Habitat preferences of juvenile and adult *Carcinus maenas* (L., 1758) under laboratory conditions

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Sammfattning

Europeiska strandkrabban *Carcinus maenas* av vadehavet lever i ett varierat utbud av habitaten. Den är mycket anpassningsbar till olika miljöer där de väljer strukturer som erbjuder skydd mot predatorer.
Dessa val av habitat påverkas också av individens ålder. Habitater som innehåller musslor och stenar fungerar som tillflyktsort och väljs oftare av juvenila krabbor. Detta har bekräftats under laboratorieförhållanden.

Abstract

The european shore crab *Carcinus maenas* of the Wadden Sea lives in a diverse range of habitats. It is highly adaptable to different environments where it chooses structures that offer protection from predators.
Its choice of habitat is also influenced by the age of the specimen. Habitats that contain mussels and rocks function as a refuge and are chosen more often by juvenile crabs. This has been confirmed under laboratory conditions.

Introduction

The european shore crab *Carcinus maenas* is an ubiquitous organism in the Wadden Sea, native to Europe and northern Africa. It has been widely introduced to shores all around the world as an invasive species. It can be encountered in a diverse range of habitats: hard substrates of the outer coast, hard and soft substrates in protected embayments, as well as sheltered areas such as rocky intertidal, unvegetated intertidal, subtidal mud and sand, saltmarshes and seagrasses. (Klassen & Locke, 2007) Its wide range of distribution and habitats indicates that *C. maenas* is highly adaptable to changing conditions.

Juvenile crabs not only seem to cover themselves with seagrass, as adults do, but also hide near rocks and shell hash in the intertidal zone (Jensen et al., 2002). In Sylt, Thiel and Dernedde (1994) observed a drastic increase of juvenile shore crabs after the addition of shell to beach. It has been suggested, that the increase in mussel culturing and therefore higher abundance of mussel clumps on the tidal flats of the Wadden Sea improved the habitat availability for *C. maenas* juveniles. The mussel clumps serve as shelters and stabilise the population of juvenile crabs during autumn. On bare tidal flats, the number of young crabs decreased substantially (Thiel & Dernedde, 1994).

Our aim was to examine whether differences in habitat preferences of juvenile and adult crabs in the wild can be observed as different choices under laboratory conditions and to determine how individual factors influence the behaviour.

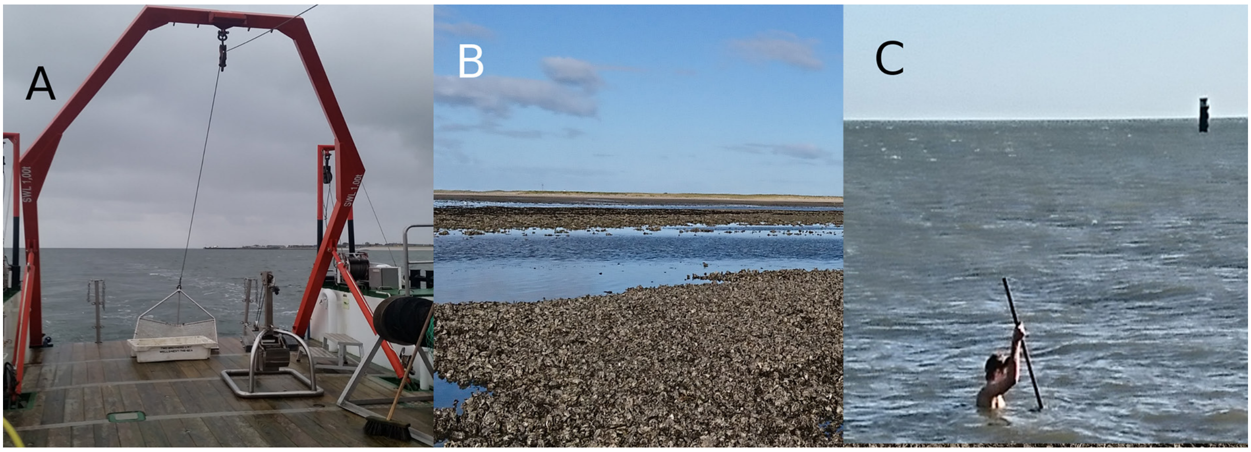

Fig.1. Sampling sites of *C. maenas*. A) Depicting the Dredge net of the MYA II. B) The local oyster bed at List. C) Sampling the Sylter Beach in front of "Naturgewalten".

Material and Methods

Habitat preference experiments were conducted on two consecutive days in October 2017 in Sylt/ Germany. *Carcinus maenas* individuals were collected at three different sites over the course of five days. Firstly, using a dredge on board the "Mya II" in the Wadden Sea of Sylt (04.10. 2017) (Fig.1A), secondly, during low tide at the oyster bank in List/ Sylt (08.10.2017) (Fig. 1B) and lastly, using a dip net during high tide on the local beach in front of "Erlebniswelten Naturgewalten/List/Sylt (06.10.2017) (Fig. 1C). In order to compare the specimens's behaviour according to their age three size categories were determined: small (≤3,5 cm), medium (>3,5 ≤ 4,5 cm) and large (>4,5 cm). By measuring the carapace width point-to-point (Klassen, 2017) (Fig. 2) crabs were allocated to size categories and kept in dark grey storage boxes with flow through seawater until tested. The experiments were conducted in transparent plastic boxes with the dimensions of 44 cm x 23.5 cm x 26 cm in height. The boxes were filled with seawater to a height of 10 cm. In total, four different experimental settings were tested. To prevent distraction of the crabs from outside and create similar lighting conditions, the boxes were separated by white plastic containers. The negative control was performed in an empty box with two identical sides to identify potential bias due to side preferences other than the variable parameters (Fig. 3A). For a colour preference test, a dark and a light surface was offered (Fig. 3B). In the third setting, specimens were offered the choice between an empty side and a side with two large oyster shells to test for the preference for shelter. The oyster shells were cleaned and boiled beforehand for five minutes to eliminate any residues of mussel flesh or other edible material hence interesting material for the crab, which could bias the side choice (Fig. 3C). To test preferences for surface structures a sand-filled box without water was used (Fig. 3D).

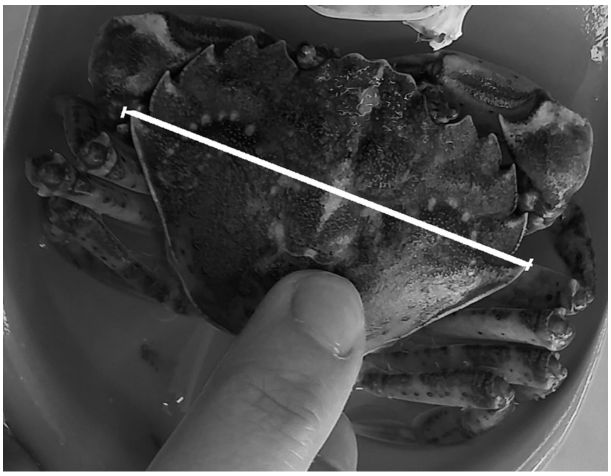

Fig. 2. *Carcinus maenas*. The white line indicates the width measured to determine the carapace size.

Sand was compared to an empty sand-coloured surface to exclude influences of colour. In this experimental setting, digging behaviour was observed qualitatively. The crab showed "digging" behaviour, if it buried its pleon in the sand.
The amount of time spent at each side of every experiment was measured quantitatively for five minutes, using stopwatches and a timer. Individuals were transferred to the test box using a small plastic container and placed randomly on the middle line of the testing area. The first 60 seconds were not observed to let the specimen adjust to the new environment. For the following 300 s only the time spent on the right side of the box was measured. As the criterion for the whereabouts of the crab, the location of its right eye was used. Overall, 36 *C. maenas* were tested in groups of 12 per size category.
To eliminate sources of errors, each crab was tested in each experimental setting. Also, the sequences of settings were rotated in case of changes in behaviour due to exhaustion or long-term stress. For consistency, one crab was handled by one experimentator at all experimental settings and each experimentator handled half of the total sampling population. Also, the crabs were rotated by age group to diminish influences of lighting conditions at different times of day.

In this study, *Carcinus maenas* were tested in behavioural experiments. Since the animals used for this study were captured on different days, bias due to different levels of long-term stress has to be taken into account. However, we aimed to prevent our experiment from internal and external bias as much as possible.

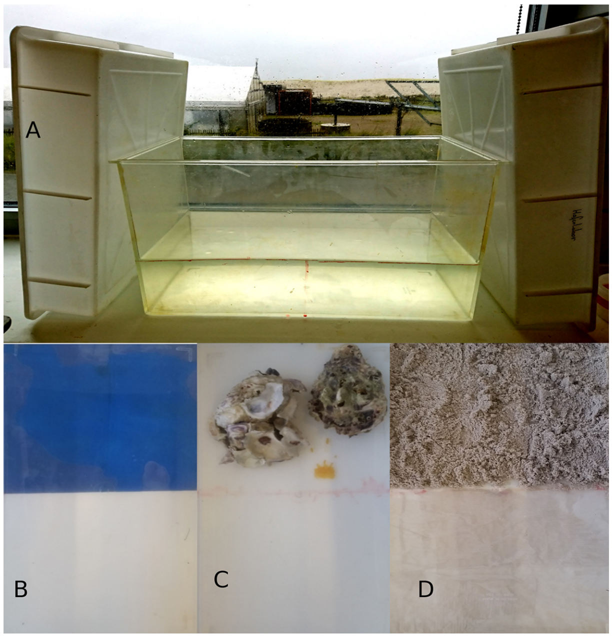

Fig. 3. Study design. A) The negative control. Surrounding the testing box are two white plastic boxes to act as a barrier

Results

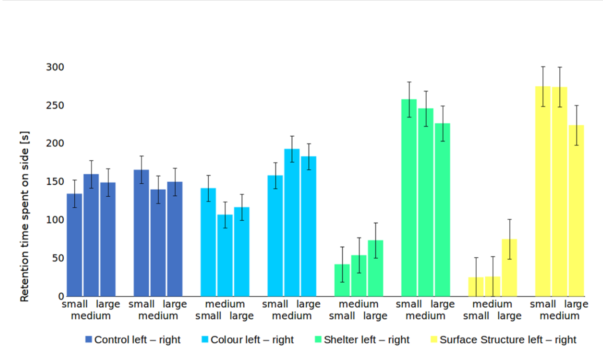

Fig. 4. Average time spent on left or right side of each experiment for each size category. Notably, all groups do not lean towards either of the control sides. Furthermore, all groups of crabs highly prefer the sheltered mussel side (Fig. 3C) respectively the sandy side (Fig. 3D) to the empty side.

Carcinus maenas was given a choice of habitats in four different experimental settings.

In the control setting, the 36 specimen did not show a preference for either left or right side. On each side, crabs spent on average about 150 s (left: 147 s, right: 152 s). The standard error of the mean (SEM) was 10 s (Fig. 4).

The colour preference experiment revealed a slight tendency to favour the darker side of the testing area. Here, on average 187 s were spent compared to 121 s on the brighter side. Again, the SEM was 10 s. Crabs preferred the darker area and SEM error bars did not overlap comparing results for bright and dark surfaces.

An even more prominent preference for one side of the testing area was observed in the “no shelter / shelter” experiment. Here, the average time spent on the empty side was 56 s ± 13 s whereas C. maenas spent on average 243 s ± 13 s on the oyster side.

The smooth to rough surface test revealed a strong tendency for the sandy side. Crabs spent an average time of 257 s ± 15 s on the sand, whereas the mean time spent on the sand coloured, yet smooth surface was 41 s ± 15 s.

Considering the three size categories of shore crabs, some difference in habitat preference could be observed (S1. see attachment for detailed results.) The control experiment did not show a leaning towards one specific side in any of the tested crab sizes (retention time of small crabs 134 s : 166 s ± 16 s on left and right side respectively).

There was no preferred side in the colour preference experiment in small crabs (average retention time on dark surface 142 s : 158 s ± 17 s). However, both medium and large sized crabs did prefer the dark side of the testing box (64 % in medium sized crabs and 61% of large crabs). Here, only the small crabs did not respond positively to the darker surface.

For the shelter preference a dominant preference for both the oyster side and the sandy side was observed in all groups.

In total, small crabs stayed on the oyster side of the shelter preference test for about 86 % of the time (mean duration on oyster side 258 s ± 20 s).

Medium sized crabs showed an average retention time of 82 %, compared to large crabs who spend only 76 % of the time amongst the oyster.

On the sandy surface, the small crabs stayed for 92 % of the time or 275 s ± 19 s.

A similar pattern was observed within the medium sized crabs who explored the oyster side for 76 % of their time.

For the surface structure test, a preference for the sandy side was observed in all tested crab sizes. Notably, the small crabs showed a higher tendency to select the sandy side than the medium and large crabs. They occupied the sandy side in 92 %, 91%, and 75% of the cases respectively. Furthermore, the interaction with sand was registered.

While all 12 small crabs showed burying behavior immediately upon being placed in the aquarium, only 80 % (ten of 12 crabs) of the medium sized crabs did so and even fewer of the large crabs. Here, only six of 12 crabs buried themselves in the sand (Fig. 5).

and create as similar conditions as possible on both sides.
B) The colour preference test. A dark blue microfiber tissue under one side (the right) of the box results in a dark side. C) The shelter preference test. Two oysters provide shelter. D) The surface structure test. One half of the testing box is covered with sand and can be used by the crabs to burrow themselves.

Discussion

This study investigated the behaviour of a total of 36 *Carcinus maenas* crabs in four different experimental settings. As a very common and highly invasive species, the habitat preference of C. maenas is a cornerstone to further understand the success of this crustacean. Also, we wanted to understand whether juveniles and adults differ in their behaviour.

As the age of *C. maenas* correlates with the carapace‘s width, we determined three groups with an average carapace width of 3,0 cm (small), 4,0 cm (medium) and 5,4 cm (large).

The body size of *C. maenas* increases by 20-33 % per moult. It takes about ten moults to grow to a carapace width of 2.0 cm in the first year. After the first year, the shore crab moults more than once per year although the rate decreases as the crab reaches maturity to about once a year. (MarLIN, 2006). The lifespan of *Carcinus maenas* is about 5-7 years in Europe.

In the experiments all 36 crabs showed a similar behaviour. The negative control revealed no tendency to either one of the two sides of the box. We therefore conclude that there is no specific bias to neither left nor right side (Fig.4).

As a result, the outcome of the three other behavioural experiments is unbiased by the position of the “sides” of each test.

Interestingly, we did observe some behavioural differences between small, medium and large crabs. Although all crabs showed a tendency for the dark side, small crabs spend nearly as much time here as on the bright side.

A very prominent difference occurred when comparing the shelter behaviour in all three groups. Although each group preferred the sheltered side with oysters, the largest crabs did so the least. We suspect, that the large and therefore oldest and strongest crabs are not as dependent on the safety offered by a shelter as the smaller crabs are. Larger adult crabs leave this intertidal habitat to avoid the higher risk of physiological stress caused by fluctuations of temperature and salinity. Smaller, younger crabs cope with these changing abiotic factors since the danger of predation by adult shore crabs, shrimps and fish is diminished here (Thiel & Dernedde, 1994).

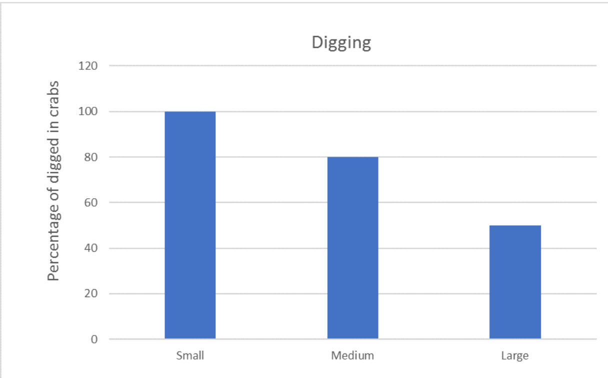

Fig. 5. Digging behaviour of small, medium and large *C. maenas* in percent. Per group, 12 crabs of both sexes were tested.

Similarly, in the percentage of “digging behaviour” a clear trend was notable. While all small crabs digged themselves in, only half of the large crabs did. This is not due to too little sand in the testing box, because some of the large crabs did dig themselves in. We therefore conclude, that the larger crabs are less vulnerable to predation and do not need to hide as well as smaller *Carcinus maenas* need to.

Supportingly, when collecting the crabs from the oyster Bank of Odde Watt, we found larger crabs in a higher abundance than small crabs. More often, the large crabs were found on sandy ground with no to few sheltered space in the immediate vicinity.

Since we encountered the invasive species *Hemigrapsus sanguineus* (De Haan, 1853) in the same habitat as *C. maenas* it seems likely that the two species compete for a similar ecological niche. This competition is especially prominent in young *C. maenas* due to the smaller size of *H. sanguineus*. Studies show that adults of the asian shore crab drive out exclude nearly all juvenile *C. maenas* of a similar size from shelters such as mussel shells and rocks. These competitive interactions could limit the distribution and impact as an invasive species of the shore crab since it may lead to its displacement from its favoured habitats. This could lead to an increased risk of predation and decreased access to food. As a result, a bottleneck to population growth could be generated (Jensen et al. 2002). Therefore, as a subsequent study, it would be interesting to also investigate the behaviour of *Hemigrapsus sanguineus* under the same conditions as we did.

References

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Supplementary Material

S1: Complete data set for the behaviour of *C. maenas* observed during the different experimental settings, separated by age group and pooled.

Size		Standard Error of the Mean	Average	Absolute time [s]	%	Standard Deviation
small	Control left	16	134	1612	0,45	56
	Control right		166	1988	0,55	56
				3600		
	Bright	17	142	1700	0,47	
	Dark		158	1900	0,53	59
				3600		59
	Empty	20	42	505	0,14	
	Oyster		258	3095	0,86	
				3600		68
	Empty	19	25	302	0,08	68
	Sand		275	3298	0,92	
				3600		
medium	Control left	21	160	1920	0,53	73
	Control right		140	1680	0,47	73
				3600		
	Bright	15	107	1284	0,36	53
	Dark		193	2316	0,64	53
				3600		
	Empty	24	54	648	0,18	85
	Oyster		246	2952	0,82	85
				3600		
	Empty	25	26	313	0,09	86
	Sand		274	3287	0,91	86
			9	3600		
large	Control left	17	150	1795	0,50	61
	Control right		150	1805	0,50	61
				3600		
	Bright	20	117	1401	0,39	68
	Dark		183	2199	0,61	68
				3600		
	Empty	26	74	882	0,25	90
	Oyster		227	2718	0,76	90
				3600		
	Empty	33	75	904	0,25	116
	Sand		225	2696	0,75	116
				3600		
all sizes	Control left	10	156	5621	0,49	61
	Control right		161	5779	0,51	61
				11400		
	Bright	10	386	4634	0,41	59
	Dark		564	6766	0,59	59
				11400		
	Empty	13	178	2131	0,19	78
	Oyster		772	9269	0,81	78
				11400		
	Empty	15	131	1570	0,14	90
	Sand		819	9830	0,86	90
				11400		