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Copepods associated with the red king crab *Paralithodes camtschaticus* (Tilesius, 1815) in the Barents Sea

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Abstract

Background: There are no data on copepod occurrence, infestation indices, and localization on red king crabs in the Russian part of the Barents Sea. For this reason, we examined the species composition, infestation indices, and localization patterns of copepods colonizing the red king crab *Paralithodes camtschaticus* in Dalnezelenetskaya Bay, a small gulf in the Barents Sea.

Results: In total, nine species of Copepoda were found on hosts in July of 2009 ~ 2011. Typical planktonic copepods (*Calanus*, *Microsetella*, and *Microcalanus*) and some benthopelagic and benthic species (*Ectinosoma*, *Cyclopina*, and *Zaus*) were found in low occurrences; their prevalences did not exceed 5%, and these copepods should be considered incidental visitors. The benthopelagic harpacticoid copepods *Tisbe*, *Harpacticus*, and *Dactylopusia* were the most abundant, with high prevalences and mean intensities of infestation, and should be classified as commensals. Infestation indices were correlated with the host size and shell condition. The majority of copepods were found on host gills (90% ~ 100%). We suggest that the recorded absence of symbiotic copepods from crab branchial chambers in our previous studies in August of 2004 ~ 2008 may be explained by predation or competition with the symbiotic amphipod *Ischyrocerus commensalis*, which was confirmed by analysis of amphipod gut contents.

Conclusions: This report expands our knowledge on the colonization of different fouling and symbiotic species on red king crabs. Our study has management and aquaculture implications as it sheds light on possible interactions between copepods and their crab hosts. Colonization of crabs in general may be beneficial for the copepod species because it enhances food acquisition, increases their mobility, and affords them a degree of protection from predators. Conversely, this association may have negative effects on the crab host due to a decrease in respiratory function.

Keywords: *Paralithodes camtschaticus*; Associated copepods; Barents Sea; Infestation indices; Localization patterns

Background

The red king crab *Paralithodes camtschaticus* (Tilesius, 1815), originally native to sea of the Far East, was introduced into the Barents Sea in the 1960s and has formed a self-sustaining population. It is intensively studied due to its commercial importance (Kuzmin and Gudimova 2002); however, little is known about the epibiotic and symbiotic species that colonize *P. camtschaticus* in the Barents Sea (Bakay et al. 1998; Jansen et al. 1998). Copepods were found on red king crabs in Varanger-fjord in Norway (Haugen et al. 1998; Jansen et al. 1998). In previous studies, we described associates of the red king crab in some eastern coastal areas of the Barents Sea during late summer (August to September), but we recorded no copepod species on red king crabs (Dvoretsky and Dvoretsky 2009a, 2010a). Thus, there are no data on copepod occurrences, infestation indices, and localization on red king crabs in the Russian part of the Barents Sea. To expand our knowledge on the colonization of different fouling and symbiotic species on red king crabs, we have continued to study epifaunal species associated with anomuran and brachyuran crabs in the Barents Sea (Dvoretsky and Dvoretsky 2008, 2009a, 2010a). Such studies have management implications as they shed light on possible parasitic interactions between copepods and their crab hosts (Ho et al. 2011). The specific objectives of this

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study were to (1) survey crab-associated copepod species in Dalnezelenetskaya Bay, (2) describe their infestation indices, (3) determine distribution patterns of commonly associated copepods on *P. camtschaticus*, and (4) compare the new data with previous studies.

Methods

In total, 272 crabs were studied for associated copepods during three coastal expeditions in Dalnezelenetskaya Bay (Figure 1), a small semi-open gulf on the eastern Murman coast of the Barents Sea, in July of 2009 ~ 2011. Crabs were collected by scuba divers at depths of 5 ~ 36 m. Each

specimen was sexed, and the carapace length (CL) was measured. CL is the straight-line distance across the carapace from the posterior margin of the right eye orbit to the medial-posterior margin of the carapace. In addition, the molting stage was determined using a method adapted for lithodid crabs (Donaldson and Byersdorfer 2005). Crabs were divided into small (CL < 90 mm) and large (CL > 90 mm) size classes for analysis. This division roughly corresponds with the onset of sexual maturity in *P. camtschaticus* in the Barents Sea (Sokolov and Milyutin 2006).

Associated organisms were removed from the crabs and preserved in 4% formalin (Dvoretsky and Dvoretsky

Figure 1 Map of the study area.
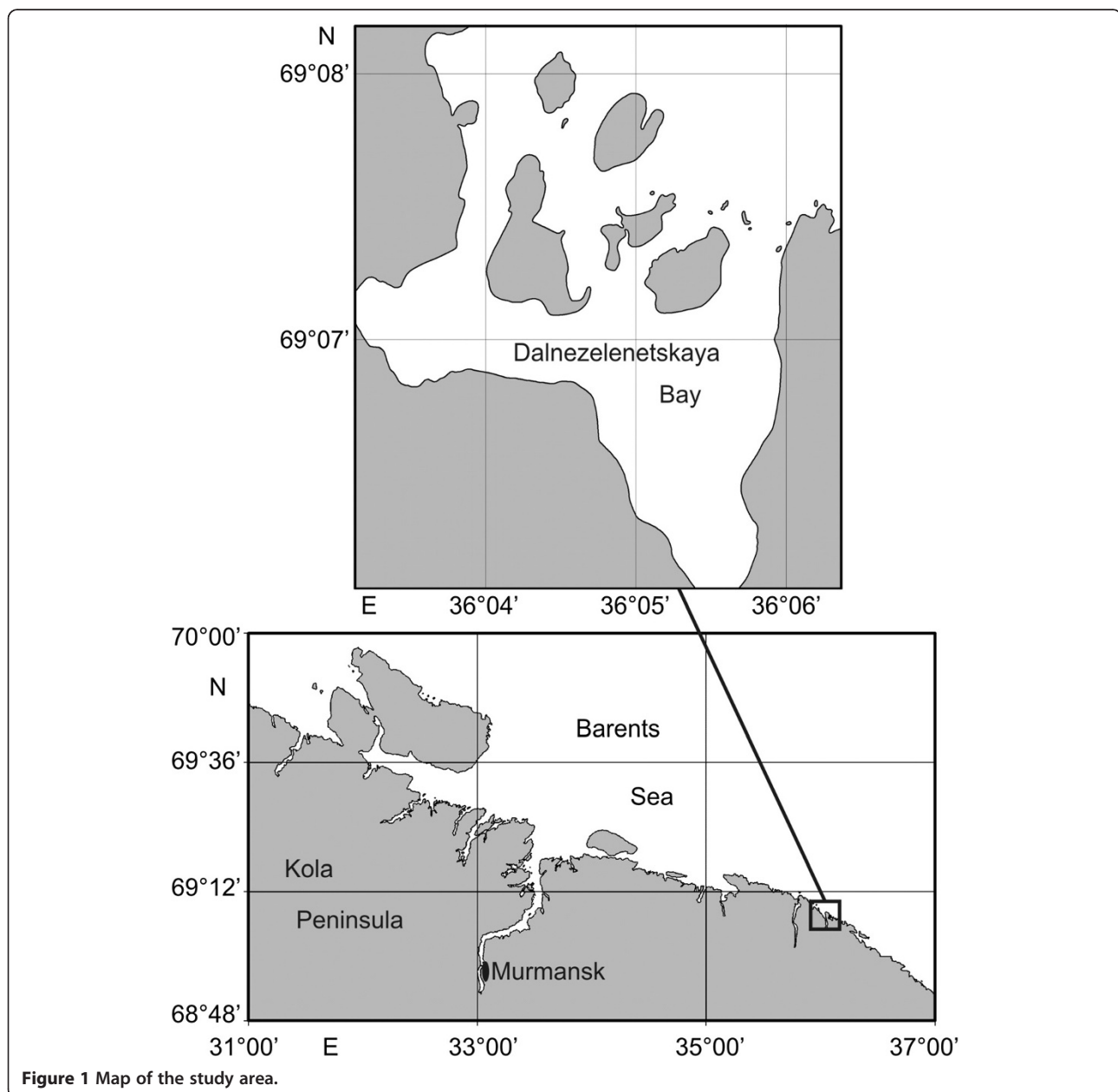
2010a). Their positions on the crab (carapace, limbs, abdomen, mouthparts, gills, and eggs) were recorded. Localization patterns were determined for each species. In the laboratory, associated organisms were counted and identified. Copepods were divided into habitat distribution groups (pelagic, benthopelagic, or benthic) according to our recent checklist of Barents Sea zooplankton (Dvoretsky and Dvoretsky 2010b) and other sources (Lang 1948; Chislenko 1967; Kornev and Chertoprud 2008).

To quantify infestations of crabs, the following standard indices were used: (1) the prevalence which is equivalent to the proportion (%) of infested crabs and (2) the intensity which is the mean number of fouling specimens per infested crab (Bush et al. 1997). Differences in crab size frequency distributions and infestation prevalences between different crab size classes and crabs with different shell conditions were examined with chi-square tests. However, differences in infestation intensities with crab size were examined using a Kruskall-Wallis test because the data failed a normality test (Zar 1984).

The nature of interspecific relationships between red king crab gill commensals (i.e., amphipod-copepod interactions) was assessed by collecting amphipods (*Ischyrocerus commensalis* Chevreux, 1900) from crab branchial chambers and examining their gut contents under a microscope. For this analysis, we used five small and five large amphipods, with respective body lengths of 5.5 ~ 7.4 mm and 8.5 ~ 10.8 mm. All statistical analyses were performed using STATISTICA software, version 6 (http://www.statsoft.com/).

Results

The size frequency distribution of red king crabs caught in Dalnezelenetskaya Bay is presented in Table 1. The proportion of small crabs to large crabs was similar between 2009 (62.9%:37.1%) and 2010 (56.4%:43.6%) ($d.f. = 1$, $\chi^2 = 0.738$, $p = 0.390$). In contrast, the prevalence of large crabs in 2011 (84.4%) was significantly higher than in 2009 or 2010 ($d.f. = 1$, $\chi^2 = 33.106$, $p < 0.001$; and $d.f. = 1$, $\chi^2 = 33.465$, $p < 0.001$, respectively).

Table 1 Size class distributions of red king crabs (number / percent) in Dalnezelenetskaya Bay in July of 2009 to 2011

Carapace length (mm)	Year					
	2009		2010		2011	
	Male	Female	Male	Female	Male	Female
1 to 45	10 / 16.1	6 / 9.7	6 / 4.5	3 / 2.3	2 / 2.6	6 / 7.8
46 to 90	9 / 14.5	14 / 22.6	44 / 33.1	22 / 16.5	2 / 2.6	2 / 2.6
91 to 135	3 / 4.8	11 / 17.7	6 / 5.5	23 / 17.3	3 / 3.9	29 / 37.6
>135	1 / 1.7	8 / 12.9	1 / 0.8	28 / 21.0	1 / 1.3	32 / 41.6

Sex ratios of small crabs (combined data for the 3-year study period) did not significantly vary from an expected level of 1:1 ($d.f. = 1$, $\chi^2 = 3.175$, $p = 0.075$). The sex ratio of large crabs was, however, strongly biased toward females ($d.f. = 1$, $\chi^2 = 92.164$, $p < 0.001$).

In total, nine species of Copepoda belonging to three orders (Calanoida, Cyclopoida, and Harpacticoida) were found on *P. camtschaticus* in Dalnezelenetskaya Bay. Infestation indices for the associated copepods are presented in Table 2. All collected copepods were classified as being either pelagic (calanoid copepods *Calanus finmarchicus* and *Microcalanus pusillus* and the harpacticoid copepod *Microsetella norvegica*), benthopelagic (harpacticoid copepods *Ectinosoma normani*, *Tisbe furcata*, *Harpacticus uniremis*, and *Dactylopusia vulgaris* and the cyclopoid copepod *Cyclopina gracilis*), or benthic (the harpacticoid copepod *Zaus abbreviatus*).

Proportions of crabs colonized by *T. furcata* significantly varied among study years ($d.f. = 2$, $\chi^2 = 47.733$, $p < 0.001$), although levels in 2009 and 2010 were similar ($d.f. = 1$, $\chi^2 = 0.091$, $p = 0.763$). Significant differences in the prevalence of *T. furcata* on large crabs were observed ($d.f. = 2$, $\chi^2 = 12.566$, $p = 0.002$), with respective levels of 82.6%, 71.6%, and 95.4% in 2009, 2010, and 2011. The prevalence of *H. uniremis* also strongly varied among study years ($d.f. = 2$, $\chi^2 = 13.544$, $p = 0.001$), although levels in 2010 and 2011 were similar ($d.f. = 1$, $\chi^2 = 0.010$, $p = 0.920$). Mean intensities of *T. furcata* were similar in 2010 and 2011, but both were significantly lower than in 2009 (Kruskall-Wallis test, $d.f. = 2$, $H = 15.012$, $p < 0.001$). Additionally, the mean intensity of *H. uniremis* was two times higher in 2010 than in 2011 (data for 2009 were not included in the analysis due to a low sample size, $n = 2$).

Infestation indices for the two most common symbiotic copepods (*T. furcata* and *H. uniremis*) were correlated with the host size. For crabs with the same shell conditions (new shell, 2 ~ 12 months post-ecdysis), prevalences of both *T. furcata* and *H. uniremis* were significantly higher in larger size classes (combined data for 2009 ~ 2011, $d.f. = 2$, $\chi^2 = 129.742$, $p < 0.001$; and $d.f. = 2$, $\chi^2 = 27.977$, $p < 0.001$, respectively, Figure 2). Similarly, the mean intensities of both species also increased with crab size (Kruskall-Wallis test, $d.f. = 2$, $H = 16.196$, $p < 0.001$; and $d.f. = 1$, $H = 8.294$, $p = 0.004$, respectively, Figure 2).

The mean intensity of *T. furcata* (±SE) on crabs with new shells (considering large crabs only) was 33.6 ± 3.5 individuals (ind.)/crab, while on crabs with old shells (12 ~ 24 months post-ecdysis), it was as high as 406.0 ± 176.5 ind./crab (Kruskall-Wallis test, $d.f. = 1$, $H = 5.818$, $p = 0.016$). There were no significant differences between the intensities of *H. uniremis* on crabs with new and old shells: 7.0 ± 1.6 vs. 10.5 ± 5.3 ind./crab, respectively (Kruskall-Wallis test, $d.f. = 1$, $H = 5.818$, $p = 0.016$).

Table 2 Species composition, prevalence, and mean intensity of copepods found on the red king crabs studied

Species	Year								
	2009			2010			2011		
	Pr	Int		Pr	Int		Pr	Int	
		Mean	Range		Mean	Range		Mean	Range
C. finmarchicus (Gunner, 1765)	4.8	1.0	1 ~ 1	-	-	-	-	-	-
C. gracilis (Claus, 1863)	-	-	-	-	-	-	1.3	1.0	1-1
D. vulgaris (GO Sars, 1905)	-	-	-	-	-	-	20.8	2.4	1-9
E. normani (T and A Scott, 1894)	3.2	73.0	2-144	0.8	1.0	1-1	3.9	3.0	1-3
H. uniremis (Krøyer, 1842)	3.2	14.5	3-26	24.1	12.3	1-90	24.7	6.0	1-21
M. pusillus (GO Sars, 1903)	-	-	-	-	-	-	1.3	1.0	1-1
M. norvegica (Boeck, 1865)	-	-	-	-	-	-	1.3	1.0	1-1
T. furcata (Baird, 1837)	33.9	216.8	1-1607	36.1	19.5	1-107	81.8	33.8	1-145
Z. abbreviatus (GO Sars, 1904)	-	-	-	-	-	-	2.6	1.5	1-2

Pr, prevalence; Int, intensity.

The majority of commonly associated copepods were found in crab gills, except for *D. vulgaris*, which was prevalent on crab bodies (Table 3). Other commensals were also found in the gills of *P. camtschaticus*; in particular, the highest infestation levels were recorded for the amphipod *I. commensalis*, which is larger in body size than any of the associated copepod species. Harpacticoid copepod remnants were found in the gut contents of larger *I. commensalis* individuals, although no such remnants were found in the gut contents of smaller *I. commensalis*.

Discussion

The size distribution and sex ratio of *P. camtschaticus* in Dalnezelenetskaya Bay were consistent with values found in other red king crab populations in the coastal zone of the Barents Sea (Sokolov and Miliytin 2006). Juvenile crabs and ovigerous females aggregate in shallow waters in summer, while larger males migrate to deep-water parts of the sea after mating in spring (Kuzmin and Gudimova 2002), and most of the results from our study corroborate such population demographics. However, in contrast to data from 2009 to 2010, relatively few small immature crabs were observed at the study site in 2011. This may have been associated with extreme environmental conditions (low water temperatures) observed in winter 2010 (Matishov et al. 2012) that may have resulted in high mortality of young crab instars.

This study primarily emphasized the presence of copepods associated with red king crabs in the Barents Sea, and we found nine copepod species in total. However, many of these species had low infestation prevalences and intensities. These low-prevalence/low-intensity species included typical planktonic species (*C. finmarchicus*, *M. pusillus*, and *M. norvegica*), rare benthopelagic species (*E. normani* and *C. gracilis*), and a typical benthic

species (*Z. abbreviatus*). The majority of these species were found attached to the crabs' gills, which trap plankton and seston. Combined with their low prevalence and intensity, this suggests that these species should be considered incidental visitors. In contrast, the harpacticoid copepods *T. furcata*, *H. uniremis*, and *D. vulgaris* had relatively higher prevalences and mean intensities and should thus be classified as commensals.

The most prevalent copepods, *T. furcata* and *H. uniremis*, are commonly associated with macrophytes (Johnson and Olson 1948; Jewett and Feder 1977; Webb and Parsons 1992). These species were also found attached to the body and gills of the great spider crab *Hyas araneus* in Dalnezelenetskaya Bay. In the Sea of Okhotsk, *T. furcata* was found in the gills of the golden king crab *Lithodes aequispinus* (Karmanova, personal communication). Therefore, *T. furcata* and *H. uniremis* seem to have a low specificity for potential hosts. Colonization of crabs in general may be beneficial for these copepod species because it enhances food acquisition, increases their mobility, and affords them a degree of protection from predators, as was suggested for a number of other similar associations (Wahl 1989; McGaw 2006; Fernandez-Leborans 2010). Conversely, this association may have negative effects on the crab host. Colonization of the gills may impart a physiological cost on *P. camtschaticus* individuals due to a decrease in respiratory function. Similar effects were established for ectocommensal barnacles attached to the gills of the blue crab *Callinectes sapidus* (Gannon and Wheatly 1992).

Previous studies reported finding the copepod *Tisbe* sp. on large red king crabs in the Varanger-fjord area of northern Norway (Haugen et al. 1998; Jansen et al. 1998). In Jansen et al. (1998), presence data were based on a limited sample size (*n* = 15), and the authors only discussed the prevalence of *Tisbe* sp. (66.7%). In Haugen et al.
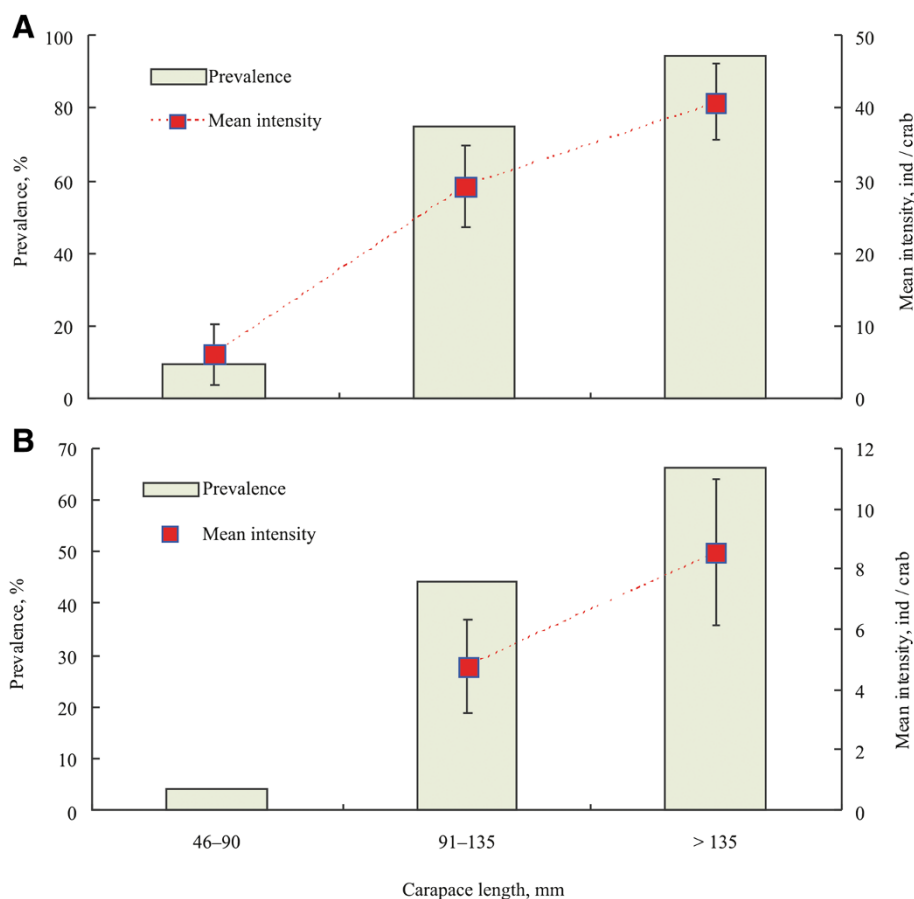
Figure 2 Infestation levels of *T. furcata* (A) and *H. uniremis* (B) on red king crabs of different size classes. Vertical bars show standard errors. The intensity of *H. uniremis* in 2009 is not included due to the low sample size.

(1998), both the prevalence and mean intensity of *Tisbe* sp. on the gills of 77 crabs were reported: 94% and 21.0 (range, 1 ~ 146) ind./crab in spring and 100% and 26.7 (1 ~ 88) ind./crab in autumn. Additionally, prevalences of this copepod on female egg clutches were 11% in spring and 30% in autumn (Haugen et al. 1998). Prevalence data reported for *P. camtschaticus* in previous studies were similar to our results for *T. furcata*-colonized large crabs. However, it is difficult to compare our data for *Tisbe* intensity on crabs in Dalnezelenetskaya Bay with the Varanger-fjord data because the Norwegian authors counted copepods only from 1 pair of gills (the fourth pair from the rear, Haugen et al. (1998)), while we examined all 11 pairs of gills.

The maximum prevalence of the most commonly associated copepod (*T. furcata*) was observed in 2011. This result may be explained by the low occurrence of small

Table 3 Percent occurrence of copepods at each of the five locations on the bodies of *P. camtschaticus*

Species	Number	Carapace	Limbs	Abdomen	Gills	Mouthparts
C. finmarchicus	5	0.00	0.00	0.00	100.00	0.00
C. gracilis	1	0.00	0.00	0.00	0.00	100.00
D. vulgaris	39	35.90	53.85	2.56	0.00	7.69
E. normani	172	0.00	9.30	0.00	90.70	0.00
H. uniremis	622	0.00	2.25	0.00	97.11	0.64
M. pusillus	1	0.00	0.00	0.00	100.00	0.00
M. norvegica	1	0.00	0.00	0.00	100.00	0.00
T. furcata	7,587	0.25	0.21	0.03	99.42	0.09
Z. abbreviatus	3	0.00	66.67	0.00	33.33	0.00

crabs in that year. The maximum mean intensity level of *T. furcata* in 2009 may be explained by the relatively high occurrence of crabs with old shells in that year. In addition, competition between copepods or between copepods and other commensals may also be a driver of these patterns. For example, the mean intensity of *H. uniremis* in 2011 (the year with the maximum prevalence of *T. furcata*) was significantly lower than that in 2010. Overall, these significant variations in the prevalence and mean intensity of associated copepods also support their having low specificity for the red king crab host.

The proportion of colonized hosts depends on host size, with larger crabs being more susceptible to infestation. Similarly, the mean intensity of the harpacticoid copepod *T. furcata* tended to increase with crab carapace age. High infestation levels of associated species on larger crabs appear to be common among crustaceans (Key et al. 1999; Mantelatto et al. 2003; McGaw 2006; Villegas et al. 2006; Dvoretsky and Dvoretsky 2010a): large crabs provide larger targets for both settling larvae and mobile species. Larger crabs also molt less frequently (Donaldson and Byersdorfer 2005), allowing more time for commensal species to colonize, potentially in greater numbers. In particular, red king crabs with old shells (i.e., males) can skip a molt, and the age of their exoskeleton can reach 4 years (Kuzmin and Gudimova 2002; Donaldson and Byersdorfer 2005).

The location of different organisms on the host strongly depends on the settlement patterns of larvae (McGaw 2006) and on host-associate relationships (Dvoretsky and Dvoretsky 2010a). Mobile organisms can select specific areas on hosts according to their preference. Colonization of the gills provides commensals with aeration and protection from predators; therefore, many copepods, including all but one of the species observed in this study, concentrate in the branchial chambers of their host. In contrast, the harpacticoid copepod *D. vulgaris* was not found in crab gills. This was probably due to differences in the biotic requirements of this species. *T. furcata* and *H. uniremis* usually inhabit a water layer far from the bottom and are frequently found in zooplankton samples (Johnson and Olson 1948; Jewett and Feder 1977; Dvoretsky and Dvoretsky 2010b); thus, they may infest crabs as a result of being drawn into the gills during host respiration activity. *D. vulgaris*, in contrast, is a more benthic species (Kornev and Chertoprud 2008) and more often infests the host when *P. camtschaticus* moves along the sea floor.

During our previous long-term study on associated species of red king crabs in Dalnezelenetskaya Bay (August ~ September of 2004 ~ 2008), we found no copepod species on the hosts (Dvoretsky and Dvoretsky 2009b, 2010a). The absence of copepods in that study was probably not connected with a mismatch in copepod and crab life histories as the majority of crab-associated species have several generations per year, and August is a period with average or even optimal conditions for copepod reproduction. We suggest that an explanation for the absence of copepods on red king crabs is in their interspecific relationships with other species of associated organisms. One possible mechanism for the absence of copepods on crabs in August is interspecific competition between medium- and small-sized amphipods and copepods when population densities of these species are high, as was reported for other symbiotic associations (Lindberg and Stanton 1988; Tsuchiya and Yonaha 1992; Baeza et al. 2001). In addition, we showed in previous studies that the amphipod *I. commensalis* was the most common gill symbiont of the red king crab in August (Dvoretsky et al. 2007; Dvoretsky and Dvoretsky 2009b). At that time, this amphipod had relatively high infestation prevalences and intensities, and its mean body length was significantly greater in August than in July (Dvoretsky and Dvoretsky 2011). The analysis of *I. commensalis* guts showed that large specimens could eat harpacticoid copepods. Thus, it is reasonable to hypothesize that predation by *I. commensalis* was the main cause of the observed absence of copepods from crab gills in August.

Conclusions

This report expands our knowledge on the colonization of different fouling and symbiotic species on red king crabs. Our study has management and aquaculture implications as it sheds light on possible interactions between copepods and their crab hosts. Colonization of crabs in general may be beneficial for the copepod species because it enhances food acquisition, increases their mobility, and affords them a degree of protection from predators. Conversely, this association may have negative effects on the crab host due to a decrease in respiratory function.

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