Aquatic Invasions (2017) Volume 12, Issue 2: 201–212
DOI: https://doi.org/10.3391/ai.2017.12.2.08

Open Access

Research Article

Cross-species comparison of parasite richness, prevalence, and intensity in a native compared to two invasive brachyuran crabs

M. Anouk Goedknegt*, Jarco Havermans, Andreas M. Waser, Pieternella C. Luttikhuizen, Estefania Velilla, Kees (C. J.) Camphuysen, Jaap van der Meer and David W. Thieltges

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

E-mail addresses: Anouk.Goedknegt@nioz.nl (AG), j-havermans@live.nl (JH), Andreas.Waser@nioz.nl (AMW), Pieternella.Luttikhuizen@nioz.nl (PCL), estefania.velilla@gmail.com (EV), Kees.Camphuysen@nioz.nl (KC), Jaap.van.der.Meer@nioz.nl (JVDM), David.Thieltges@nioz.nl (DT)

**Corresponding author*

Received: 13 July 2016 / Accepted: 11 January 2017 / Published online: 15 February 2017

Handling editor: April M.H. Blakeslee

Abstract

An introduced species' invasion success may be facilitated by the release of natural enemies, like parasites, which may provide an invader with a competitive advantage over native species (enemy release hypothesis). Lower parasite infection levels in introduced *versus* native populations have been well documented. However, any potential competitive advantage will depend on whether native competitors exhibit higher parasite loads than introduced hosts and whether native hosts suffer more (e.g., reduced reproduction or growth) from parasite infections than introduced hosts. In this study, we compared macroparasite richness, prevalence, and intensity in sympatric populations of one native and two introduced brachyuran crab hosts in the centre of their European range. While the native green crab *Carcinus maenas* (Linnaeus, 1758) hosted three parasite groups (acanthocephalans, microphallid trematodes, rhizocephalans), the two invasive crab species *Hemigrapsus sanguineus* (De Haan, 1835) and *H. takanoi* Asakura and Watanabe, 2005 were only infected with acanthocephalans. All acanthocephalans were molecularly identified (COI) as the native *Profilicollis botulus* (Van Cleave, 1916). Prevalence and intensities of *P. botulus* were generally lower in the introduced than in the native crabs. Metacercariae of microphallid trematodes were only found in the native *C. maenas*, with mean infection levels of 100–300 metacercariae per host, depending on geographical location. Likewise, the castrating rhizocephalan barnacle *Sacculina carcini* Thompson, 1836 was only found in *C. maenas* at a few locations with low prevalences (< 3%). This first study on infection levels in invasive *Hemigrapsus* species in Europe indicates that these invasive crabs indeed experience lower infection levels than their native competitor *C. maenas*. Future experiments are needed to investigate whether this difference in infection levels leads to a competitive advantage for the invasive crab species.

Key words: enemy release, *Carcinus maenas*, *Hemigrapsus sanguineus*, *Hemigrapsus takanoi*, *Profilicollis botulus*, parasite spillback, Wadden Sea

Introduction

One of the mechanisms potentially facilitating the invasion success of introduced species is the release from natural enemies during the process of translocation (*enemy release hypothesis*; Elton 1958; Keane and Crawley 2002). During translocation, various barriers can reduce the number of predators and parasites that are co-introduced to the species' new range (Keane and Crawley 2002; Torchin et al. 2003; Colautti et al. 2004). This reduction in or release from enemies can result in direct fitness benefits for introduced populations when a species is negatively affected by the lost enemies in its native region (*regulatory release*; Colautti et al. 2004). In addition, a reduced set of enemies in the introduced range may release physiological resources otherwise invested in defence mechanisms (e.g. immune system) leading to increased fitness of the introduced host (*compensatory release*; Colautti et al. 2004). The two types of release are not mutually exclusive and may lead to a competitive advantage for introduced

species over native species (Keane and Crawley 2002; Grosholz and Ruiz 2003; Mitchell and Power 2003; Torchin et al. 2003; Parker et al. 2013).

Regarding parasites, a general reduction of parasite burdens in introduced hosts has been well documented and seems to be particularly strong in aquatic ecosystems (Torchin et al. 2003; Torchin and Lafferty 2009; Blakeslee et al. 2013). A review by Torchin et al. (2003) showed that parasite richness (number of species) and prevalence (proportion of hosts infected) are, on average, 2–3 times lower in hosts in their introduced compared to their native range. In general, the level of parasite reduction seems to differ among parasite groups. For example, in marine ecosystems, rhizocephalan parasites seem to be regularly lost during the process of introduction, while other parasite groups, like cestodes, are usually lost at a lower frequency (Blakeslee et al. 2013). It is important to note that the parasite richness of introduced hosts often consists of co-introduced parasites, but also of native or previously established parasites that have been acquired by the introduced species in the invasive range (Torchin and Mitchell 2004). This parasite acquisition may ultimately amplify the population size of these parasites and increase parasite loads in native hosts (*parasite spillback*; Kelly et al. 2009). Regardless of the parasite origin and level of reduction, the generality of the observed patterns suggests that many introduced hosts may have a competitive advantage over native species due to regulatory and compensatory release. However, a potential competitive advantage will depend on whether native competitors actually exhibit higher parasite loads than introduced hosts and whether native hosts suffer more (e.g., reduced reproduction or growth; Calvo-Ugarteburu and McQuaid 1998a, b; Bachelet et al. 2004; Byers 2000) from parasite infections than introduced hosts (Torchin and Mitchell 2004; Hatcher et al. 2006; Torchin and Lafferty 2009; Dunn et al. 2012). Studies comparing local infection levels between competing native and introduced hosts (community studies or cross-species comparisons, *sensu* Colautti et al. 2004; Torchin and Mitchell 2004) suggest that parasite richness, prevalence, and abundance are indeed often higher in native compared to introduced host species (Georgiev et al. 2007; Dang et al. 2009; Roche et al. 2010; Gendron et al. 2012). However, for most introduced host species, such cross-species comparisons between introduced and native competitors are lacking. This is also true for brachyuran crab species, some of which have been globally introduced into coastal waters and have been studied in respect to parasite release. The most prominent case is the European green crab *Carcinus maenas* (Linnaeus, 1758), which has been introduced to North America, Australia, Tasmania and parts of Japan and South Africa (Carlton and Cohen 2003). In a seminal study, Torchin et al. (2001) investigated infection levels in global *C. maenas* populations and found that crabs in native populations generally harboured more parasite species and showed higher infection levels than populations in areas where the crab species had been introduced. However, how this general parasite release of introduced *C. maenas* compares to parasite infection levels in native competitors has not been investigated to date. Another invasive crab species, the Asian shore crab *Hemigrapsus sanguineus* (De Haan, 1835), has been introduced from the North-West Pacific (with a native range from Russia along the coasts of Japan, Korea and China up to Hong-Kong; Epifanio 2013) to the North Atlantic coasts of North America (Williams and McDermott 1990; Epifanio 2013) and Europe (Dauvin et al. 2009). In North America, only three parasite species have been found in introduced populations of *H. sanguineus* (Torchin et al. 2001; Blakeslee et al. 2009; Kroft and Blakeslee 2016), while the crab species is infected with nine parasite species in native locations (reviewed in Blakeslee et al. 2009; McDermott 2011). Furthermore, infection intensities were much lower in populations in the introduced compared to the native range (Blakeslee et al. 2009; McDermott 2011). In comparison to other crab species at the Atlantic coast of North America, there was no significant difference in parasite richness and prevalence between the invasive *H. sanguineus* and two native crab species (Kroft and Blakeslee 2016), but compared to another invader, *C. maenas*, richness and prevalence were relatively lower in *H. sanguineus* (Blakeslee et al. 2009). However, whether *H. sanguineus* also shows enemy reduction in Europe is presently unknown. A third species, the brush-clawed shore crab *Hemigrapsus takanoi* Asakura and Watanabe, 2005, has been introduced to Europe from the same region as *H. sanguineus* and now occupies the same range in Europe as its congener (northern Spain to Sweden; Dauvin et al. 2009; NORSAS 2012; Markert et al. 2014). As *H. takanoi* was only recently identified as a pseudocryptic sibling species of *H. penicillatus* (Takano et al. 1997; Asakura and Watanabe 2005), literature records on parasite infections from native and introduced populations do not exist.

This study conducted a cross-species comparison of macroparasite infection levels in the two *Hemigrapsus* species (*H. sanguineus* and *H. takanoi*) introduced to Europe, with infection levels in their main native competitor (*C. maenas*). By sampling sympatric populations of the three species in the Dutch Wadden Sea, located in the centre of the invasive

Table 1. Habitat, sampling dates and sample sizes of native (*Carcinus maenas*) and invasive (*Hemigrapsus* spp.) crabs collected in the Wadden Sea around the island of Texel.

Location	Habitat	Sampling dates	*Carcinus maenas*		*Hemigrapsus sanguineus*		*Hemigrapsus takanoi*	
			Females	Males	Females	Males	Females	Males
1	Dyke	18, 19 June	10	56	40	11	7	27
2	Mussel/oyster bed	19, 29 Jun; 16 Aug	38	94	3	0	0	3
3	Mussel/oyster bed	14 Aug	4	22	17	1	5	19
4	Dyke	4 Jun; 2 Jul	7	5	28	24	5	26
5	Dyke	30 May; 1, 5, 12 Jun; 5 Jul	21	16	29	16	5	23
6	Mussel/oyster bed	24 May; 5 Sep	62	22	10	2	5	15
7	Dyke	7, 20, 21 Jun	14	23	25	12	8	33
8	Channel	20 Jun	5	31	0	0	0	0
9	Sand flat	18 Jun	4	14	0	0	0	0
10	Sand flat	18 Jun	3	4	0	0	0	0
Total			168	287	152	66	35	146

European range of the two species of *Hemigrapsus*, the study aimed to answer two main questions: 1) Is there evidence for parasite reduction in European populations of the two introduced *Hemigrapsus* species?; and 2) how do parasite richness and infection levels of the introduced crabs compare with those levels of their main native competitor *C. maenas*? As *Hemigrapus* spp. is currently expanding its range, and negative impacts of invasion have been documented in the US where it is also invasive (e.g., Lohrer and Whitlatch 2002; Tyrell et al. 2006; Brousseau et al. 2014), this first investigation on parasite infections in introduced *Hemigrapsus* species in Europe contributes to the understanding of the magnitude and relevance of parasite release for native and introduced host populations.

Material and methods

Sampling and dissection

Sampling of crabs was carried out between May and September 2012 at ten locations around the island of Texel in the southern Wadden Sea in the Netherlands (Figure 1, Table 1). In the intertidal zone, three habitats (dykes reinforced with rocks, epibenthic bivalve beds composed of invasive Pacific oysters (*Crassostrea gigas*) and native blue mussels (*Mytilus edulis*), and sandy tidal flats) were sampled at low tide by collecting crabs (>1 cm carapace width) by hand and by setting crab traps which were retrieved the following low tide. Previous studies indicated that crabs can be collected without a size class bias by these methods (Landschoff et al. 2013). In the subtidal zone, a single location was sampled by collecting crabs caught in a kom-fyke net used by the NIOZ Royal Netherlands Institute for Sea Research for long-term monitoring of fish and macroinvertebrates (Campos et al. 2010; Van der Veer et al. 2015).

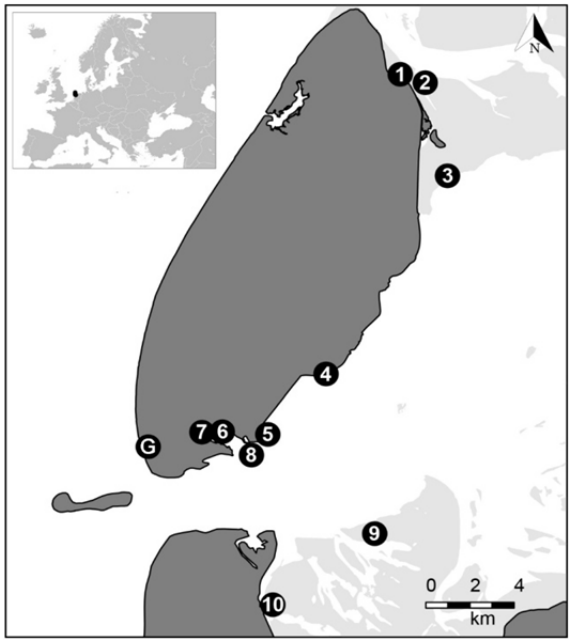

Figure 1. Sampling locations of crabs (1−10) around the island of Texel in the southern Wadden Sea in the Netherlands (black dot in the small insert left top corner) as well as sampling location of gull colony on Texel (G) from which additional acanthocephalans were sourced from gulls for molecular identification.

Sample sizes depended on local abundances of crabs and generally differed among locations and the three crab species (Table 1).

After collection, all crabs were brought to the laboratory and stored frozen at −18 ºC for later dissections. The dissection protocol for crabs was similar as the one described by Torchin et al. (2001). Prior to dissection, sex was determined for each crab, identified to species level, and carapace width (CW in mm) measured between the fifth spines on the dorsal side of the carapace. Before removing the

carapace, crabs were checked for infection by the rhizocephalan *Sacculina carcini* Thompson 1836 (visible externa). As early infections without a visible externa could not be detected with this approach, our estimates of rhizocephalan infection levels are conservative. The carapace was then opened, the internal carapace tissue carefully removed and squeezed between two large glass plates and examined under a stereomicroscope. All macroparasites found were identified and counted. Acanthocephalans found in *Carcinus maenas* and the two *Hemigrapsus* species were carefully removed from the tissue and stored in pure ethanol for molecular analysis.

Molecular identification

To identify potentially introduced acanthocephalans, a sub-set of acanthocephalans found in the two invasive crab species (*Hemigrapsus takanoi* n = 14, *Hemigrapsus sanguineus* n = 10) and of the ones found in *C. maenas* (n = 17; one acanthocephalan per individual crab) were molecularly identified (Supplementary material Table S1). To compare the data from larval stages collected from crab hosts with adult stages from local definitive hosts, we also added two adult parasites that were retrieved from two herring gull (*Larus argentatus*; Pontoppidan, 1763) chicks from a breeding colony on Texel (e.g. Camphuysen 2013) (Figure 1; see Appendix 1 for dissection protocol).

Parasite genomic DNA was extracted using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma) according to the manufacturer's instructions. DNA concentrations were determined using spectrophotometry (ND-1000, NanoDrop Technologies). New primers were designed based on an alignment of COI sequences for the acanthocephalans *Profilicollis botulus* (Van Cleave, 1916) and *Polymorphus minutus* (Zeder, 1800) (Genbank accession numbers EF467862 and EF467865, respectively). With the help of the primers AcaCOf (TGATATATGTTTT GGTTAGGTTRTGAA) and AcaCOr (CACCYCCT GTAGGATCAAAA), a portion of the cytochrome-c-oxidase I (COI) gene was amplified in a total volume of 50 µl containing 1× PCR buffer, 0.25 mM of each dNTP, 1 µM of each primer and 1 unit Biotherm+ DNA polymerase, using 2 µl undiluted DNA extract. Initial denaturation was performed at 94 °C for 2 min., followed by 35 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C and extension for 1 min. at 72 °C, with a final extension step of 72 °C for 10 min. Sequencing of the PCR products was carried out at Macrogen, Korea. Sequences were aligned manually in BioEdit 7.2.5 (Hall 1999) and compared to published acanthocephalan COI sequences. Genetic distances were estimated with MEGA 6 (Tamura et al. 2013), and minimum spanning networks among all haplotypes detected was constructed using the R package Pegas version 0.8-2 (Paradis 2010).

Phylogenetic trees were constructed in MEGA 7 (Kumar et al. 2016) by adding as outgroup two acanthocephalan sequences from different species (EF467865 from *P. minutus* and KF835320 from *Profilicollis altmani* (Perry, 1942) Van Cleave, 1947). A condensed Maximum Parsimony Tree was produced by using ten random addition trees and 500 bootstrap replicates. For the Maximum Likelihood Tree the best nucleotide substitution model was selected to be HKY+G (Hasegawa-Kishino-Yano with gamma distribution) based on both the AIC (Akaike Information Criterion) and the BIC (Bayesian Information Criterion) criteria, and 500 bootstrap replicates were run.

Statistical analyses

For each location and crab species, prevalence (proportion of infected crabs) and mean intensities (no. of parasites per infected crab) were calculated. Differences in prevalence between species or between locations were tested with likelihood-ratio tests (G-tests). Differences in intensity of acanthocephalans among the three hosts and the sampling sites were tested with general linear models (GLM) with intensities (log-transformed) as response variable and location and crab species as fixed factors. These analyses included all locations at which the three crabs co-occurred. Differences in intensities of trematodes in *C. maenas* among locations were tested with a GLM with intensity as response variable and location as fixed factor. Test assumptions were verified by inspecting residual plots. Relationships between intensity and host size as well as between prevalence and mean intensity per location among the three crab species were tested with Spearman correlations. All analyses were performed using the statistical software R v3.2.1. (R Development Core Team 2015).

Results

We sampled 854 crabs from ten locations: 455 were *Carcinus maenas,* 218 were *Hemigrapsus sanguineus,* and 181 were *Hemigrapsus takanoi* (Table 1). Although not quantified, *C. maenas* seemed more abundant on mussel/oyster beds than *Hemigrapsus* spp., while along dykes it was the opposite. *Hemigrapsus* spp. were absent at the subtidal location (location 8) and the two sandflat locations (locations 9 and 10).

Carcinus maenas was infected by three parasite taxa: acanthocephalans, trematodes, and rhizocephalans (Figure 2). In contrast, the *Hemigrapsus* species were only infected by acanthocephalans. In these two invasive crab species, prevalence was generally lower than in the native *C. maenas* (G-test, G = 218.68, P < 0.001). All acanthocephalans (cystacanth stage) molecularly identified (sequences deposited at Genbank, accession numbers KX279893-KX279935) from the two *Hemigrapsus* species, *C. maenas,* and herring gull chicks were *Profilicollis botulus* (Van Cleave, 1916) (García-Varela and Pérez-Ponte de León 2008). The maximum p-distance among any two sequences was 0.0243, while the smallest distance to any sequence in Genbank except *P. botulus* was p = 0.1739 to *Profilicollis altmani* (Perry, 1942) Van Cleave, 1947 (KF835320) (see also Figures S1 and S2). The trematodes found in *C. maenas* were metacercarial stages of microphallids, with a mix of two probably native species, *Maritrema subdolum* Jägerskiöld, 1909 and *Microphallus claviformis* (Brandes, 1888), based on previous investigations in the study region (Thieltges et al. 2008a). However, more detailed molecular analyses are pending.

Within individual sampling locations, acanthocephalan prevalence was significantly higher in native *C. maenas* than in the two invasive *Hemigrapsus* species in all but one location (Figure 3A; location 2: G-test, G = 3.050, P = 0.218, for all others P < 0.01). Within species, prevalence differed among the sampling locations in *C. maenas* (G-test, G = 39.271, P < 0.001) but not in *H. sanguineus* (P = 0.088) and *H. takanoi* (P = 0.107). Intensity of acanthocephalan infection significantly differed between species, with highest infection levels in *C. maenas* (Figure 3B; GLM, $F_{2,242}$ = 6.172, P < 0.01). Although mean intensities tended to differ among locations (Figure 3B), this was not statistically significant (GLM, $F_{6,236}$ = 0.793, P = 0.570). There was also no statistically significant interaction between location and crab species (GLM, $F_{12,224}$ = 0.626, P = 0.819). Intensity of acanthocephalan infections in individuals of the three host crab species did not significantly increase with host size (Figure 4, Spearman correlation, all P > 0.170). Both females and males of the three crab species were infected, and the size of infected individuals ranged between 14–72 mm CW for *C. maenas* and between 14–25 mm CW and 15–25 mm CW for *H. sanguineus* and *H. takanoi*, respectively (Figure S3). Mean prevalence per location was positively correlated between *H. sanguineus* and *H. takanoi* (Spearman correlation, Spearman's ρ = 0.86, P = 0.014) but not between *C. maenas* and *H. sanguineus* (Spearman's ρ = 0.36, P = 0.432) or *H. takanoi* (Spearman's ρ = 0.11, P = 0.819; Figure S4). Similarly, mean intensity at the different locations was not correlated between *C. maenas* and *H. sanguineus* (Spearman's ρ = −0.20, P = 0.672) nor between *C. maenas* and *H. takanoi* (Spearman's ρ = −0.32, P = 0.491), but positively correlated between the two *Hemigrapsus* species (Spearman's ρ = 0.96, P < 0.001; Figure S5).

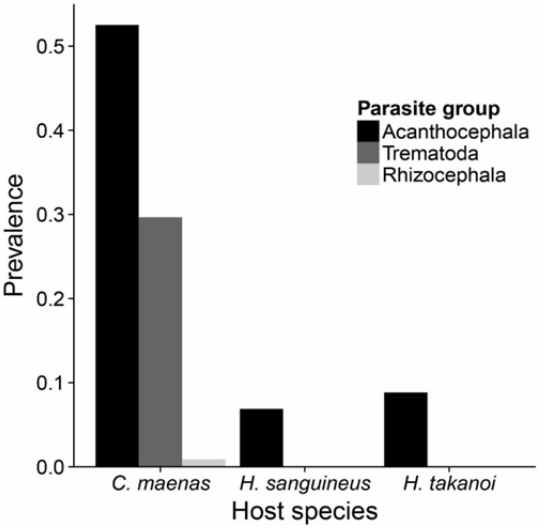

Figure 2. Overall prevalence of the three parasite groups (Acanthocephala, Trematoda, Rhizocephala) found in the three crab host species *Carcinus maenas* (n = 10 sampling locations), *Hemigrapsus sanguineus* and *Hemigrapsus takanoi* (both n = 7 sampling locations).

Prevalence of trematode infection in the native *C. maenas* varied significantly among locations (G-test, G = 51.501, P < 0.001), with the two sand flat habitats (locations 9 and 10) showing highest prevalences (Figure 5A). In contrast, intensities did not differ significantly between the ten locations (Figure 5B; GLM, $F_{9,125}$ = 0.480, P = 0.886). Crabs were, on average, infected with 100–300 metacercariae of microphallid trematodes depending on location (Figure 5B), and individual crabs were infected with up to 1,400 metacercariae. Like acanthocephalan infections, trematodes infected both sexes of *C. maenas* and the size of infected individuals (14–67 mm CW) was similar to that of uninfected crabs (13–75 mm CW, Figure S6). Mean intensity in infected crabs did not significantly correlate with crab size (Spearman's correlation, Spearman's ρ = 0.15, P = 0.080, Figure 6A). Moreover, the intensity of trematodes was independent of acanthocephalan intensity (Spearman's ρ = −0.12, P = 0.364; Figure 6B). Prevalences of infections of *C. maenas* with the rhizocephalan *S. carcini* (based on visible externa) were generally very low (< 3 %) and only occurred at four of the ten locations (Figure S7).

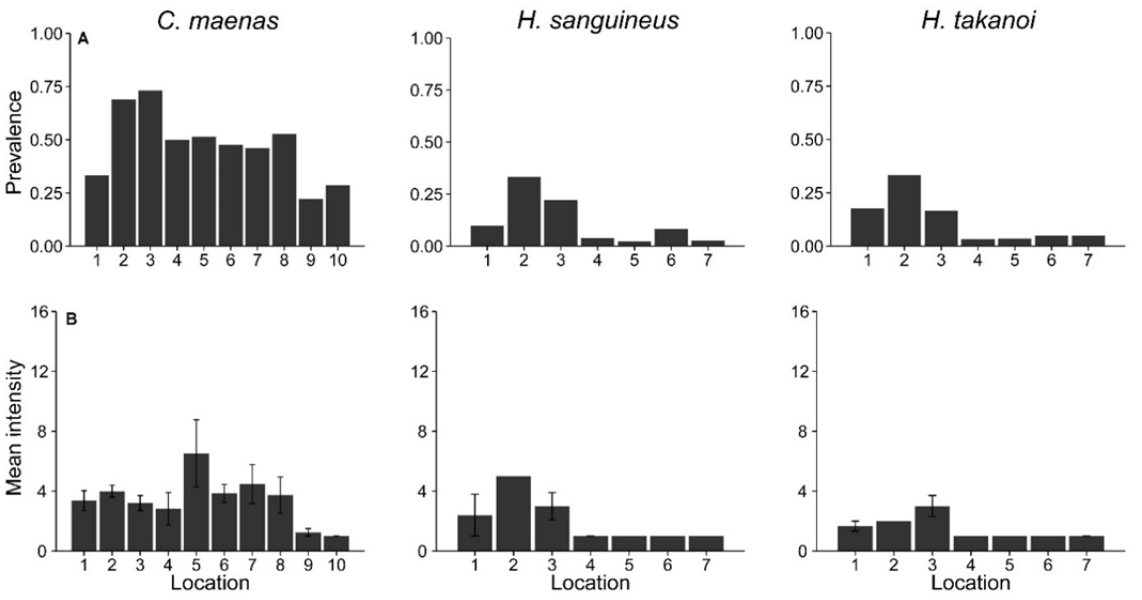

Figure 3. A) prevalence and B) mean intensity (± SE) of acanthocephalan infections in the three crab host species at the sampling locations. For sample size per location see Table 1. Note that both *Hemigrapsus* species were only present at locations 1–7.

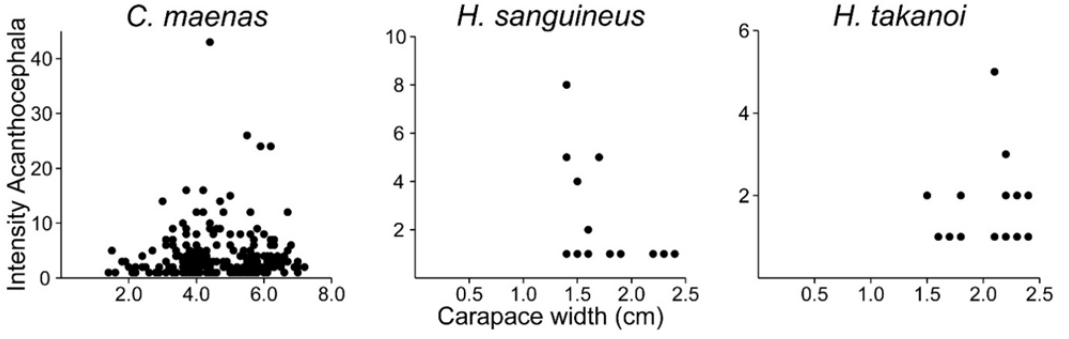

Figure 4. Intensity of acanthocephalans in infected individuals of the three host crab species (*Carcinus maenas*; n = 239, *Hemigrapsus sanguineus*; n = 15, *Hemigrapsus takanoi*; n = 16) depending on host size (carapace width). Note the different axes scales.

Discussion

In this study, we compared parasite richness, prevalence, and intensities in sympatric populations of a native and two introduced brachyuran crab host species in the centre of their European range. While the native green crab *Carcinus maenas* hosted three parasite groups (acanthocephalans, microphallid trematodes, rhizocephalans), the two invaders (*Hemigrapsus sanguineus* and *H. takanoi*) were only infected with one group (acanthocephalans). All acanthocephalans were identified as *Profilicollis botulus*.

In this first study on parasite richness in the two invasive crab species in Europe, we found fewer parasite species compared to findings from invasive *H. sanguineus* populations in North America, where the crabs are infected with three parasite species (an unidentified nematode, a microphallid trematode, and an acanthocephalan – most likely *P. botulus*; Torchin et al. 2001; Blakeslee et al. 2009; Kroft and Blakeslee 2016). In contrast to invasive populations in America and Europe, *H. sanguineus* is infected by at least nine microsporidian, rhizocephalan, or trematode parasite species in its native range (Blakeslee et al. 2009; McDermott 2011). Hence, *H. sanguineus* seems to have a reduced set of parasites in its introduced range in Europe (parasite reduction), similar to observations in North America and corresponding

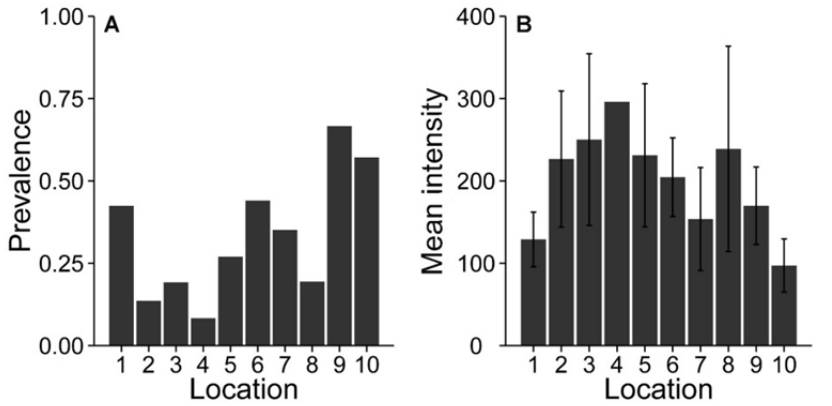

Figure 5. A) prevalence -and B) mean intensity (± SE) of trematode infections in *Carcinus maenas* (n = 135) at the 10 sampling locations. For sample size per location see Table 1.

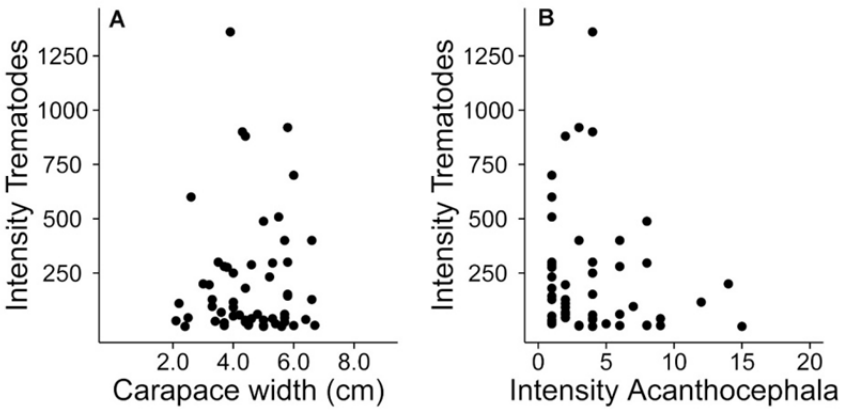

Figure 6. A) intensity of trematodes in infected *Carcinus maenas* crabs depending on host size (n = 135) and B) intensity of acanthocephalan infections (n = 57).

with findings in introduced populations of other crab species like *C. maenas* (Torchin et al. 2001). The other introduced crab species, *H. takanoi*, was also only infected with acanthocephalans. As *H. takanoi* was only recently identified as a sibling species of *Hemigrapsus penicillatus* (De Haan, 1835) (Asakura and Watanabe 2005), no literature records on parasite infections from native populations exist. However, for the sibling species *H. penicillatus*, at least eight parasite species have been reported from its native range, with many of the species also infecting *H. sanguineus* (McDermott 2011). This suggests that parasite escape is also likely for the invasive populations of *H. takanoi* in Europe. However, data on infection levels within the native range of this species will be needed for a final assessment of the existence of parasite release.

While the two introduced crab species, *H. takanoi* and *H. sanguineus*, have escaped their native parasites, they have recently acquired an acanthocephalan parasite species in their introduced European range. Our molecular analyses indicated that all acanthocephalans belonged to the same species (*P. botulus*), which has never been recorded in the native range of *Hemigrapsus* spp. (McDermott 2011) and, therefore, was unlikely to be co-introduced by the invasive crabs. However, whether *P. botulus* is native in Europe, a recent invader from North America, or native to both regions is difficult to ascertain.

The acanthocephalan *P. botulus* has been recorded extensively in the northeast Atlantic, but has also been found in the northwest Atlantic (van Cleave 1916) and the northeast Pacific (Ching 1989). Our own sequences show that all sequences known to date, which originate from herring gulls (Wadden Sea area), three crab species (Wadden Sea area), and two waterfowl species (mallard *Anas platyrhynchos* Linnaeus, 1758 from Pacific North America and common eider *Somateria mollissima* Linnaeus, 1758 from Denmark), all group together in one haplotype network (Figure S1). Phylogenetic analyses with an outgroup also demonstrate that all our sequences belong to the same cluster and that there is no support for separate clades within the *P. botulus*

sequences (Figure S2). We can therefore be confident that all acanthocephalans encountered belong to the same species. However, we cannot be certain that the *P. botulus* we identified from the Wadden Sea is native to the area. Alternatively, *P. botulus* is native to North America and may have recently been introduced from there to the northeast Atlantic and our study area. In addition, it is also possible that the species has a wide natural distribution without population differentiation, perhaps as a result of natural dispersal vectors such as migratory birds or widely distributed species such as herring gull. A more detailed phylogeographic study is needed to distinguish among these possibilities. The level of variability we observed is rather high (Figure S1), which may be interpreted in favour of the parasite being native to the study area. Hence, we tentatively assume that *P. botulus* is most likely native in the study region.

Introduced and native crabs differed in infection levels of the acanthocephalans, with generally lower prevalences and intensities in the introduced than in the native crabs. It is unlikely that this is only due to the size difference among the crab species, as crab size was not a significant predictor of infection intensity, suggesting that other factors are more important in determining the differences in infection levels between native and invasive crabs. Given that prevalence in both *Hemigrapsus* species was strongly correlated and that this was not the case between *Hemigrapsus* species and *C. maenas*, the underlying mechanisms may be the same for the two *Hemigrapsus* species. Besides size, host age may explain differences in infection intensity between invasive and native hosts, as host age is usually correlated with the actual parasite exposure over time, suggesting higher intensities in older crabs. Based on published maximum carapace width (75 mm CW; Klein Breteler 1976; Wolf 1998; Waser et al. 2016) and ages (Dries and Adelung 1982; Lützen 1984) of native *C. maenas* in Europe, the 20–70 mm CW of sampled shore crabs corresponded with ages between 2–4 years. Similarly, based upon studies in the European introduced range, the 15 to 25 mm CW of both invasive *Hemigrapsus* crabs corresponded with an age of 2–3 years (Dauvin 2009; Gothland et al. 2014). Hence, the native green crab *C. maenas* sampled was probably slightly older and had the potential to acquire more parasite infections over time. Therefore, age may be one contributor to the differences in infection intensity between native and invasive crabs. Similarly, it may be that the actual exposure (*sensu* Combes 2001) to infective stages shed by bird definitive hosts into the environment differs between the native *C. maenas* and the two invasive *Hemigrapsus* species. While *C. maenas* occupies both subtidal and intertidal zones and regularly migrates between the two zones (e.g., Silva et al. 2014), *Hemigrapsus* spp. are often found in between boulders and rocks higher in the intertidal zone (Lohrer et al. 2000 and references therein; Dauvin 2009), which may result in a different likelihood of parasite encounters for invasive crabs. However, in our study, crabs were collected at locations where all species occurred in close sympatry (i.e., on oyster beds and dykes); hence differences in tidal exposure cannot explain differences in infection levels. Nevertheless, the microhabitat use of invasive and native crabs within locations may differ. Due to their small size, both invasive crab species can be expected to hide deeper in mussel and oyster beds or within the boulders and pebbles at the bottom of dykes, potentially reducing exposure to infective stages of acanthocephalans. Physical structures and ambient organisms have been shown in other studies to reduce parasite transmission as a result of interference, predation, or other means (Thieltges et al. 2008b; Johnson and Thieltges 2010), and deserve further experimental study in our system. Alternatively, the lower acanthocephalan prevalences and intensities in invasive compared to native crabs, may result from the fact that both *Hemigrapsus* species do not share an evolutionary history with *P. botulus,* which our evidence suggests is native in the study area. Consequently, the parasite may show a preference for the native crab species, resulting in higher parasite prevalences, intensities, and abundances compared to invasive crabs as observed in other species (Georgiev et al. 2007; Dang et al. 2009; Roche et al. 2010; Gendron et al. 2012). However, given the passive transmission process from *P. botulus* eggs to crabs, this does not seem very likely. The eggs of the acanthocephalan are released via bird faeces into the water column and infection occurs via accidental ingestion of eggs by crabs (Thompson 1985), making the potential for parasite preferences in determining infection levels in crab species rather small. The potential mechanisms discussed above are not mutually exclusive and further experiments and analyses are needed to disentangle the underlying mechanisms of differential infection levels in native and invasive crabs.

Surprisingly, metacercariae of microphallid trematodes were only found in the native *C. maenas* but not in the two invasive crab species, while invasive populations of *H. sanguineus* and *C. maenas* in North America each harbour a microphallid trematode species: *Gynaecotyla adunca* (Linton, 1905) in *H. sanguineus*; and *Microphallus similis* (Jägerskiöld, 1900) Nichol, 1906 in *C. maenas* (Blakeslee et al.

2009; Kroft and Blakeslee 2016). Also in its native range, *H. sanguineus* is commonly infected with several species of trematodes (Blakeslee et al. 2009; McDermott 2011). This suggests that invasive populations of *Hemigrapsus* in Europe may not serve as suitable hosts for local trematode parasites, although the crabs are, in principle, suitable hosts for trematodes as indicated by infections in their native range and in North America. This absence of trematode infections in *Hemigrapsus* species may again relate to differences in parasite exposure and/or host susceptibility and further experiments will be needed to clarify this. In contrast to *Hemigrapsus*, individuals of *C. maenas* were, on average, infected with 100–300 metacercariae per host at the various locations. Such high infection levels have been previously reported from the wider study region (Thieltges et al. 2008a; Zetlmeisl et al. 2011) and from other native populations in Europe (Zetlmeisl et al. 2011).

In addition to trematodes, visible externa of the rhizocephalan barnacle *Sacculina carcini* were also only observed in *C. maenas,* at a few locations with low prevalence (< 3%), and never in either of the two *Hemigrapsus* species. Such low prevalences in this range have previously been reported from the wider study region (Zetlmeisl et al. 2011; Waser et al. 2016). In its native range, *H. sanguineus* is infected by three species of rhizocephalans (reviewed by McDermott 2011), indicating a parasite escape of this group of parasites in European populations of the species. Such a complete loss of rhizocephalan parasites in the course of introductions seems to be a general pattern in marine invasions (Blakeslee et al. 2013).

The observed reduced set of parasites infecting the two invasive *Hemigrapsus* species in the centre of their European range suggests the potential for a competitive advantage of the invasive crabs over the native *C. maenas*. Theoretically, invasive species that escaped their parasites might invest physical resources on host fitness parameters (e.g., reproduction and growth) that might otherwise be spent on immune responses to parasites, enhancing the competitiveness of invasive species (Calvo-Ugarteburu and McQuaid 1998a, b; Bachelet et al. 2004; Byers 2000). However, it is unclear whether the impact of the various native parasite species on the native *C. maenas* is strong enough to mediate competition with the invasive *Hemigrapsus* spp. Castrating parasites like *S. carcini* can substantially reduce the testes weight of green crabs (Zetlmeisl et al. 2011), and the loss of these parasites has been associated with faster growth, greater longevity, and/or greater biomass of invasive green crab populations (Torchin et al. 2001). Nevertheless, the low prevalences with *S. carcini* in our and wider study regions (North Sea and Wadden Sea; Zetlmeisl et al. 2011; Waser et al. 2016) suggest that very few individuals are affected by rhizocephalan castration, which is unlikely to translate into sweeping population level effects. Furthermore, a study on the effects of trematode and acanthocephalan infections on the reproduction index of native *C. maenas* could not find any negative effect of the parasites on crab testes weight (Zetlmeisl et al. 2011). In addition, in introduced populations of *C. maenas*, Blakeslee et al. (2015) did not find strong effects of trematode infections on the physiology or behaviour of infected crabs; however, crabs may respond differently in the native range and this remains to be studied. The effects of *P. botulus* infections on the two invasive *Hemigrapsus* species have not been tested, and experiments are needed to investigate whether the observed lower parasite load of invasive crabs compared to the native green crab actually leads to a competitive advantage. Although evidence for effects of the acanthocephalan on native and invasive crabs is lacking, the addition of both invasive *Hemigrapsus* crab species to the host range of *P. botulus* in Europe might have pronounced effects on native birds, the definitive host of the parasite. An increase in the number of competent intermediate host species potentially leads to an amplification of the population size of *P. botulus* in crabs, ultimately resulting in an increase in acanthocephalan infections in bird species that have brachyuran crabs in their diet (parasite spillback). Birds are known to suffer from *P. botulus* infections (e.g. mass mortalities reported for eider ducks (*S. mollissima*) in Europe and the US, reviewed in Garden et al. 1964) and therefore the inclusion of *Hemigrapsus* spp. in *P. botulus*' host range has the potential to impact higher trophic levels via these parasite spillback effects.

In conclusion, this first study on parasite infection levels in invasive *Hemigrapsus sanguineus* and *H. takanoi* in Europe indicates parasite reduction/escape and lower infection prevalences and intensities in the two invasive crabs compared to their native competitor, the green crab. Although this suggests a potential competitive advantage for invasive crabs, there is limited evidence to date that the fitness of native *C. maenas* is compromised by native parasites. Hence, whether a competitive advantage due to parasite mediated competition for invasive crabs actually exists in these invader-native pairings is questionable and deserves further experimental study. Such community studies or cross-species comparisons are a valuable approach in understanding the actual relevance of enemy release for local communities of native and invasive competitors.

Acknowledgements

We are grateful to the Netherlands Organization for Scientific Research (NWO) and the German Bundesministerium für Bildung und Forsuchung (BMBF) for funding (NWO-ZKO project 839.11.002). AMW acknowledges support through the project 'Mosselwad' which is funded by the Waddenfonds (WF 203919), the Dutch Ministry of Infrastructure and the Environment and the provinces of Fryslân and Noord Holland. We furthermore thank Ewout Adriaans (RV 'Stern') and Rob Dekker for transportation and assistance on intertidal sampling locations, Anneke Bol for assistance with molecular analysis and Hans Witte for providing subtidal samples. Furthermore, we thank the three anonymous reviewers and A.M.H. Blakeslee for constructive feedback on an earlier version of our manuscript.

References

Asakura A, Watanabe S (2005) *Hemigrapsus takanoi*, new species, a sibling species of the common Japanese intertidal crab *H. penicillatus* (Decapoda: Brachyura: Grapsoidea). *Journal of Crustacean Biology* 25: 279–292, https://doi.org/10.1651/C-2514

Bachelet G, Simon-Bouhet B, Desclaux C, Garcia-Meunier P, Mairesse G, de Mountaudouin, X, Raigné H, Randriambao K, Sauriau PG, Viard F (2004) Invasion of the eastern Bay of Biscay by the nassariid gastropod *Cyclope neritea*: origin and effects on resident fauna. *Marine Ecology Progress Series* 276: 147–159, https://doi.org/10.3354/meps276147

Blakeslee AMH, Keogh CL, Byers JE, Kuris AM, Lafferty KD, Torchin ME (2009) Differential escape from parasites by two competing introduced crabs. *Marine Ecology Progress Series* 393: 83–96, https://doi.org/10.3354/meps08225

Blakeslee AMH, Fowler AE, Keogh CL (2013) Marine invasion and parasite escape: updates and new perspectives. *Advances in Marine Biology* 66: 87–169, https://doi.org/10.1016/B978-0-12-408096-6.00002-X

Blakeslee AMH, Keogh CL, Fowler AE, Griffen BD (2015) Assessing the effects of trematode infection on invasive green crabs in Eastern North America. *PLoS ONE* 10: e0128674, https://doi.org/10.1371/journal.pone.0128674

Brousseau DJ, Goldberg R, Garza C (2014) Impact of predation by the invasive crab *Hemigrapsus sanguineus* on survival of juvenile blue mussels in Western Long Island Sound. *Northeastern Naturalist* 21: 119–133, https://doi.org/10.1656/045.021.0110

Byers JE (2000) Competition between two estuarine snails: implycations for invasions of exotic species. *Ecology* 81: 1225–1239, https://doi.org/10.1890/0012-9658(2000)081[1225:CBTESI]2.0.CO;2

Calvo-Ugarteburu G, McQuaid CD (1998a) Parasitism and introduced species: epidemiology of trematodes in the intertidal mussels *Perna perna* and *Mytilus galloprovincialis*. *Journal of Experimental Marine Biology and Ecology* 220: 47–65, https://doi.org/10.1016/S0022-0981(97)00083-X

Calvo-Ugarteburu G, McQuaid CD (1998b) Parasitism and invasive species: effects of digenetic trematodes on mussels. *Marine Ecology Progress Series* 169: 149–163, https://doi.org/10.3354/meps169149

Camphuysen CJ (2013) A historical ecology of two closely related gull species (Laridae): multiple adaptations to a man-made environment. PhD thesis, University of Groningen, Groningen, The Netherlands, pp 421

Campos J, Bio A, Cardoso JFMF, Dapper R, Witte JIJ, Veer HW van der (2010) Fluctuations of brown shrimp *Crangon crangon* abundance in the western Dutch Wadden Sea. *Marine Ecology Progress Series* 405: 203–219, https://doi.org/10.3354/meps08493

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*. *Journal of Biogeography* 30: 1809–1820, https://doi.org/10.1111/j.1365-2699.2003.00962.x

Ching HL (1989) *Profilicollis botulus* (Van Cleave, 1916) from diving ducks and shore crabs of British Columbia. *Journal of Parasitology* 75: 33–37, https://doi.org/10.2307/3282930

Cleave HJ van (1916) *Filicollis botulus* n. sp. with notes on the characteristics of the genus. *Transactions of the American Microscopical Society* 35: 131–134, https://doi.org/10.2307/3221563

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters* 7: 721–733, https://doi.org/10.1111/j.1461-0248.2004.00616.x

Combes CC (2001) Parasitism. The Ecology and evolution of intimate interactions. The University of Chicago Press, Chicago, USA, 728 pp

Dang C, de Montaudouin X, Bald J, Jude F, Raymond N, Lanceleur L, Paul-Pont I, Caill-Milly N (2009) Testing the Enemy Release Hypothesis: Trematode parasites in the non-indigenous Manila clam *Ruditapes philippinarum*. *Hydrobiologia* 630: 139–148, https://doi.org/10.1007/s10750-009-9786-9

Dauvin JC (2009) Asian Shore Crabs *Hemigrapsus* spp. (Crustacea: Brachyura: Grapsoidea) continue their invasion around the Cotentin Peninsula, Normandy, France: Status of the *Hemigrapsus* population in 2009. *Aquatic Invasions* 4: 605–611, https://doi.org/10.3391/ai.2009.4.4.6

Dauvin JC, Tous Rius A, Ruellet T (2009) Recent expansion of two invasive crab species *Hemigrapsus sanguineus* (de Haan, 1835) and *H. takanoi* Asakura and Watanabe 2005 along the Opal Coast, France. *Aquatic Invasions* 4: 451–465, https://doi.org/10.3391/ai.2009.4.3.3

Dries M, Adelung D (1982) The Schlei as a model for the distribution of the green shore crab, *Carcinus maenas*. *Helgolander Meeresuntersuchung* 35: 65–77, https://doi.org/10.1007/BF02289835

Dunn AM, Torchin ME, Hatcher MJ, Kotanen PM, Blumenthale DM, Byers JE, Coon CAC, Frankel VM, Holt RD, Hufbauer RA, Kanarak AR, Schierenbeck KA, Wolfe LM, Perkins SE (2012) Indirect effects of parasites in invasions. *Functional Ecology* 26: 1262–1274, https://doi.org/10.1111/j.1365-2435.2012.02041.x

Elton CS (1958) The Ecology of Invasions by Animals and Plants. Methuen, London, UK, 181 pp, https://doi.org/10.1007/978-1-4899-7214-9

Epifanio CE (2013) Invasion biology of the Asian shore crab *Hemigrapsus sanguineus*: A review. *Journal of Experimental Marine Biology and Ecology* 441: 33–49, https://doi.org/10.1016/j.jembe.2013.01.010

García-Varela M, Perez-Ponce de Leon G (2008) Validating the systematic position of *Profilicollis* Meyer, 1931 and *Hexaglandula* Petrochenko, 1950 (Acanthocephala: Polymorphidae) using cytochrome C oxidase (cox 1). *Journal of Parasitology* 94: 212–217, https://doi.org/10.1645/GE-1257.1

Garden EA, Rayski C, Thom VM (1964) A parasitic disease in Eider Ducks. *Bird Study* 11: 280–287, https://doi.org/10.1080/00063656409476078

Gendron AD, Marcogliese DJ, Thomas M (2012) Invasive species are less parasitized than native competitors, but for how long? The case of the round goby in the Great Lakes –St. Lawrence Basin. *Biological Invasions* 14: 367–384, https://doi.org/10.1007/s10530-011-0083-y

Georgiev BB, Sanchez MI, Vasileva GP, Nikolov PN, Green AJ (2007) Cestode parasitism in invasive and native brine shrimps (*Artemia* spp.) as a possible factor promoting the rapid invasion of *A. franciscana* in the Mediterranean region. *Parasitology Research* 101: 1647–1655, https://doi.org/10.1007/s00436-007-0708-3

Gothland M, Dauvin JC, Denis L, Dufossé F, Jobert S, Ovaert J, Pezy JP, Tous Rius A, Spilmont N (2014) Biological traits explain the distribution and colonization ability of the invasive shore crab *Hemigrapsus takanoi*. *Estuarine Coastal Shelf Science* 142: 41–49, https://doi.org/10.1016/j.ecss.2014.03.012

Grosholz ED, Ruiz GM (2003) Biological invasions drive size increases in marine and estuarine invertebrates. *Ecology Letters* 6: 700–705, https://doi.org/10.1046/j.1461-0248.2003.00495.x

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* 41: 95–98

Hatcher MJ, Dick JTA, Dunn AM (2006) How parasites affect interactions between competitors and predators. *Ecology Letters* 9: 1253–1271, https://doi.org/10.1111/j.1461-0248.2006.00964.x

Johnson PTJ, Thieltges DW (2010) Diversity, decoys and the dilution effect: how ecological communities affect diseases risk. *Journal of Experimental Biology* 213: 961–970, https://doi.org/10.1242/jeb.037721

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution* 17: 164–170, https://doi.org/10.1016/S0169-5347(02)02499-0

Kelly DW, Paterson RA, Townsend CR, Poulin R, Tompkins DM (2009) Parasite spillback: A neglected concept in invasion ecology? *Ecology* 90: 2047–2056, https://doi.org/10.1890/08-1085.1

Klein Breteler WCM (1976) Migration of the shore crab, *Carcinus maenas*, in the Dutch Wadden Sea. *Journal of Sea Research* 10: 338–353, https://doi.org/10.1016/0077-7579(76)90010-7

Kroft KL, Blakeslee AMH (2016) Comparison of parasite diversity in native panopeid mud crabs and the invasive Asian shore crab in estuaries of northeast North America. *Aquatic Invasions* 11: 287–301, https://doi.org/10.3391/ai.2016.11.3.07

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874, https://doi.org/10.1093/molbev/msw054

Landschoff J, Lackschewitz D, Kesy K, Reise K (2013) Globalization pressure and habitat change: Pacific rocky shore crabs invade armored shorelines in the Atlantic Wadden Sea. *Aquatic Invasions* 8: 77–87, https://doi.org/10.3391/ai.2013.8.1.09

Lohrer AM, Fukui Y, Wada K, Whitlatch RB (2000) Structural complexity and vertical zonation of intertidal crabs, with focus on habitat requirements of the invasive Asian shore crab, *Hemigrapsus sanguineus* (de Haan). *Journal of Experimental Marine Biology and Ecology* 244: 203–217, https://doi.org/10.1016/S0022-0981(99)00139-2

Lohrer AN, Whitlatch RB (2002) Relative impacts of two exotic brachyuran species on blue mussel populations in Long Island Sound. *Marine Ecology Progress Series* 227: 135–144, https://doi.org/10.3354/meps227135

Lützen J (1984) Growth, reproduction, and life span in *Sacculina carcini* Thompson (Cirripedia: Rhizocephala) in the Isefjord, Denmark. *Sarsia* 69: 91–105, https://doi.org/10.1080/00364827.1984.10420595

Markert A, Raupach MJ, Segelken-Voigt A, Wehrmann A (2014) Molecular identification and morphological characteristics of native and invasive Asian brush-clawed crabs (Crustacea: Brachyura) from Japanese and German coasts: *Hemigrapsus penicillatus* (De Haan, 1835) versus *Hemigrapsus takanoi* Asakura & Watanabe 2005. *Organisms Diversity and Evolution* 14: 369–382, https://doi.org/10.1007/s13127-014-0176-4

McDermott JJ (2011) Parasites of shore crabs in the genus *Hemigrapsus* (Decapoda: Brachyura: Varunidae) and their status in crabs geographically displaced: a review. *Journal of Natural History* 45: 2419–2441, https://doi.org/10.1080/00222933.2011.596636

Mitchell CE, Power AG (2003) Release of invasive plants from fungal and viral pathogens. *Nature* 421: 625–627, https://doi.org/10.1038/nature01317

NORSAS (2012) North Sea Alien Species Database (NORSAS). North Sea Alien Species Database (online). http://www.norsas.eu/observation/hemigrapsus-takanoi-h-penicillatus/1580/chetan/2012-07-17-1800

Paradis E (2010) Pegas: An R package for population genetics with an integrated-modular approach. *Bioinformatics* 26: 419–420, https://doi.org/10.1093/bioinformatics/btp696

Parker JD, Torchin ME, Hufbauer RA et al. (2013) Do invasive species perform better in their new ranges? *Ecology* 94: 985–994, https://doi.org/10.1890/12-1810.1

R Development Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org

Roche DG, Leung B, Mendoza Franco EF, Torchin ME (2010) Higher parasite richness, abundance, and impact in native versus introduced cichlid fishes. *International Journal of Parasitology* 40: 1525–1530, https://doi.org/10.1016/j.ijpara.2010.05.007

Silva ACF, Boaventura DM, Thompson RC, Hawkins SJ (2014) Spatial and temporal patterns of subtidal and intertidal crab excursions. *Journal of Sea Research* 85: 343–348, https://doi.org/10.1016/j.seares.2013.06.006

Takano M, Ikeda M, Kijima A (1997) Biochemical and morphological evidence of two sympatric forms, interpreted as sibling species, in the estuarine grapsid crab *Hemigrapsus penicillatus* (de Haan). *Benthos Research* 52: 111–117, https://doi.org/10.5179/benthos1996.52.2_111

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics: analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729, https://doi.org/10.1093/molbev/mst197

Thieltges DW, Hussel B, Herrmann J, Jensen KT, Krakau M, Taraschewski H, Reise K (2008a) Parasites in the northern Wadden Sea: a conservative ecosystem component over 4 decades. *Helgoland Marine Research* 62: 37–47, https://doi.org/10.1007/s10152-007-0091-6

Thieltges DW, Jensen KT, Poulin R (2008b) The role of biotic factors in the transmission of free-living endohelminth stages. *Parasitology* 135: 407–426, https://doi.org/10.1017/S0031182007000248

Thompson AB (1985) Transmission Dynamics of *Profilicollis botulus* (Acanthocephala) From Crabs (*Carcinus maenas*) to Eider Ducks (*Somateria mollissima*) on the Ythan Estuary, N.E. Scotland. *Journal of Animal Ecology* 54: 605–616, https://doi.org/10.2307/4502

Torchin ME, Lafferty KD, Kuris AM (2001) Release from parasites as natural enemies: increased performance of a globally introduced marine crab. *Biological Invasions* 3: 333–345, https://doi.org/10.1023/A:1015855019360

Torchin ME, Lafferty KD, Dobson AP, McKenzie VJ, Kuris AM (2003) Introduced species and their missing parasites. *Nature* 421: 628–630, https://doi.org/10.1038/nature01346

Torchin ME, Mitchell CE (2004) Parasites, pathogens, and invasions by plants and animals. *Frontiers in Ecology and the Environment* 2: 183–190, https://doi.org/10.1890/1540-9295(2004)002[0183:PPAIBP]2.0.CO;2

Torchin ME, Lafferty KD (2009) Escape from parasites. In: Rilov G, Crooks JA (eds), Biological invasions in marine ecosystems. Springer-Verlag Berlin Heidelberg, Germany, pp 203–214, https://doi.org/10.1007/978-3-540-79236-9_11

Tyrell MC, Guarino PA, Harris LG (2006) Predatory impacts of two introduced crab species: Interferences from microcosms. *Northeastern Naturalist* 13: 375–390, https://doi.org/10.1656/1092-6194(2006)13[375:PIOTIC]2.0.CO;2

Veer HW van der, Dapper R, Henderson PA, Jung AS, Philippart CJM, Witte JI, Zuur AF (2015) Changes over 50 years in fish fauna of a temperate coastal sea: Degradation of trophic structure and nursery function. *Estuarine Coastal Shelf Science* 155: 156–166, https://doi.org/10.1016/j.ecss.2014.12.041

Waser AM, Goedknegt MA, Dekker R, McSweeney N, Witte JIJ, van der Meer J, Thieltges DW (2016) Tidal elevation and parasitism: patterns of infection by the rhizocephalan parasite *Sacculina carcini* in shore crabs *Carcinus maenas*. *Marine Ecology Progress Series* 545: 215–225, https://doi.org/10.3354/meps11594

Williams AB, McDermott JJ (1990) An eastern United States record for the western Indo-Pacific crab, *Hemigrapsus sanguineus* (Crustacea: Decapoda: Grapsidae). *Proceedings of the Biological Society of Washington* 103: 108–109

Wolf F (1998) Red and green colour forms in the common shore crab *Carcinus maenas* (L.) (Crustacea: Brachyura: Portunidae): theoretical predictions and empirical data. *Journal of National History* 32: 1807–1812, https://doi.org/10.1080/00222939800771311

Zetlmeisl C, Hermann J, Petney T, Glenner H, Griffiths C, Taraschewski H (2011) Parasites of the shore crab *Carcinus maenas* (L): implications for reproductive potential and invasion success. *Parasitology* 138: 394–401, https://doi.org/10.1017/S0031182010001344

Supplementary material

The following supplementary material is available for this article:

Table S1. Sources of acanthocephalans for the molecular (COI) identification.
This material is available as part of online article from:
http://www.aquaticinvasions.net/2017/Supplements/AI_2017_Goedknegt_etal_TableS1.xls

Figure S1. Minimum spanning network among partial COI haplotypes of *Profilicollis botulus*.
Figure S2. Rooted phylogenetic trees of *Profilicollis botulus* based on partial COI sequences.
Figure S3. Frequency distribution of crab host sizes of the three crab species.
Figure S4. Correlations of prevalences of acanthocephalan infections at 7 sampling locations.
Figure S5. Correlations of mean intensities of acanthocephalan infections at 7 sampling locations.
Figure S6. Frequency distribution of *Carcinus maenas* carapace sizes separated into males and females.
Figure S7. Prevalence of the rhizocephalan *Sacculina carcini* in *Carcinus maenas* crab hosts at the 10 sampling locations.
This material is available as part of online article from:
http://www.aquaticinvasions.net/2017/Supplements/AI_2017_Goedknegt_etal_Supplementary_Figures.pdf

Appendix 1. Dissection protocol of herring gull *Larus argentatus* chicks for *Acanthocephala* parasites.
This material is available as part of online article from:
http://www.aquaticinvasions.net/2017/Supplements/AI_2017_Goedknegt_etal_Appendix1.pdf