIRO Marine Laboratories,
428 Hobart, p 76–79

429 Mouritsen KN, Poulin R (2002) Parasitism, community structure and biodiversity in intertidal
430 ecosystems. Parasitology 124 Suppl:S101–S117

431 O'Brien J, Wyk P Van (1985) Effects of crustacean parasitic castrators (epicaridean isopods
432 and rhizocephalan barnacles) on growth of crustacean hosts. In: Wenner AM (ed)
433 Crustacean Issues: factors in adult growth. A.A. Balkema, Rotterdam, p 191–218

434 Øksnebjerg B (2000) The Rhizocephala (Crustacea: Cirripedia) of the Mediterranean and
435 Black seas: Taxonomy, biogeography, and ecology. Isr J Zool 46:1–102

436 Pebesma E, Bivand R (2015) sp: Classes and Methods for Spatial Data. R package version
437 1.1-1. https://cran.r-project.org/web/packages/sp/sp.pdf

438 Pietrock M, Marcogliese DJ (2003) Free-living endohelminth stages: At the mercy of
439 environmental conditions. Trends Parasitol 19:293–299

440 R Development Core Team. 2015. R: a language and environment for statistical computing. R
441 Foundation for Statistical Computing, Vienna, Austria.

442 Rainbow PS, Ford MP, Hepplewhite I (1979) Absence of gregarious settling behaviour by
443 female cypris larvae of British parasitic Rhizocephalan barnacles. J Mar Biol Assoc UK
444 59:591–596

445 Rasmussen E (1959) Behaviour of Sacculinized Shore Crabs (*Carcinus maenas* Pennant).
446 Nature 183:479–480

447 Reid DG, Abelló P, Kaiser MJ, Warman CG (1997) Carapace colour, inter-moult duration and
448 the behavioural and physiological ecology of the shore crab *Carcinus maenas*. Estuar
449 Coast Shelf Sci 44:203–211

450 Reise K (1985) Tidal Flat Ecology. Springer Berlin Heidelberg, Berlin, Heidelberg

451 Rubiliani C, Rubiliani Durozoi M, Payen GG (1980) Effets de la sacculine sur les gonades, les
452 glandes androgenes et le systeme nerveux central des crabes *Carcinus maenas* (L.) et C.
453 mediterraneus Czerniavsky. Bull Soc Zool Fr 105:95–100

454 Smith KD, Hall NG, Lestang S De, Potter IC (2004) Potential bias in estimates of the size of
455 maturity of crabs derived from trap samples. ICES J Mar Sci 61:906–912

456 Styrishave B, Rewitz K, Andersen O (2004) Frequency of moulting by shore crabs *Carcinus*
457 *maenas* (L.) changes their colour and their success in mating and physiological
458 performance. J Exp Mar Bio Ecol 313:317–336

459 Thiel M, Dernedde T (1994) Recruitment of shore crabs *Carcinus maenas* on tidal flats:
460 mussel clumps as an important refuge for juveniles. Helgol Mar Res 48:321–332

461 Thieltges DW, Jensen KT, Poulin R (2008) The role of biotic factors in the transmission of
462 free-living endohelminth stages. Parasitology 135:407–426

463 Thieltges DW, Reise K (2007) Spatial heterogeneity in parasite infections at different spatial
464 scales in an intertidal bivalve. Oecologia 150:569–581

465 Thresher RE (1996) Environmental tolerances of larvae of the European parasitic barnacle,
466 *Sacculina carcini* (Rhizocephala: Sacculinidae): implications for use as a biological
467 control agent against the introduced European crab, *Carcinus maenas*. Centre for
468 Research on Introduced Marine Pests. Technical Report Number 7. CSIRO Marine
469 Laboratories, Hobart, p 1–23

470 Torchin ME, Høeg JT (2008) Rhizocephalan Barnacles. In: Denny MW, Gaines SD (eds)
471 Encyclopedia of Tidepools and Rocky Shores. University of California Press, p 465–468

472 Torchin ME, Lafferty KD, Kuris AM (2001) Release from parasites as natural enemies:
473 Increased performance of a globally introduced marine crab. Biol Invasions 3:333–345

474 van Aken HM (2008) Variability of the salinity in the western Wadden Sea on tidal to
475 centennial time scales. J Sea Res 59:121–132

476 Wickham H (2009) ggplot2: Elegant Graphics for Data Analysis. Springer New York, New
477 York, NY

478 Werner M (2001) Prevalence of the parasite *Sacculina carcini* Thompson 1836 (crustacea,
479 rhizocephala) on its host crab *Carcinus maenas* (L.) on the West Coast of Sweden.
480 Ophelia 55:101–110

481 Williams MJ, Hill BJ (1982) Factors influencing pot catches and population estimates of the
482 portunid crab Scylla serrata. Mar Biol 71:187–192

483

Table 1: Names of locations and location codes 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, coded W) and 2013 (East, coded E). For each location, the number of hauls in subtidal gullies and on intertidal bare sand flats as well as the number of traps set on mussel beds are given. Numbers in parenthesis show the total number of crabs investigated per habitat. For details of sampling design see text.

Code	Location	Coordinates	Date	n mussel bed	n intertidal	n subtidal
W001	De Cocksdorp	53°9'31.97"N, 4°53'20.81"E	7 & 8 June	30 (593)	13 (659)	5 (613)
W007b	Vlieland	53°16'14.45"N, 5°1'19.64"E	6 & 7 June	19 (379)	7 (248)	6 (401)
W012	Krassekeet	53°6'51.77"N, 4°55'11.85"E	5 June	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 June	17 (319)	10 (858)	6 (690)
W017	Napoleondam	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 June	17 (571)	10 (504)	8 (1292)
E013	Roode Hoofd	53°26'9.10"N, 6°9'59.02"E	18 June	8 (110)	5 (263)	8 (756)
E015	Schiermonnikoog	53°27'51.16"N, 6°10'53.07"E	19 June	16 (611)	10 (570)	8 (1675)
E022	Ternaard south	53°23'56.33"N, 5°56'14.33"E	11 June	10 (55)	10 (844)	8 (1622)
E032	Ternaard north	53°24'38.45"N, 5°57'34.47"E	11 June	10 (51)	7 (556)	Same as E022
E031	Kromme Balg	53°22'37.16"N, 5°39'33.29"E	12 June	16 (583)	5 (514)	7 (186)

Table 2: Results of the general mixed effects model (binomial error structure) analysing the prevalences of infected crabs and ovigerous females in different habitats. Table is showing predictor estimates, standard errors (SE), *z* and p values. The model included habitat as a fixed factor and location as a random factor. The habitat intertidal mussel bed is included in the intercept.

	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

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, $p < 0.001$).

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

	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		

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

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

512

513

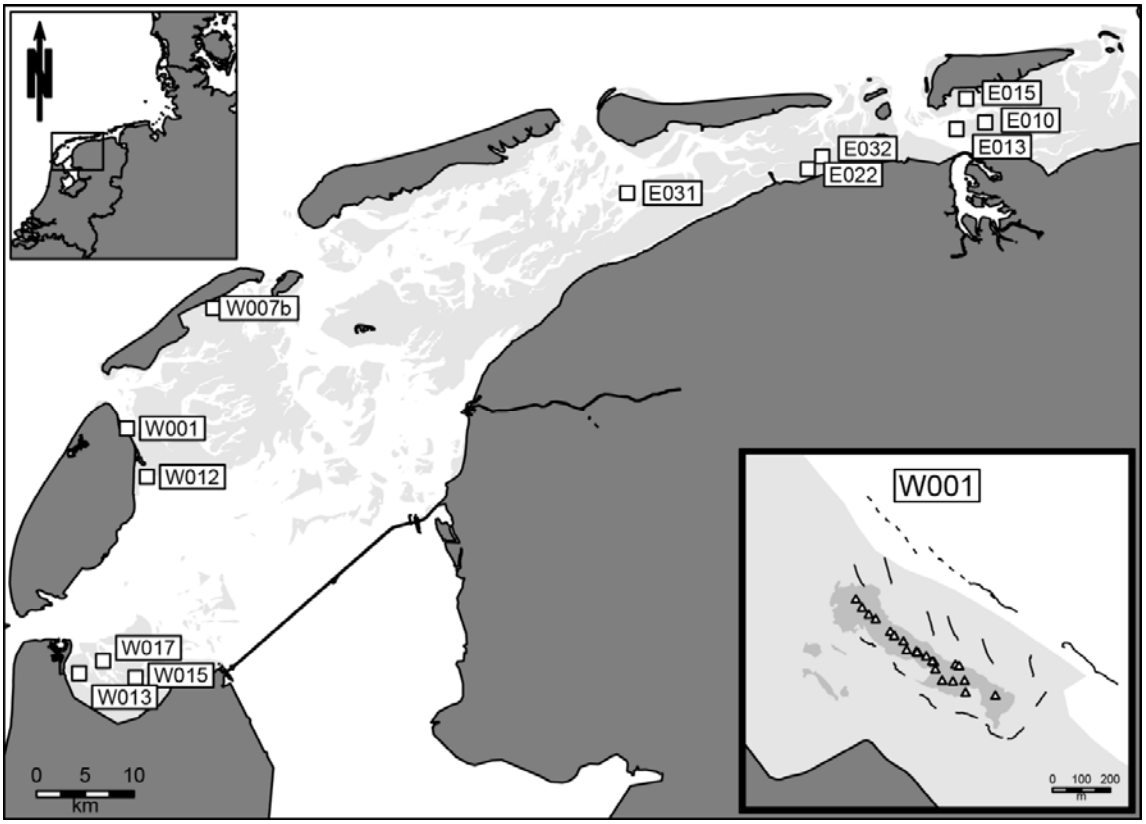


Fig. 1: Sampling locations in the Dutch Wadden Sea. At each location (white squares), intertidal mussel beds and adjacent intertidal bare sand flats and subtidal gullies were sampled. For location names and numbers of crabs sampled see Table 1. White areas represent the subtidal, light grey areas indicate intertidal flats exposed during low tide, intermediate grey indicates mussel beds and land is represented by dark grey. An example of the specific sampling design of one site is given on the bottom right. In this detail map, white triangles represent positions of traps and lines represent the hauls taken by beam trawl. Dashed lines indicate hauls at low tide and full lines the ones at high tide.

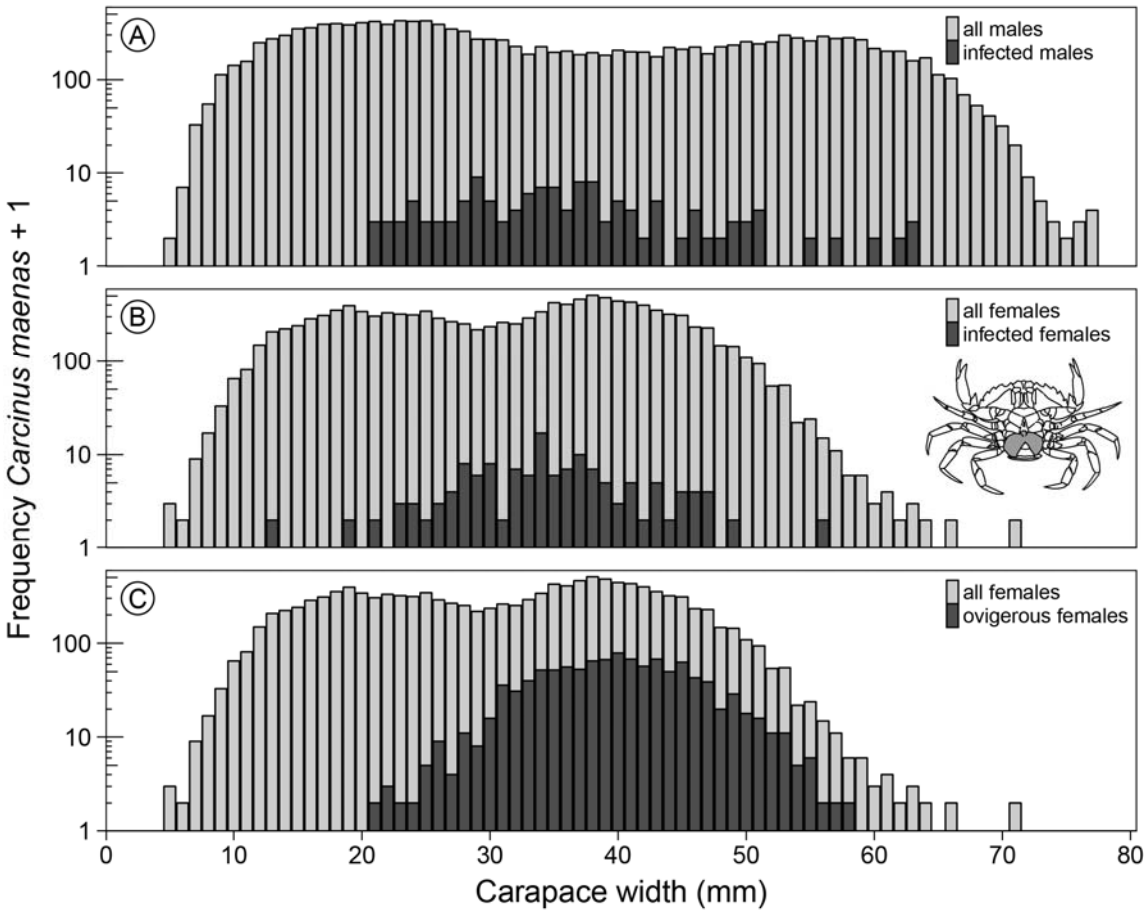
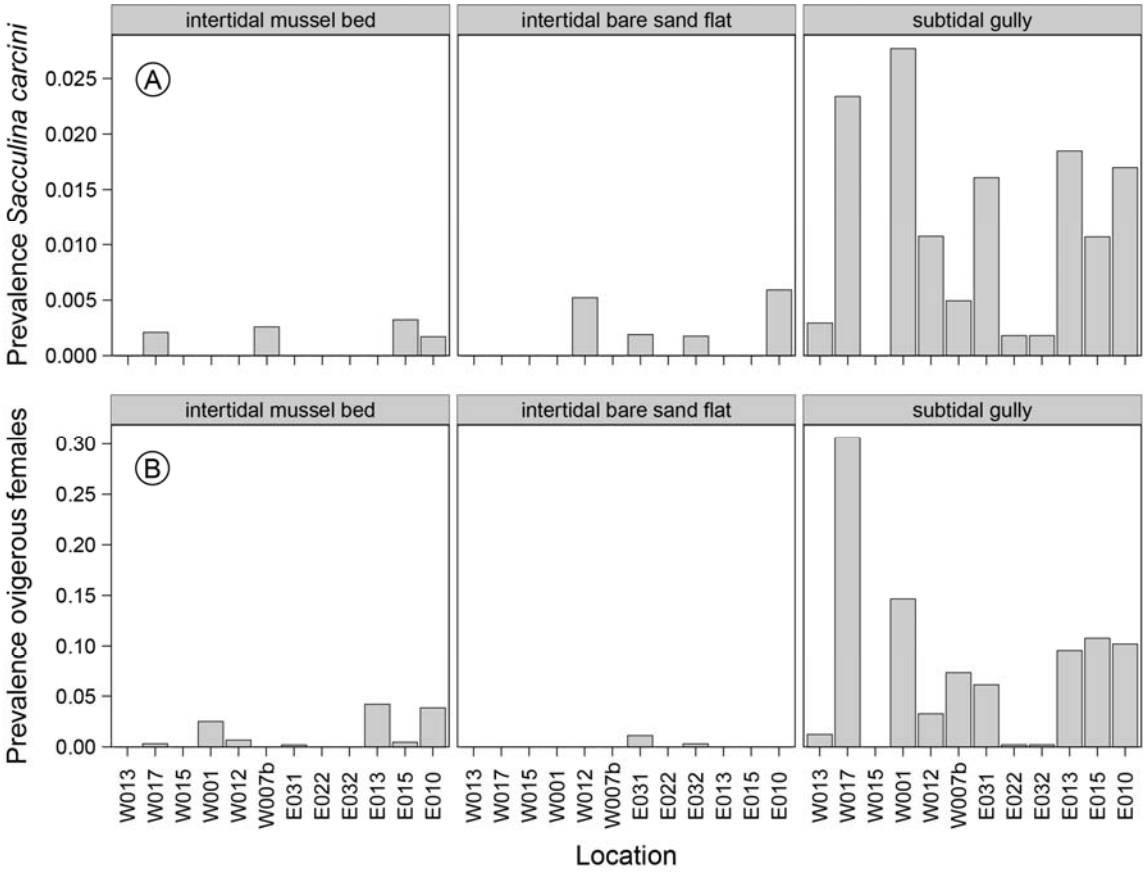


Fig. 2: Size frequency distributions of *Carcinus maenas* (on a log-scale) summed for all locations and habitats: A) male crabs infected with *Sacculina carcini* in comparison to all male crabs caught; B) female crabs infected with *S. carcini* and the total of caught female crabs; and C) ovigerous female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



528

529 **Fig. 3:** Prevalence of shore crabs infected with *Sacculina carcini* A) and prevalence of

530 ovigerous females B) in three habitats at 12 locations in the Dutch Wadden Sea. For location

531 codes and sample sizes see Table 1. Note that mussel beds E022 and E032 shared the same

532 subtidal gully and hence the same value.

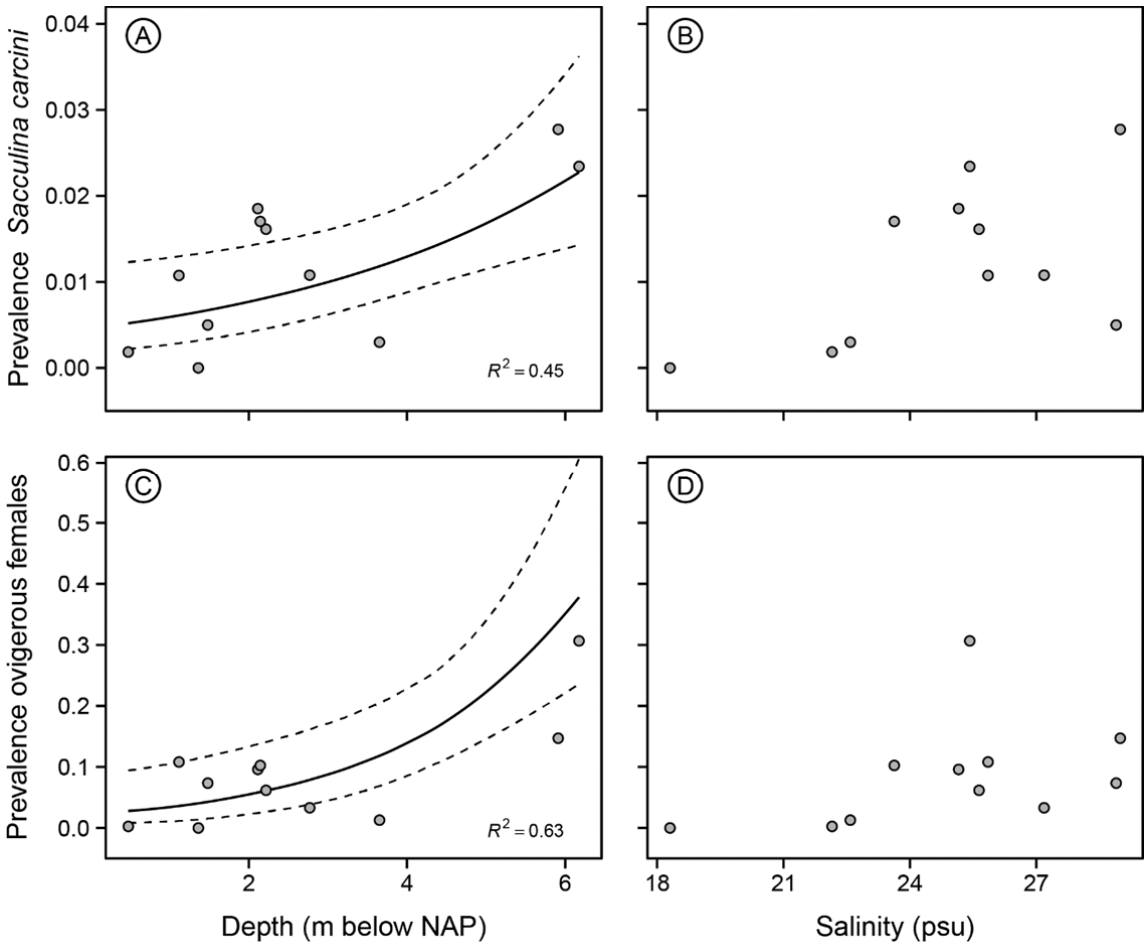


Fig. 4: Prevalence of shore crabs infected with *Sacculina carcini* in subtidal gullies depending on A) water depth (m below MTL) and B) salinity (psu). Prevalence of ovigerous females in subtidal gullies depending on C) water depth and D) salinity. Plots show observed values (dots) and fitted values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

Electronic appendix

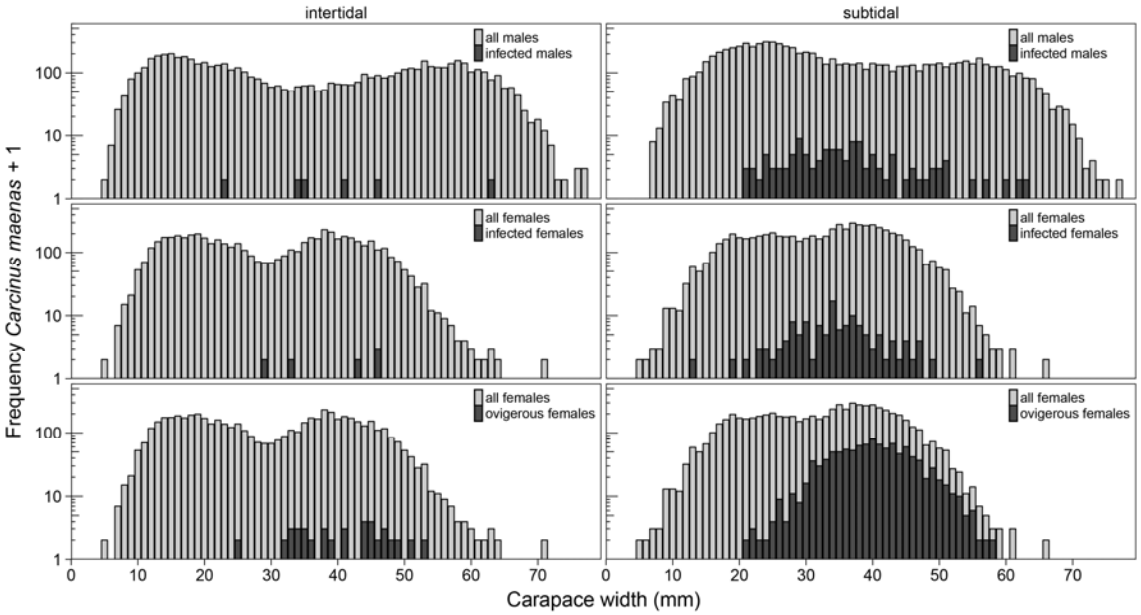


Fig. S1: Size frequency distributions of *C. maenas* (on a log-scale) in intertidal (left) and subtidal (right) habitats summed for all locations: male crabs infected with *S. carcini* in comparison to all male crabs caught in the intertidal (top left) and in the subtidal (top right); female crabs infected with *S. carcini* and the total of caught female crabs in the intertidal (middle left) and in the subtidal (middle right); and ovigerous female crabs compared to all female crabs caught in the intertidal (bottom left) and in the subtidal (bottom right).

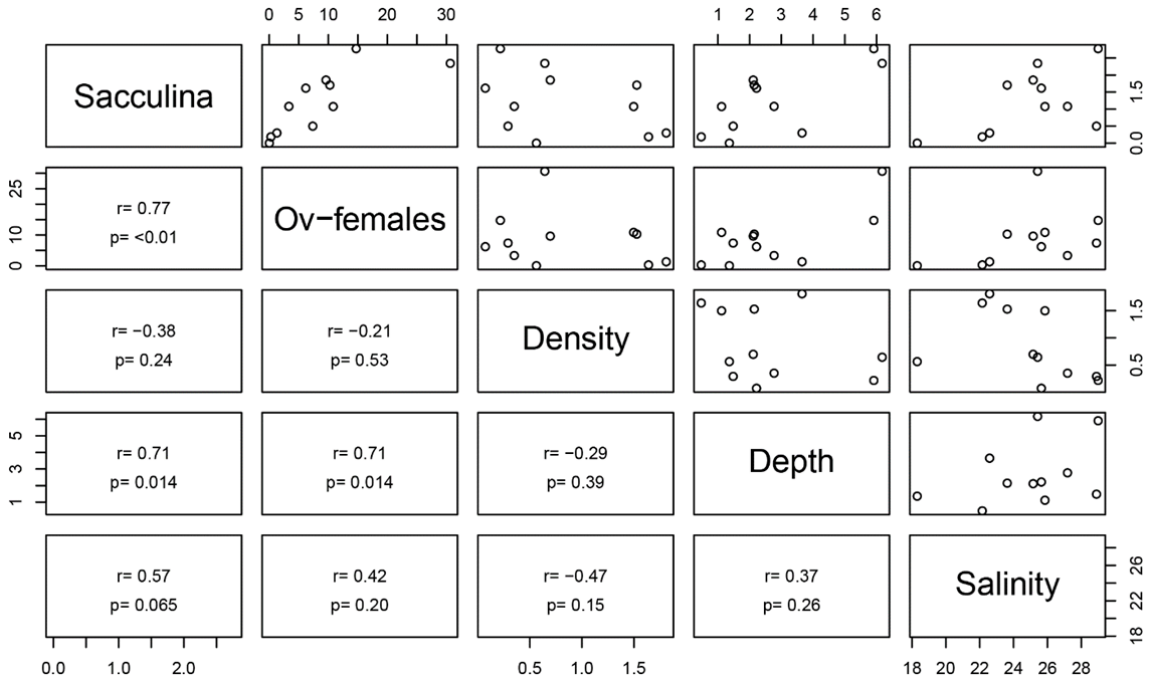


Fig. S2: Pearson correlations between the various variables obtained from samples from the subtidal gullies (n = 11 locations: same gully for E022 and E032): prevalence of the rhizocephalan parasite *Sacculina carcini* (Sacculina), prevalence of ovigerous females (Ov-females), density of crabs (Density), water depth (Depth) and salinity (Salinity).