

Comparing the boldness and exploration of co-occurring invasive marine crabs (*Hemigrapsus sanguineus* and *Carcinus maenas*)

by

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## Abstract

Boldness (i.e., the propensity to take risks) and exploratory behaviours can influence an animal's ability to acquire resources, thus affecting competitive success. The Asian shore crab, *Hemigrapsus sanguineus*, and European green crab, *Carcinus maenas*, co-occur as highly invasive species in coastal areas of the United States, and more recently, in Atlantic Canada. Both crabs use structurally complex rocky intertidal habitat and consume similar macroalgal and invertebrate prey, such that they likely compete for resources. Competitive interactions between co-invaders could influence the success of either species, affecting their impacts on the ecosystem. Yet, there is a gap in research comparing behaviours, such as boldness or exploration, between co-invaders such as these crabs. Here, the boldness of *H. sanguineus* and *C. maenas* are compared by investigating their tendencies to exit preferred rocky habitat with shelter to explore an open and brightly lit area. The crabs' exploratory tendencies are also compared, by evaluating if the species spontaneously alternate (i.e., preferentially explore regions that they have least recently explored), an indicator of efficient exploration. The two species had similar latencies when exiting the sheltered habitat, but *C. maenas* spent more time in the open area overall. Both crabs also tended to spontaneously alternate, but *H. sanguineus* had a greater tendency to do so than *C. maenas*, revealing for the first time the existence of significant differences in spontaneous alternation behaviour between invasive species. If the strong exploration ability of *H. sanguineus* can improve the species' ability to find resources, it may have a competitive advantage over *C. maenas* in regions where the crabs co-occur.

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**Table of Contents**

<b>Abstract.....</b>	<b>i</b>
<b>Acknowledgements .....</b>	<b>ii</b>
<b>List of Tables.....</b>	<b>iv</b>
<b>List of Figures.....</b>	<b>v</b>
<b>Introduction.....</b>	<b>1</b>
<b>Materials and Methods.....</b>	<b>6</b>
<b>Results .....</b>	<b>15</b>
<b>Discussion .....</b>	<b>25</b>
<b>Literature Cited .....</b>	<b>31</b>
<b>Appendix A: Spontaneous alternation score calculation.....</b>	<b>42</b>
<b>Appendix B: Chance level alternation score calculation.....</b>	<b>43</b>
<b>Appendix C: Relationships between temperature and carapace width and behaviours .....</b>	<b>44</b>

**List of Tables**

<i>Table 1.</i> The change points detected in the spontaneous alternation performance of male adult <i>Hemigrapsus sanguineus</i> and juvenile <i>Carcinus maenas</i> during 60 minutes of exploration in a plus-maze .....	25
<i>Table C1.</i> Results of Spearman's rank order correlation tests for relationships between temperature and carapace width and the behaviours of male adult <i>Hemigrapsus sanguineus</i> and juvenile <i>Carcinus maenas</i> .....	44

## List of Figures

<i>Figure 1.</i> Field site at Bear Cove, Nova Scotia, Canada where male adult <i>Hemigrapsus sanguineus</i> and juvenile <i>Carcinus maenas</i> were collected as needed in July 2024 .....	7
<i>Figure 2.</i> Diagram of boldness test arena consisting of an open area, a structurally complex habitat, and a brightly lit open passageway connecting the two.....	9
<i>Figure 3.</i> Diagram and dimensions of the plus-maze apparatus.....	10
<i>Figure 4.</i> Boldness of male adult <i>Hemigrapsus sanguineus</i> and juvenile <i>Carcinus maenas</i> in the boldness experiment.....	17
<i>Figure 5.</i> Hesitancy of male adult <i>Hemigrapsus sanguineus</i> and juvenile <i>Carcinus maenas</i> in the boldness experiment.....	18
<i>Figure 6.</i> Number of times male adult <i>Hemigrapsus sanguineus</i> and juvenile <i>Carcinus maenas</i> exited the complex habitat to enter the passageway in the boldness arena during the 60-minute trials.....	19
<i>Figure 7.</i> Time spent outside of the complex habitat in the boldness arena by male adult <i>Hemigrapsus sanguineus</i> and juvenile <i>Carcinus maenas</i> in the 60 minute boldness experiment.	20
<i>Figure 8.</i> Alternation performances of male adult <i>Hemigrapsus sanguineus</i> and juvenile <i>Carcinus maenas</i> in a plus-maze .....	22
<i>Figure 9.</i> Willingness to explore of male adult <i>Hemigrapsus sanguineus</i> and juvenile <i>Carcinus maenas</i> in a plus-maze .....	23
<i>Figure 10.</i> Activity of male adult <i>Hemigrapsus sanguineus</i> and juvenile <i>Carcinus maenas</i> .....	24

## Introduction

An animal's behavioural traits are important in shaping its interactions with its biotic and abiotic environment (Ward et al. 2004; Kabalan et al. 2024). In some species, behaviour varies among individuals in a manner that is consistent over time and across contexts, and this is often referred to as personality (Gosling 2001; Biro and Stamps 2008). An individual's tendency to take risks, especially to increase the likelihood of finding resources (e.g., food or habitat) or mating, is a trait referred to as boldness (Ward et al. 2004). Boldness exists on a continuum, the bold-shy axis, with bold individuals having a high propensity for risk-taking and shy individuals avoiding risks (Ward et al. 2004). For instance, bold individuals might spend less time using shelter or be more active under predation risk than shy individuals (Ward et al. 2004; Cote et al. 2010; Su et al. 2019). The tendency to explore novel environments is also sometimes considered boldness, or otherwise a trait that often coincides with boldness (Wilson et al. 1993; Patrick et al. 2017; Adeli et al. 2024).

Boldness might influence trade-offs related to resource acquisition (Ward et al. 2004; Patrick et al. 2017), such as life-history trade-offs, which occur between survival, growth, and reproduction due to resource limitations (Biro and Stamps 2008; Dhellemmes et al. 2020). Growth-mortality trade-offs could be particularly affected by boldness, since bold individuals tend to accept greater risks to obtain essential resources (Ward et al. 2004; Stamps 2007; Wolf et al. 2007). For instance, bold three-spined sticklebacks (*Gasterosteus aculeatus*), that foraged soon after a single stimulated predation threat, fed more and grew larger than shy sticklebacks (Ward et al. 2004). Due to the association between boldness and exploring novel environments, boldness might also be linked to trade-offs between gathering information about novel resource patches through exploration and exploiting known resources (Patrick et al. 2017). For example, bold albatrosses (*Diomedea exulans*), where boldness was characterized by their responsiveness towards

humans, were more exploratory of unfamiliar regions during foraging trips, suggesting that they may have an advantage in competing for heterogeneously distributed resources (Patrick et al. 2017). Rather than exploring, shy albatrosses spent more time feeding on known resource patches, which may increase the risk of resource depletion (Patrick et al. 2017).

While most studies evaluate intraspecific variation in boldness and exploration (e.g., Ward et al. 2004; Patrick et al. 2017), stable interspecific differences in these traits can also occur (Ingleby et al. 2014a, 2014b), and bold or exploratory species might be more likely to establish non-native populations and become successfully invasive (Rehage and Sih 2004; Balci et al. 2014; Damas-Moreira et al. 2019). Some invasive species have been found to be bolder or more exploratory than co-occurring native species (Balci et al. 2014; Damas-Moreira et al. 2019). For example, invasive Italian wall lizards (*Podarcis sicula*) were bolder than native co-occurring green Iberian wall lizards (*Podarcis virescent*), as measured by their shorter latency to exit an undesirable shelter (Damas-Moreira et al. 2019). Additionally, Balci et al. (2014) evaluated the tendency of invasive European green crabs (*Carcinus maenas*), native blue crabs (*Callinectes sapidus*), and native fiddler crabs (*Uca pugnax*) to spontaneously alternate, meaning to innately (e.g., without reinforcement such as food rewards) explore regions that were least-recently explored (Ramey et al. 2009; Balci et al. 2014). The invasive *C. maenas* was more exploratory of the least-recently visited regions of a maze than both native crabs (Ramey et al. 2009; Balci et al. 2014). The tendencies to be bold and explore novel areas have been linked to increased dispersal (Trinidad killifish, *Rivulus hartii*: Fraser et al. 2001; signal crayfish, *Pacifastacus leniusculus*: Galib et al. 2022), suggesting that they could increase the likelihood of a species' introduction to a non-native environment, along with subsequent spread. If bold invaders can more successfully find

resources (Ramey et al. 2009; Patrick et al. 2017), they may also have a competitive advantage over shyer native species.

Anthropogenic activities have increased the rate of non-native species introductions, such that communities often contain more than one invader (Jackson and Grey 2012; Jackson 2015; Castro et al. 2022). Despite the large literature base on invasive species and their interactions with native species, relatively few studies have considered interactions among co-invaders, particularly for marine animals (Lowry et al. 2013; Jackson 2015). Co-invaders could interact mutualistically or could negatively impact each other through competition, such that the environmental impact of co-invaders could be greater or lesser than predicted based on the impacts of the individual species (Simberloff and Von Holle 1999; Jackson 2015). For animals, interactions among co-invaders might be influenced by the species' boldness or exploratory behaviours, particularly if the invaders compete for resources.

The Asian shore crab, *Hemigrapsus sanguineus*, was introduced in the North Atlantic on the east coast of the United States (USA) in 1988 (Williams and Mcdermott 1990), where its invaded range overlaps with that of the invasive *C. maenas* (reported in Massachusetts, USA in 1817; Carlton and Cohen 2003; Klassen and Locke 2007). *Hemigrapsus sanguineus*, native to the coasts of eastern Asia, spends most of its life cycle in the intertidal zone (Klassen 2012; Kraemer 2019). *Carcinus maenas* is native to coastal Europe and Northern Africa, and juveniles occupy the intertidal zone, whereas adults primarily inhabit the subtidal zone (Klassen and Locke 2007; Landschoff et al. 2013). In Canada, *H. sanguineus* was first identified and officially reported in 2020 in Nova Scotia (Ramey-Balci et al. in prep), where it co-occurs in the mid-high intertidal zone with juveniles of the longer established (~1950s, Klassen and Locke 2007) *C. maenas* at small spatial scales of 0.25 m<sup>2</sup> (Bharti et al. 2023). Both crabs are omnivorous and likely predate

upon native species of green and brown macroalgae, mussels (*Mytilus edulis*), and other marine invertebrates (DeGraaf and Tyrrell 2004; Bharti et al. 2023; Saborowski et al. 2023). Due to the crabs' similar resource use, they are also likely competitors (Hobbs et al. 2017b; Bleile and Thieltges 2021). The outcomes of this competition could influence the population dynamics or distribution of existing *C. maenas* populations (Hobbs et al. 2017b; Baillie and Grabowski 2019), or the continued spread and establishment of *H. sanguineus*. Understanding the behavioural factors that mediate the interactions between these highly invasive species is valuable in predicting the outcomes of this co-invasion on their introduced ecosystems.

Research to-date regarding the behaviour of *H. sanguineus* and *C. maenas* has largely focused on their resource use and aggressive or avoidant behaviours (Lord and Dalvano 2015; Hobbs et al. 2017b), and there has been limited work directly comparing their boldness or exploration. Preliminary experiments found that juvenile *C. maenas* from Nova Scotia were quicker to exit a dark shelter and enter a brightly lit and open area than co-occurring adult *H. sanguineus*, suggesting that *C. maenas* may be bolder than *H. sanguineus* (DeJaegher et al. in prep). However, it is unknown if these results remain consistent under more ecologically relevant conditions, such as if the crabs had the opportunity to shelter in rocky substrate as they might in their natural habitat (Jensen et al. 2002). Furthermore, though adult *C. maenas* have been found to spontaneously alternate, a likely indicator of efficient exploration (Ramey et al. 2009), the alternation behaviour of *H. sanguineus* and juvenile *C. maenas* has not yet been evaluated, and this behaviour is yet to be compared between two invasive species. Mark-recapture experiments,

however, have indicated that *H. sanguineus* is highly mobile and exhibits low site fidelity (Brousseau et al. 2002), suggesting that the species is exploratory.

### *Research objectives and hypotheses*

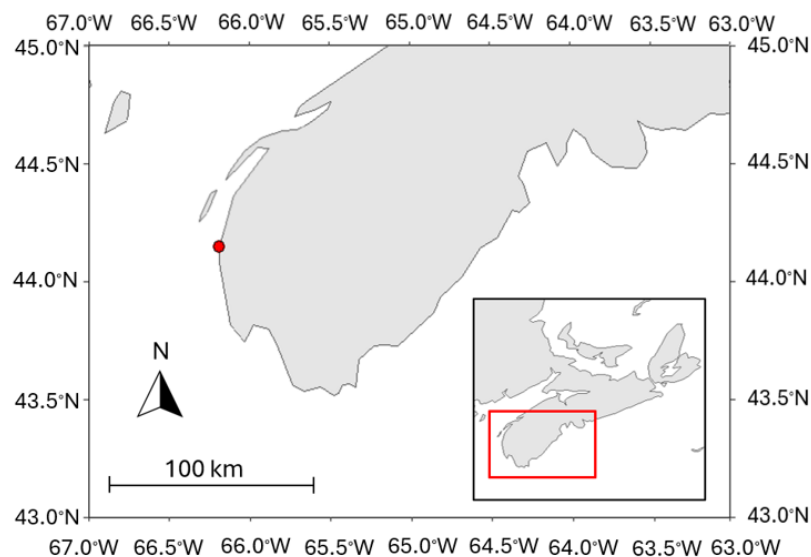
The objectives of this research are to: (1) compare the boldness of adult *H. sanguineus* and juvenile *C. maenas* by assessing their tendencies to take a risk by exiting a structurally complex habitat to explore an open and brightly lit area, (2) determine if adult *H. sanguineus* and juvenile *C. maenas* spontaneously alternate, and (3) compare the species' exploration efficiencies by examining their alternation performances. If interspecific differences in the boldness of adult *H. sanguineus* and juvenile *C. maenas* exist, then how long each species waits before exiting a complex habitat with shelter to explore an open (i.e., without shelter) and lit area will be different. As juvenile *C. maenas* were quicker to exit a dark shelter than adult *H. sanguineus* (DeJaegher et al. in prep), *C. maenas* are also predicted to be quicker to explore the open area, and to thus be bolder, than *H. sanguineus*. In terms of spontaneous alternation, if adult *H. sanguineus* and juvenile *C. maenas* spontaneously alternate, then they should enter the least-recently visited arms of a plus-maze at a rate greater than chance, such that their alternation scores (i.e., alternation performances) are greater than chance level. This is predicted due to *H. sanguineus*' low-site fidelity and the tendency of adult *C. maenas* to spontaneously alternate (Brousseau et al. 2002; Ramey et al. 2009). If interspecific differences in exploration efficiency exist between the crabs, then they will have different likelihoods of entering arms of a plus-maze that they have least-recently visited, leading to different spontaneous alternation scores (with a higher score indicating greater exploration efficiency). Specifically, *C. maenas* are predicted to have higher alternation scores than *H. sanguineus*, due to the findings that *C. maenas* may be bolder than *H. sanguineus* and the positive correlations between boldness and exploration

reported in other species (Trinidad killifish: Fraser et al. 2001; albatrosses: Patrick et al. 2017; bluegills, *Lepomis macrochirus*: Adeli et al. 2024).

## Materials and Methods

### *Field collection and test subjects*

Adult male *H. sanguineus* (carapace width, CW, 12–40 mm: Jungblut et al. 2018; Kraemer 2019) and juvenile male *C. maenas* (CW 15–35 mm: Mascaró and Seed 2001) were collected between July 16 and July 30, 2024 from the mid to high intertidal zone at Bear Cove, located near the mouth of St. Mary’s Bay, Nova Scotia (44.145275, -66.193882; Fig. 1). Male crabs were selected since adult female *H. sanguineus* are commonly ovigerous during July (McDermott 1998; Hobbs et al. 2017a), and ovigerous *H. sanguineus* were previously found to have a lower frequency of injuries than non-ovigerous females, suggesting the potential for altered boldness (Hobbs et al. 2017a). Only crabs in the calcified intermoult stage that were motile (i.e., capable of immediately turning themselves right-side up after being placed on the back of their carapace, Dimant and Maldonado 1992) were used in experiments. In total, 26 *H. sanguineus* (CW  $18.04 \pm SE 0.48$ ) and 29 *C. maenas* (CW  $22.48 \pm SE 0.90$ ) were tested in the boldness experiment, and 38 *H. sanguineus* (CW  $18.02 \pm SE 0.44$ ) and 40 *C. maenas* (CW  $22.02 \pm SE 0.70$ ) were tested in the spontaneous alternation experiment.



*Figure 1.* Field site at Bear Cove, Nova Scotia, Canada (44.145275, -66.193882) where male adult *Hemigrapsus sanguineus* and juvenile *Carcinus maenas* were collected as needed in July 2024.

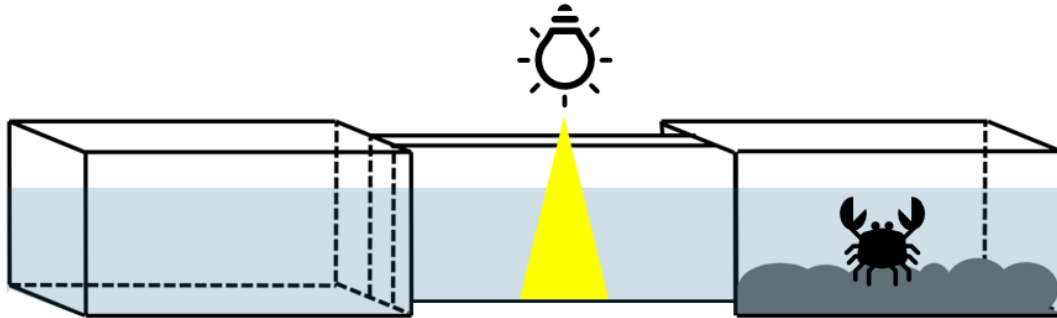
#### *Experimental protocol and apparatuses*

Experiments were conducted between July 18 and August 1, 2024. Prior to the experiments, crabs were held in minnow traps in a small kiddie pool with seawater, together with conspecifics, and fed local mussels (*Mytilus* spp.) and brown macroalgae (*Ascophyllum nodosum*, *Fucus* spp.) *ad libitum*. Following experiments, all crabs were euthanized by freezing. A license to fish for scientific purposes pursuant to Section 52 of the Fishery (General) Regulations SOR 93-53 (Atlantic region) was received to collect the crabs (DFO #364431), and no ethics or animal care permits were required.

### *Boldness task*

To examine boldness, a test arena consisting of two square habitats (20 x 20 x 15 cm, LxWxH) connected by a brightly lit passageway (20 x 8 cm x 15 cm, LxWxH) was built from transparent plexiglass (Fig. 2). The top of the arena was uncovered. Aquarium-safe glue was used to glue the plexiglass components of the arena together and silicone sealant was used to seal the arena so that it could hold seawater. The arena was left to dry for 24 hours following sealant application and then rinsed with filtered (63 µm) seawater prior to experiments to eliminate odours. One of the arena's square habitats contained a substrate of rocks of varying sizes with crevices, to create a structurally complex habitat, and the other square habitat and the passageway contained no substrate, creating an open unsheltered area (Fig. 2). Complex rocky regions are the preferred habitat of *H. sanguineus* and juvenile *C. maenas* when provided with choice in the absence of competition (Hobbs et al. 2017b). Structurally complex habitat can provide shelter to crabs from predators, improving survival (Gehrels et al. 2017), and exiting the complex habitat is thus considered risky. Rocks free of debris, plants, and animals were collected locally from the upper intertidal region and were rinsed in filtered seawater prior to being placed in the arena to create the complex habitat. The middle of the passageway was brightly lit from above with an LED light (VOLTAX Sonics Super Bright, 7.5 x 3.5 cm panel, lumen 900). When well fed, *H. sanguineus* and *C. maenas* reportedly avoid bright light, and crossing the lit passageway is thus assumed to increase the risk of exiting the complex habitat (Orlosk et al. 2011; Spilmont et al. 2015). The test arena was placed on a base of white plexiglass (62 x 22 cm LxW) in a dimly lit room. A cardboard divider (15 cm high) was arranged to surround the maze, to eliminate visual stimuli from the surrounding regions. The entirety of the boldness test arena was filled with filtered seawater to a depth of 6 cm. Prior to each new trial, the arena and rocks were rinsed in fresh

filtered seawater and the water in the arena was replaced. The water temperature was measured prior to each trial using a digital thermometer.

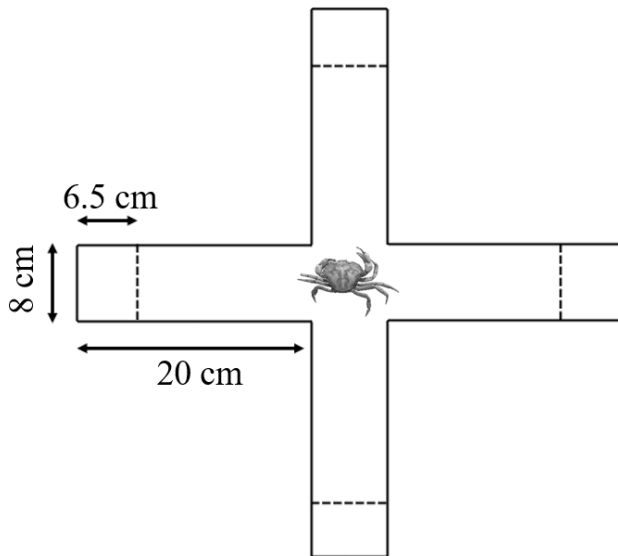


*Figure 2.* Diagram of boldness test arena consisting of an open area (20 x 20 x 15 cm, LxWxH), a structurally complex habitat (20 x 20 x 15 cm), and a brightly lit open passageway (20 x 8 x 15 cm) connecting the two.

Prior to the boldness test, individual crabs were placed in the centre of the complex habitat, held in a plexiglass cylinder with an open top and bottom (7.5 cm diameter, 12 cm height), and left to acclimate for five minutes. The cylinder was removed following acclimation, and the crabs were allowed to move freely for 60 minutes with the passageway LED light turned on. While in the arena, crabs were video recorded from above using a Panasonic HC-WXF1 video camera with an infrared lighting source. Infrared light (wavelength of approximately 780 nm to 1 mm) is assumed to not be visible to the crabs and to not affect their behaviour, because the visual pigments of other brachyuran crabs have poor absorbance of light with wavelengths  $\geq 600$  nm (Cronin and Forward 1988). Infrared cameras have also been previously used to record the behaviour of *H. sanguineus* under otherwise dark conditions (Spilmont et al. 2015).

### *Exploration task*

Two transparent plexiglass plus-mazes, each consisting of four arms (20 x 8 x 15 cm, LxWxH), with open tops were used to examine spontaneous alternation (Ramey et al. 2009; Fig. 3). Each maze was placed on a base of white plexiglass (50 x 50 cm, LxW). The plus-mazes were placed adjacently in a dimly lit room, so that two trials could be run simultaneously. A cardboard divider (15 cm high) surrounded each maze, to prevent crabs from viewing each other. The plus-mazes were filled with filtered seawater to a depth of 6 cm, the temperature of which was measured prior to experiments. Prior to each new trial, the arenas were rinsed in fresh filtered seawater and the water in the arena was replaced.



*Figure 3.* Diagram and dimensions of the plus-maze apparatus. Solid lines indicate the maze walls (15 cm height). Dashed lines depict the threshold that a crab must cross with the midpoint of its body to be considered an arm entry.

Prior to each experiment, individual crabs were placed in the centre of the plus-maze, held in a plexiglass cylinder with an open top and bottom (7.5 cm diameter, 12 cm height), and left to

acclimate for five minutes. Following acclimation, the crabs were left alone to move freely in the maze for 60 minutes while being video recorded using infrared lighting (following Ramey et al. 2009).

### *Video analyses*

#### *Boldness task*

EthoVision behavioural video analyses software (Noldus Information Technology Inc.) was used to track the movements of each crab from the video recordings of the boldness tests. Boldness was measured as the length of time elapsed prior to the midpoint of a crab's body entering the open area, with a shorter time indicating greater boldness. To account for crabs that did not enter the open area, boldness was examined in two ways, by excluding those that did not enter the open area and by assigning these crabs the maximum open area entry time of 60 minutes. Hesitancy was measured in two ways, the first as the time at which the midpoint of a crab's body first entered the open area minus the time at which the midpoint of the crab's body first entered the passageway, with a shorter time indicating low hesitancy (Brown et al. 2005). The second hesitancy measurement was the number of times that the midpoint of the crab's body entered the passageway prior to entering the open area for the first time, with a lower count indicating low hesitancy. Crabs that did not enter the open area were excluded from hesitancy data. The total number of passageway entries (i.e., entering the passageway with at least the midpoint of the body) and the total duration of time spent outside of the complex habitat (i.e., in the passageway or open area) during the trials were also recorded, excluding individuals that never exited the complex habitat.

### *Exploration task*

With the use of the EthoVision software, each time the midpoint of a crab's body crossed a threshold of 13.5 cm into an arm of the plus-maze, the selected arm (labelled A, B, C, or D) and the time at which the entry occurred were recorded (Ramey et al. 2009). In accordance with the procedures of Ramey et al. (2009), only the first of consecutive entries into the same arm was considered during data analysis (see also Lennartz 2008). For all individuals with at least 10 recorded arm entries, disregarding repeated entries into the same arm, the alternation scores were calculated as described by Ramey et al. (2009; Appendix A). A successful alternation was defined as entering all four arms of the plus-maze within five sequential arm entries, whereas five consecutive arm entries without entering the four unique arms was an unsuccessful alternation (Ramey et al. 2009). Whether each alternation was successful or unsuccessful was evaluated for each set of five sequential and overlapping arm entries (e.g., 1–5, 2–6, 3–7, etc.) (Ramey et al. 2009). For each subject, the ratio of successful alternations over the total number of alternations, successful and unsuccessful, is equal to the alternation score (Ramey et al. 2009). Crabs that selected arms in the plus-maze according to chance would receive the chance level alternation score of 0.44 (Appendix B; Ramey et al. 2009). In contrast, crabs that spontaneously alternate, tending to visit the least-recently explored maze arms, would receive scores above the chance level ( $> 0.44$ ), and those tending to re-enter arms that have been recently visited would receive scores below chance level ( $< 0.44$ ). Crab willingness to explore was also measured in two ways, the first as the number of arm choices that had been made once all four arms of the plus-maze had been entered for the first time (i.e., fewer arm entries indicate a greater willingness to explore). The second willingness to explore measurement was the time elapsed once all four arms of the plus-maze had been entered for the first time (i.e., a shorter duration indicates a greater willingness to

explore). Activity was also measured as the total number of arm choices made within the 60 minutes.

A change point algorithm was used to examine whether alternation performance in the plus-maze changed over time (i.e., acquisition patterns), following the procedures described by Balci et al. (2014). In the spontaneous alternation experiment, a change point is a point during the trial at which a change in alternation performance occurred. A positive change point represents an increase in alternation performance, a negative change point represents a decrease in alternation performance, and a lack of change points indicates that alternation performance remained the same throughout the trial. A relative-likelihood change-point algorithm was used to determine if change points occurred during each trial (Balci et al. 2009; Balci et al. 2014). For this algorithm, successful alternations (i.e., five sequential arm entries where all four maze arms were entered) were scored as 1, and unsuccessful alternations (i.e., five sequential arm entries without entering all four maze arms) as 0. The algorithm assumes that a Bernoulli distribution determines the series of successful alternations (1) and unsuccessful alternations (0) and assesses if the alternation data is better represented by a model in which the Bernoulli parameter (i.e., probability of drawing a 1) has changed or a model in which this parameter has not changed. The algorithm used a decision criterion of 50, meaning that the odds had to favour a change in likelihood by 50:1 for a change-point to be detected (Balci et al. 2009). Matlab (Mathworks, 2024b) was used for the change point algorithm.

### *Statistical analyses*

Since water temperature varied slightly among trials, two-sample t-tests were used to compare water temperature between experiments on *H. sanguineus* and *C. maenas*. Spearman's

rank order correlation tests were also used to test for correlations between behaviours and water temperature or carapace width. The Holm-Bonferroni correction for multiple comparisons was applied separately to the temperature and carapace width correlation tests for each species.

Prior to analyses, all data were tested for the assumptions of normality and equal variances using the Shapiro-Wilk test and Levene's test, respectively. To compare behaviour between species, data for boldness, the time elapsed once all four maze arms had been entered for the first time (i.e., willingness to explore), and activity were normalized with a  $\log_{10}$  transformation and compared with independent two-sample t-tests. Data for both hesitancy measurements, alternation scores, and the number of arm choices that had been made once all four maze arms had been entered for the first time (i.e., willingness to explore) could not be normalized in both species ( $\log_{10}$  or square root transformations for hesitancy and willingness to explore, arcsine square root transformation for alternation scores), and Mann-Whitney U tests were thus applied. The assumption of equal variances was not fulfilled for the data on the time spent in the open area, and a Mann-Whitney U test was thus applied to compare this between species. The Holm-Bonferroni correction was applied separately to the interspecific hesitancy and willingness to explore comparisons. Each species' spontaneous alternation scores were also compared to the chance level score of 0.44 (Ramey et al. 2009). The *H. sanguineus* scores were normalized with an arcsine square root transformation and compared to chance level with a one-sample t-test, but the *C. maenas* scores could not be normalized with this transformation and a one-sample Wilcoxon signed rank test was thus applied. An alpha level of 0.05 was used for all relevant statistical tests. Effect sizes were measured using Cohen's *d* for t-tests and rank-biserial correlation (RBC) for Mann-Whitney U and Wilcoxon signed rank tests. Statistical analyses were conducted using jamovi (version 2.3.28) software.

## Results

### *Temperature and carapace width*

There was no significant difference in seawater temperature between species for the boldness (*H. sanguineus*:  $20.1^{\circ}\text{C} \pm \text{SE } 0.31$ ,  $n = 26$ ; *C. maenas*:  $20.2^{\circ}\text{C} \pm \text{SE } 0.24$ ,  $n = 29$ ;  $t(53) = -0.0857$ ,  $p = 0.932$ ) or exploration experiments (*H. sanguineus*:  $19.8^{\circ}\text{C} \pm \text{SE } 0.22$ ,  $n = 38$ ; *C. maenas*:  $20.0^{\circ}\text{C} \pm \text{SE } 0.22$ ,  $n = 40$ ;  $t(76) = 0.543$ ,  $p = 0.589$ ). Temperature and carapace width were not correlated with any behaviours in either species (Table C1).

### *Boldness task*

In the boldness experiment, a similar proportion of each species did not enter the open area (eight crabs of each species,  $\sim 31\%$  of tested *H. sanguineus* and  $\sim 28\%$  of tested *C. maenas*), and these crabs were excluded from subsequent statistical analyses to compare boldness and hesitancy between those that entered the open area. Thus, 18 *H. sanguineus* (CW  $17.55 \pm \text{SE } 0.61$  mm) and 21 *C. maenas* (CW  $22.05 \pm \text{SE } 1.06$  mm) were included in the boldness and hesitancy analyses. Boldness was not significantly different among species ( $t(37) = -0.821$ ,  $p = 0.417$ ; Fig. 4), and these results remained the same if crabs that did not enter the open area were included in the analysis, by assigning them an open area entry time of 60 minutes ( $U = 335$ ,  $p = 0.479$ ). For hesitancy, there was no significant difference among species in the time between the first passageway and first open area entries ( $U = 167$ ,  $p = 0.544$ ; Fig. 5) or the number of passageway entries prior to the first open area entry (*H. sanguineus*:  $1.17 \pm \text{SE } 0.09$ ,  $n = 18$ ; *C. maenas*:  $1.10 \pm \text{SE } 0.10$ ,  $n = 21$ ;  $U = 168$ ,  $p = 0.544$ ).

Three crabs of each species never entered the open area but spent time in the passageway. These crabs were included in the analyses for the total number of passageway entries and time spent outside of the complex habitat, such that 21 *H. sanguineus* (CW  $17.80 \pm SE 0.56$  mm) and 24 *C. maenas* (CW  $22.14 \pm SE 0.93$  mm) were included in these analyses. Over the entire trial duration, *C. maenas* entered the passageway more times than *H. sanguineus* (*H. sanguineus*:  $4.14 \pm SE 0.97$ ,  $n = 21$ ; *C. maenas*:  $11.00 \pm SE 2.70$ ,  $n = 24$ ;  $t(43) = -2.05$ ,  $p = 0.046$ , Cohen's  $d = 0.61$ ; Fig. 6). Additionally, *C. maenas* spent more time outside of the complex habitat than *H. sanguineus* (*H. sanguineus*:  $480.14 \pm SE 108.17$  s,  $n = 21$ ; *C. maenas*:  $1324.67 \pm SE 215.64$  s,  $n = 24$ ;  $U = 131$ ,  $p = 0.006$ , RBC = 0.48; Fig. 7).

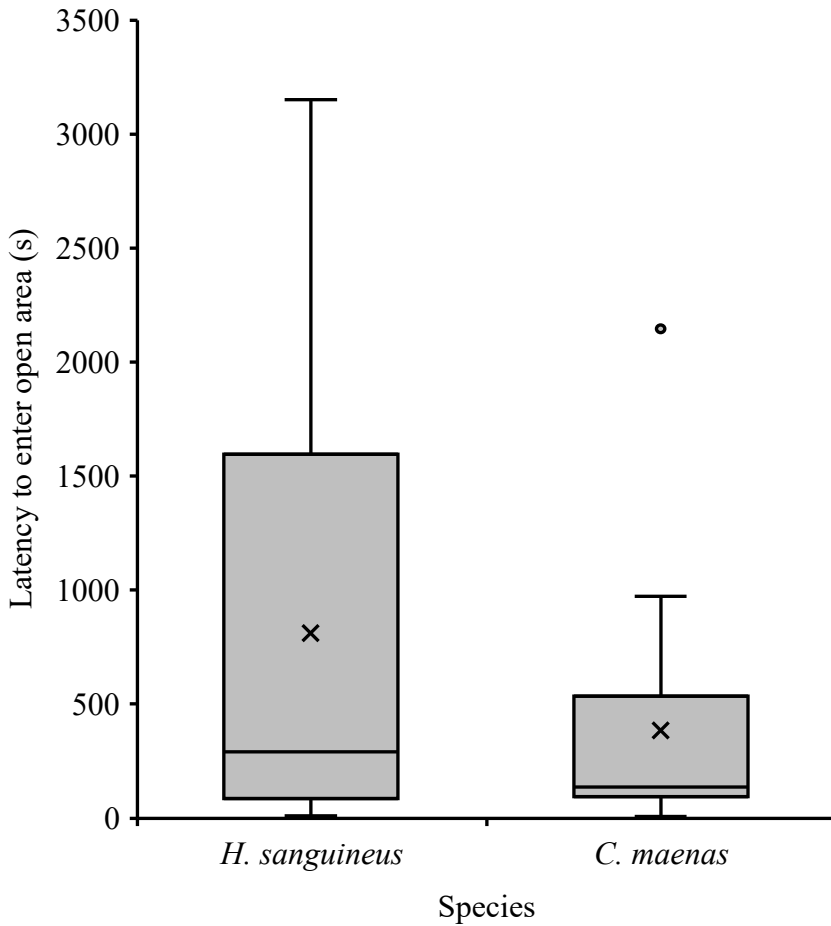


Figure 4. Boldness (i.e., latency to enter open area of the boldness test arena) of male adult *Hemigrapsus sanguineus* ( $n = 18$ ) and juvenile *Carcinus maenas* ( $n = 21$ ). Lower and upper box boundaries are the 25<sup>th</sup> and 75<sup>th</sup> percentiles and lower and upper error lines are the 10<sup>th</sup> and 90<sup>th</sup> percentiles, respectively. Medians are lines inside each box, means are an x, and circles are outliers.

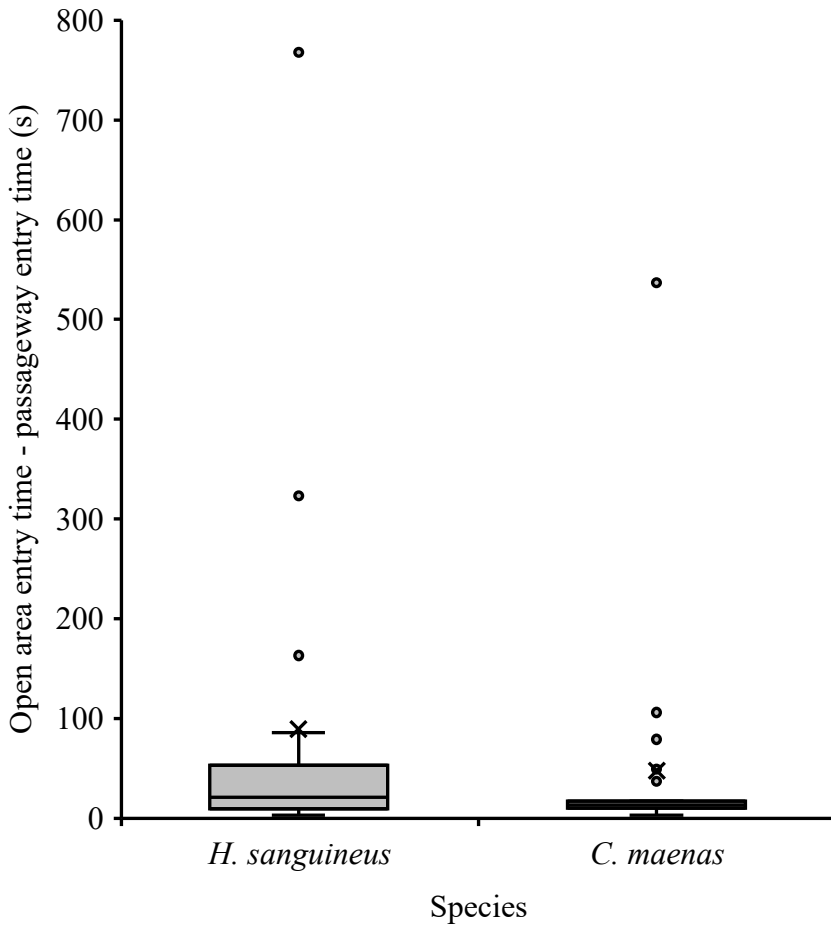


Figure 5. Hesitancy (i.e., duration between the first passageway entry and the first open area entry) of male adult *Hemigrapsus sanguineus* ( $n = 18$ ) and juvenile *Carcinus maenas* ( $n = 21$ ) in the boldness experiment. Lower and upper box boundaries are the 25<sup>th</sup> and 75<sup>th</sup> percentiles and lower and upper error lines are the 10<sup>th</sup> and 90<sup>th</sup> percentiles, respectively. Medians are lines inside each box, means are an x, and circles are outliers.

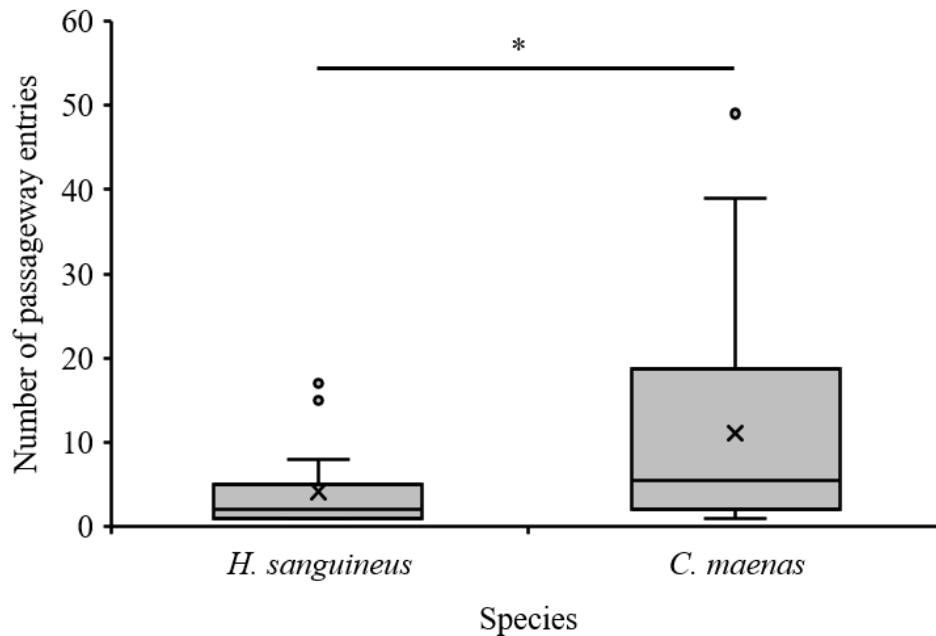


Figure 6. Number of times male adult *Hemigrapsus sanguineus* ( $n = 21$ ) and juvenile *Carcinus maenas* ( $n = 24$ ) exited the complex habitat to enter the passageway in the boldness arena during the 60-minute trials. The asterisk represents a significant difference between species. Lower and upper box boundaries are the 25<sup>th</sup> and 75<sup>th</sup> percentiles and lower and upper error lines are the 10<sup>th</sup> and 90<sup>th</sup> percentiles, respectively. Medians are lines inside each box, means are an x, and circles are outliers.

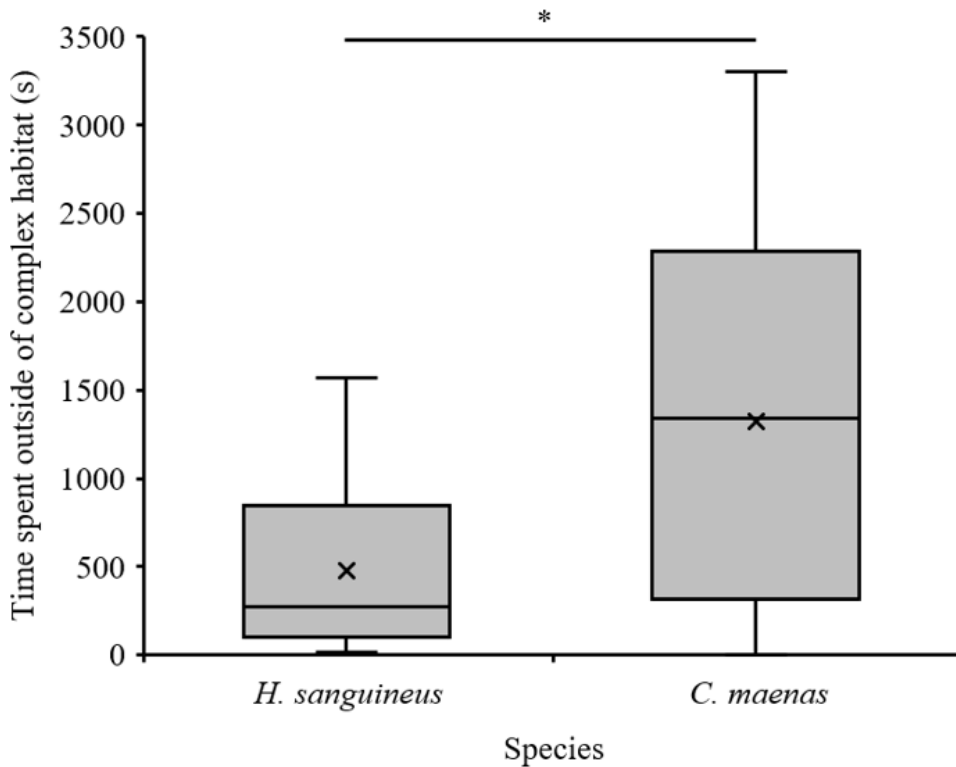


Figure 7. Time spent outside of the complex habitat in the boldness arena by male adult *Hemigrapsus sanguineus* ( $n = 21$ ) and juvenile *Carcinus maenas* ( $n = 24$ ) in the 60-minute boldness experiment. The asterisk represents a significant difference between species. Lower and upper box boundaries are the 25<sup>th</sup> and 75<sup>th</sup> percentiles and lower and upper error lines are the 10<sup>th</sup> and 90<sup>th</sup> percentiles, respectively. Medians are lines inside each box, means are an x, and circles are outliers.

#### Exploration task

In the plus-maze experiment, seven *H. sanguineus* (~18%) and five *C. maenas* (~13%) selected fewer than 10 arms and were thus excluded from subsequent statistical analyses, such that 31 *H. sanguineus* (CW  $18.10 \pm SE 0.50$  mm) and 35 *C. maenas* (CW  $21.60 \pm SE 0.73$  mm) were included. The mean *H. sanguineus* alternation score ( $0.73 \pm SE 0.03$ ,  $n = 31$ ) was greater

than chance level ( $t(30) = 8.31, p < 0.001$ , Cohen's  $d = 1.49$ ; Fig. 8) and the *C. maenas* alternation scores ( $0.65 \pm SE 0.03, n = 35$ ) were also greater than chance level ( $W = 584, p < 0.001$ , RBC = 0.85; Fig. 8). The alternation scores of *H. sanguineus* were also greater than those of *C. maenas* ( $U = 370, p = 0.027$ , RBC = 0.32). In terms of willingness to explore, *H. sanguineus* required fewer arm choices to select all four maze arms for the first time (*H. sanguineus*:  $5.55 \pm SE 0.28, n = 31$ ; *C. maenas*:  $8.54 \pm SE 1.21, n = 35$ ;  $U = 287, p = 0.002$ , RBC = 0.47; Fig. 9A), but there was no significant difference in the time required to enter all four arms for the first time ( $t(64) = 0.295, p = 0.769$ ; Fig. 9B). On average, *C. maenas* was more active than *H. sanguineus* (*H. sanguineus*:  $21.26 \pm SE 1.75, n = 31$ ; *C. maenas*:  $52.91 \pm SE 4.81, n = 35$ ;  $t(64) = 6.23, p < 0.001$ , Cohen's  $d = 1.54$ ; Fig. 10).

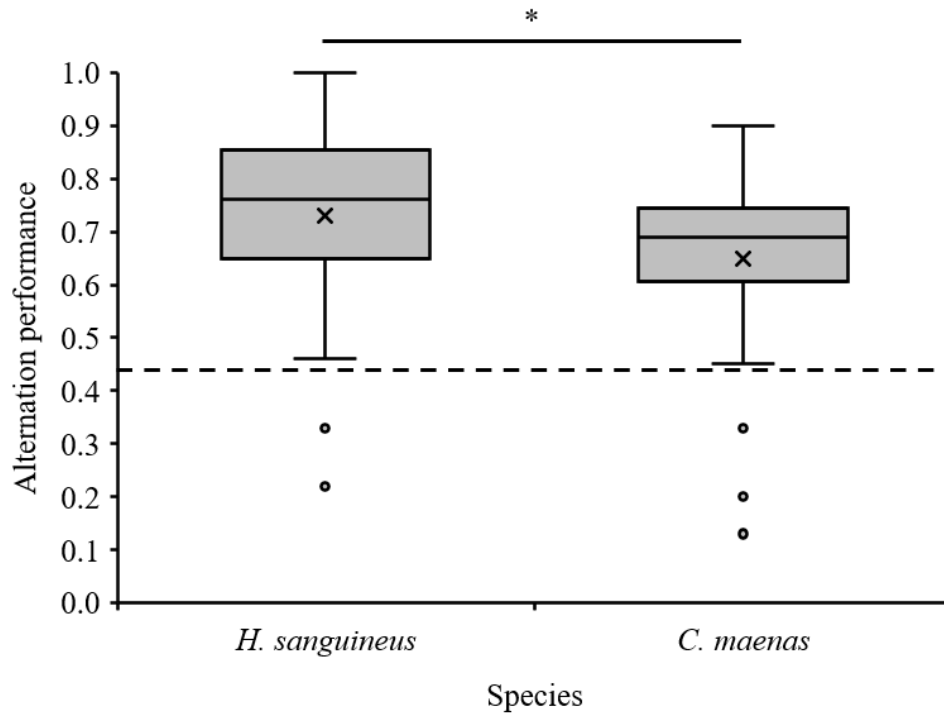


Figure 8. Alternation performances (i.e., number of successful alternations/total number of successful and unsuccessful alternations) of male adult *Hemigrapsus sanguineus* ( $n = 31$ ) and juvenile *Carcinus maenas* ( $n = 35$ ) in a plus-maze. The dashed line represents the chance level alternation score (0.44). The asterisk represents a significant difference between species. Lower and upper box boundaries are the 25<sup>th</sup> and 75<sup>th</sup> percentiles and lower and upper error lines are the 10<sup>th</sup> and 90<sup>th</sup> percentiles, respectively. Medians are lines inside each box, means are an x, and circles are outliers.

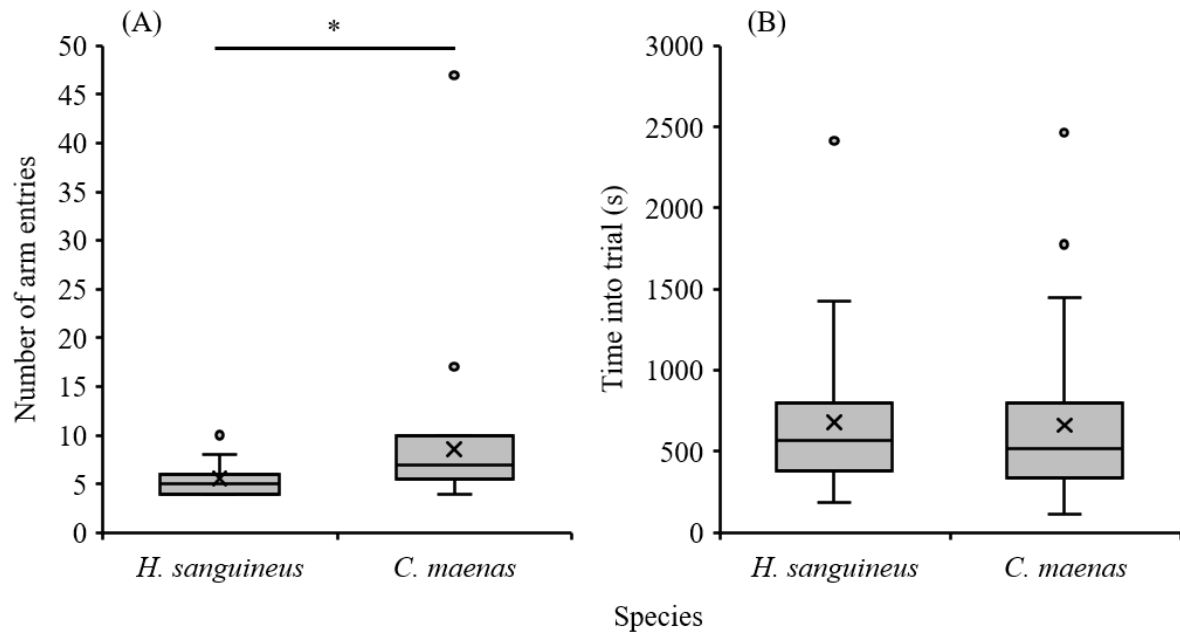


Figure 9. Willingness to explore (i.e., A: number of arm choices made once all four unique arms were entered for the first time in the plus-maze; B: time elapsed once all four unique arms were entered for the first time in the plus-maze) of male adult *Hemigrapsus sanguineus* ( $n = 31$ ) and juvenile *Carcinus maenas* ( $n = 35$ ). The asterisk represents a significant difference between species. Lower and upper box boundaries are the 25<sup>th</sup> and 75<sup>th</sup> percentiles and lower and upper error lines are the 10<sup>th</sup> and 90<sup>th</sup> percentiles, respectively. Medians are lines inside each box, means are an x, and circles are outliers.

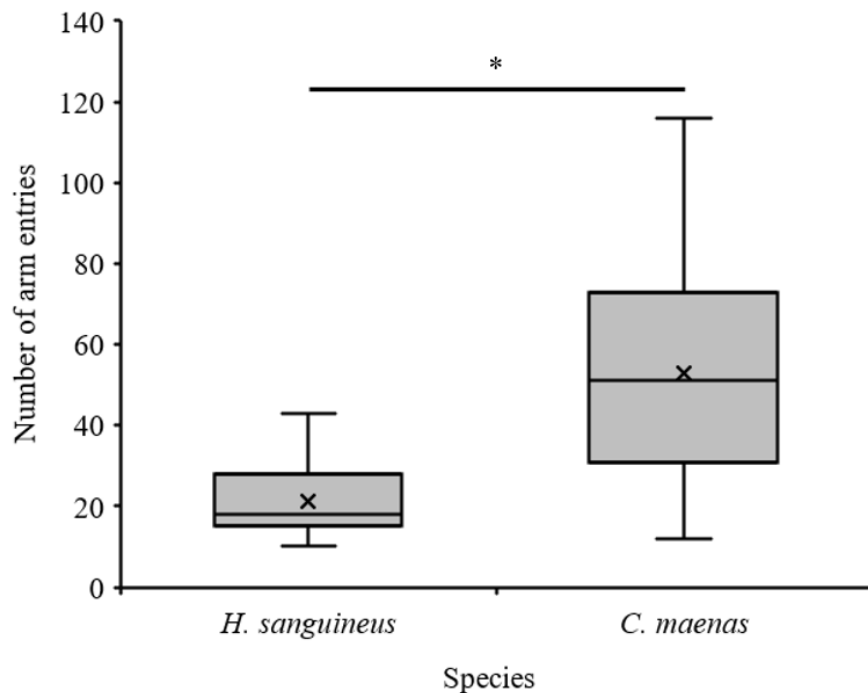


Figure 10. Activity (i.e., total number of arm choices made during 60 minutes in a plus-maze) of male adult *Hemigrapsus sanguineus* ( $n = 31$ ) and juvenile *Carcinus maenas* ( $n = 35$ ). The asterisk represents a significant difference between species. Lower and upper box boundaries are the 25<sup>th</sup> and 75<sup>th</sup> percentiles and lower and upper error lines are the 10<sup>th</sup> and 90<sup>th</sup> percentiles, respectively. Medians are lines inside each box, means are an x, and circles are outliers.

The majority (83.9%) of *H. sanguineus* spontaneous alternation trials did not have change points (i.e., the individuals had consistent alternation performances throughout the trials; Table 1). A single positive change point (i.e., improvement in alternation performance) occurred in 12.9% of *H. sanguineus* and a single negative change point (i.e., decrease in alternation performance) in 3.2% of *H. sanguineus* (Table 1). No *H. sanguineus* individuals had more than one change point during the trial (Table 1). For *C. maenas*, 45.7% of individuals had no change points, 34.3% had a single positive change point, 2.9% had a single negative change point, and 17.1% had more than one change point (Table 1).

Table 1. The change points detected in the spontaneous alternation performance of male adult *Hemigrapsus sanguineus* ( $n = 31$ ) and juvenile *Carcinus maenas* ( $n = 35$ ) during 60 minutes of exploration in a plus-maze. A positive change point signifies an improvement in spontaneous alternation performance and a negative change point signifies a decrease in performance.

	None	Positive	Negative	Positive followed by negative	Negative followed by positive	$\geq 3$ change points
<i>H. sanguineus</i>	26	4	1	0	0	0
<i>C. maenas</i>	16	12	1	1	1	4

## Discussion

The tendency of *H. sanguineus* and *C. maenas* to spontaneously alternate (i.e., alternation scores greater than chance level), suggests that both invasive crabs are efficient explorers. In contrast, native blue crabs and fiddler crabs haven't been found to spontaneously alternate (Ramey et al. 2009; Balci et al. 2014), supporting the link between exploratory behaviour and invasiveness (e.g., mosquitofish, *Gambusia* spp.: Rehage and Sih 2004; house geckos, *Hemidactylus frenatus* and *Gehyra dubia*: Nordberg et al. 2021). Both crabs consume high volumes of macroalgal and invertebrate prey (DeGraaf and Tyrrell 2004; Howard et al. 2018; Saborowski et al. 2023), much of which is sessile or could have patchy distributions (Miller and Ambrose 2000), and a strong exploration ability could increase the rate of encountering these resources. However, *H. sanguineus* explored more efficiently than *C. maenas*, due to having a greater alternation performance and making fewer arm choices before entering all four maze

arms for the first time. The alternation performances of most (83.9%) *H. sanguineus* were also consistent throughout the trial, which was not the case for *C. maenas*, indicating that *H. sanguineus* explored efficiently in a novel environment and continued to do so over time. The consistent exploratory behaviour of *H. sanguineus* might improve the species' ability to find new resources, and thus to outcompete *C. maenas* for preferred prey items, particularly when their availability is limited. Even without resource limitations, the consumption of preferred mussel prey by *C. maenas* was negatively related to *H. sanguineus* density in field cage experiments (Griffen et al. 2008). Conversely, *C. maenas* density did not affect mussel consumption by *H. sanguineus* and neither species' consumption was impacted by conspecific density (Griffen et al. 2008). Decreased availability of preferred prey such as mussels could impair energy storage (i.e., hepatopancreas mass relative to body weight, Griffen et al. 2011), growth (Griffen et al. 2008), and reproduction in *C. maenas* (Griffen et al. 2011; Griffen 2016). Increased competition for prey may thus negatively impact *C. maenas* population sizes, which may be relevant in explaining the coincident decline in *C. maenas* abundance with *H. sanguineus* population growth reported in New England, USA (Lohrer and Whitlatch 2002).

Although *C. maenas* was a less efficient explorer, it was more active than *H. sanguineus*, exploring all four maze arms within a similar amount of time, despite requiring a greater number of arm choices to do so. In the boldness experiment, *C. maenas* also exited the complex habitat more frequently than *H. sanguineus*, which could indicate greater activity. Higher activity levels might help *C. maenas* compensate for a lower exploration efficiency than *H. sanguineus* when searching for resources, though this would likely increase energy expenditure while exploring. Compared to *H. sanguineus*, *C. maenas* also tends to consume more high-energy animal prey relative to vegetation (Saborowski et al. 2023), which would be beneficial for maintaining high

activity levels. However, our findings that *C. maenas* is more active than *H. sanguineus* contrast with the results of Jungblut et al. (2018), who measured the respiratory activity of *H. sanguineus* and *C. maenas* from Helgoland, Germany and found that *H. sanguineus* was likely the more active species (i.e., had a median respiration rate closer to the species' maximal respiration rate). Though, in other decapods, activity has also been found to vary among populations collected from different locations (e.g., virile crayfish, *Faxonius virilis*, Reisinger et al. 2020).

Interestingly, the mean alternation score for juvenile *C. maenas* of 0.65 ( $\pm SE$  0.03) is similar to the scores reported for adult *C. maenas* collected from New Jersey, USA (0.62, Ramey et al. 2009; 0.67, Balci et al. 2014), suggesting that the tendency to spontaneously alternate is a repeatable characteristic of this species, and that it is consistent across life stages. However, in the current experiment, less than half (45.7%) of *C. maenas* (juvenile males from Nova Scotia) had consistent alternation performances throughout the trial durations, compared to 86.7% of adult *C. maenas* (males and females) from New Jersey (Balci et al. 2014). Furthermore, 34.3% of *C. maenas* in the current study showed an improvement in alternation performance, compared to just 13.3% of individuals in the study of Balci et al. (2014).

Since both crabs are invasive in several regions around the globe (Carlton and Cohen 2003; Blakeslee et al. 2017; Bouwmeester et al. 2019), and boldness can be associated with invasiveness (Rehage and Sih 2004), it is reasonable that the species could be similarly bold. However, although not statistically significant, *C. maenas* was on average approximately two times faster at leaving the shelter of the complex habitat than *H. sanguineus*. Moreover, previous experiments examining juvenile *C. maenas* from Nova Scotia in less ecologically relevant conditions (i.e., a dark container as shelter rather than rocks) also found that *C. maenas* had a significantly shorter latency ( $\sim 1.7$  times as fast on average) to exit a dark shelter and enter a

brightly lit open area than *H. sanguineus* (DeJaegher et al. in prep). Notably, the mean latencies herein were very similar to the previous study (*H. sanguineus*: 812 s,  $n = 21$  vs. 822 s,  $n = 31$ , respectively; *C. maenas*: 385 s,  $n = 24$  vs. 475 s,  $n = 34$ , respectively). The smaller sample size used in the present study in addition to the high variability of crab behaviour may have contributed to the lack of a statistically significant trend.

In the boldness arena, *C. maenas* also exited the complex habitat more frequently and spent a greater amount of time in the open compared to *H. sanguineus*. Similarly, the previous preliminary experiments reported that the mean time spent outside of a shelter in an open area was significantly greater in *C. maenas* than in *H. sanguineus* (DeJaegher et al. in prep). Since the survival rate of juvenile *C. maenas* when exposed to adult *C. maenas* predation has been shown to be positively associated with habitat complexity (Gehrels et al. 2017), spending more time in the open area is risky and may also be considered boldness. The survival of *H. sanguineus*, which also prefers structurally complex habitat (Hobbs et al. 2017b), should also be favoured by spending more time under shelter. However, though both crabs prefer structurally complex habitat, *C. maenas* might be less dependent on this habitat than *H. sanguineus*. When adult *H. sanguineus* and juvenile *C. maenas* from Nova Scotia were tethered in a sandy intertidal area, video footage showed that *C. maenas* burrowed in the sand to escape predation by adult *C. maenas*, but *H. sanguineus* did not (Juenke and Ramey-Balci, 2024). This burrowing behaviour has also been observed in juvenile *C. maenas* from Prince Edward Island, Canada (Gehrels et al. 2017). Furthermore, in experiments where crabs were held alone in containers with sandy substrate and offered a bivalve (*Macoma balthica*) shell as shelter, nearly all *H. sanguineus* used the shelter, compared to just 42% of *C. maenas* (Jensen et al. 2002).

In the natural environment, the greater tendency of *C. maenas* to leave rocky shelter could be amplified in the presence of competitors (Jensen et al. 2002; Hobbs et al. 2017b). Previously, *H. sanguineus* was found to tolerate co-occurring with conspecifics in preferred cobble habitat, but *C. maenas* avoided close co-occurrence with conspecific or heterospecific competitors (Hobbs et al. 2017b). High crab densities might thus drive *C. maenas* to explore new regions or occupy more open habitat. In fact, juvenile *C. maenas* from Maine, USA that did not co-occur with the shore crabs *H. sanguineus* and *H. oregonensis* were primarily (> 97%) found under rocks during field sampling, compared to approximately 20% of *C. maenas* that co-occurred with shore crabs (in California, New Jersey, and Massachusetts, USA; Jensen et al. 2002). Furthermore, the presence of a similarly sized *H. sanguineus* competitor decreased the use of a bivalve shell as shelter by *C. maenas* from 42 to 6.6% of individuals, whereas *C. maenas* did not affect *H. sanguineus* shelter use (Jensen et al. 2002). With increasing density, *H. sanguineus*' ability to compete for shelter also seems to improve, since the crab was shown to better exclude juvenile lobsters (*Homarus americanus*), an intraguild competitor, from shelters at higher population densities (Baillie and Grabowski 2018).

Overall, *H. sanguineus*' behaviour suggests that the species spends less time outside of shelter, but explores more efficiently when doing so, compared to *C. maenas*. These behaviours could be advantageous if they favour survival while still allowing *H. sanguineus* to efficiently find resources. Future research should thus investigate if, in these species, relationships exist between boldness or exploration and resource acquisition or survival. These traits should also be compared among crabs from different sites, to understand the extent to which they are influenced by phenotypic plasticity or genotypic variation. In other species, boldness can vary among populations (e.g., signal crayfish: Pintor et al. 2008; virile crayfish: Reisinger et al. 2020) and be

affected by factors such as population density during development (red swamp crayfish, *Procambarus clarkii*: Su et al. 2024). In *C. maenas*, competitive ability when foraging for a limited food resource also varied among crabs from Newfoundland, Canada compared to those from Nova Scotia and New Brunswick, Canada (Rossong et al. 2012). Among-population variation in behaviour could affect whether the results of this study apply to other regions where these crabs co-occur, such as in the USA, where both species are invasive (Carlton and Cohen 2003; Blakeslee et al. 2017), and in Europe, where *H. sanguineus* has invaded *C. maenas*' native range (Breton et al. 2002; Bouwmeester et al. 2019). Furthermore, the behaviour of juvenile *H. sanguineus*, which also co-occur with juvenile *C. maenas* (Ramey-Balci et al. in prep; Kraemer 2019), and females of either species should be considered. It would also be valuable to understand how boldness and exploration are influenced by the presence of predators, prey, or competitors, to better understand how these traits might influence resource acquisition and competition in the natural environment.

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## Appendix A: Spontaneous alternation score calculation

Calculation of spontaneous alternation scores, following the procedure of Ramey et al. (2009):

Successful alternation (1): Entering all four arms of a plus-maze in five consecutive arm entries.

Unsuccessful alternation (0): Five consecutive arm entries without entering all four arms of a plus-maze.

Each consecutive and overlapping run of five arm choices (e.g., 1–5, 2–6, 3–7, etc.) will be scored as either a successful (1) or an unsuccessful (0) alternation. The number of successful alternations will be divided by the total number of alternations (i.e., sum of successful and unsuccessful alternations).

Example arm choices: C-D-C-B-A-B-C-A-D-A

Scoring:

110011

$4/6 = 0.67/1$

Alternation score of 0.67

## Appendix B: Chance level alternation score calculation

Calculation of the chance level alternation score for a plus-maze using partial probabilities, following the procedure of Ramey et al. (2009):

To begin, the probability of entering all four arms of a plus-maze during five consecutive arm entries (i.e., completing a successful alternation) by chance is calculated. When selecting arms in the maze, arm choice one is always novel (4/4) and arm choice two is always novel (3/3), because re-entries into the same arm are disregarded. Arm choice three then has a 2/3 probability of being novel (a re-entry into arm choice two would be disregarded). If arm choice three is novel, arm choice four has a 1/3 probability of being novel (a re-entry into arm choice three would be disregarded). If arm choice four is novel, any arm can be selected for choice five and all four novel arms will have still been entered. The partial probability of entering four novel arms in this way is  $(4/4)(3/3)(2/3)(1/3)(1) = 0.22$ . If arm choice one, two, and three are novel, arm choice four could also be non-novel (2/3), and arm choice five could then be novel (1/3). The partial probability of entering four novel arms in this way is  $(4/4)(3/3)(2/3)(2/3)(1/3) = 0.15$ . After two novel arm choices, arm choice three could also be non-novel (1/3), and arm choice four would then have a 2/3 probability of being novel, and arm choice five would have a 1/3 probability of being novel. The partial probability of entering four novel arms in this way is  $(4/4)(3/3)(1/3)(2/3)(1/3) = 0.07$ . The chance level alternation score is thus calculated as  $0.22 + 0.15 + 0.07 = 0.44$ . If crabs enter arms by chance, then approximately 44% of their alternations should be successful, providing the chance level alternation score of 0.44.

### Appendix C: Relationships between temperature and carapace width and behaviours

*Table 1.* Results of Spearman's rank order correlation tests for relationships between temperature and carapace width and the behaviours of male adult *Hemigrapsus sanguineus* and juvenile *Carcinus maenas*. Hesitancy (time) is the duration between the first passageway entry and the first open area entry. Hesitancy (count) is the number of passageway entries prior to entering the open area. Willingness to explore (time) is the time elapsed once all four plus-maze arms had been entered for the first time. Willingness to explore (count) is the total number of arm choices that had been made once all four plus-maze arms had been entered for the first time. Corrected  $p$ -values were calculated using Holm-Bonferroni corrections, which were applied separately to the temperature and carapace width correlations for each species. Alpha is equal to 0.05.

Species	Variable 1	Variable 2	DF	$\rho$	$p$	Corrected $p$
<i>H. sanguineus</i>	Temperature	Boldness	16	-0.339	0.169	1.000
		Hesitancy (time)	16	-0.538	0.021	0.189
		Hesitancy (count)	16	-0.432	0.073	0.584
		Time spent outside of the complex habitat	19	0.302	0.184	0.920
		Number of times exiting the complex habitat	19	0.367	0.102	0.714
		Alternation score	29	-0.148	0.427	1.000
		Willingness to explore (time)	29	0.088	0.639	1.000
		Willingness to explore (count)	29	-0.059	0.752	0.752
		Activity	29	-0.073	0.698	1.000

Table 1 (Continued).

Species	Variable 1	Variable 2	DF	$\rho$	$p$	Corrected $p$
<i>H. sanguineus</i>	Carapace width	Boldness	16	-0.212	0.398	1.000
		Hesitancy (time)	16	-0.297	0.231	0.924
		Hesitancy (count)	16	0.014	0.955	0.955
		Time spent outside of the complex habitat	19	-0.444	0.045	0.405
		Number of times exiting the complex habitat	19	-0.297	0.191	0.955
		Alternation score	29	-0.277	0.131	0.786
		Willingness to explore (time)	29	0.083	0.657	1.000
		Willingness to explore (count)	29	0.311	0.089	0.712
		Activity	29	0.279	0.128	0.896
<i>C. maenas</i>	Temperature	Boldness	19	-0.127	0.582	1.000
		Hesitancy (time)	19	-0.427	0.053	0.477
		Hesitancy (count)	19	-0.148	0.522	1.000
		Time spent outside of the complex habitat	22	-0.116	0.589	1.000
		Number of times exiting the complex habitat	22	-0.016	0.940	0.940
		Alternation score	33	-0.155	0.374	1.000
		Willingness to explore (time)	33	-0.146	0.404	1.000

Table 1 (Continued).

Species	Variable 1	Variable 2	DF	$\rho$	$p$	Corrected $p$
<i>C. maenas</i>	Temperature	Willingness to explore (count)	33	0.028	0.871	1.000
		Activity	33	0.043	0.807	1.000
	Carapace width	Boldness	19	-0.219	0.338	1.000
		Hesitancy (time)	19	-0.363	0.105	0.945
		Hesitancy (count)	19	-0.074	0.750	1.000
		Time spent outside of the complex habitat	22	-0.168	0.431	1.000
		Number of times exiting the complex habitat	22	-0.064	0.795	1.000
		Alternation score	33	0.211	0.224	1.000
		Willingness to explore (time)	33	0.079	0.654	1.000
		Willingness to explore (count)	33	0.007	0.967	0.967
		Activity	33	-0.169	0.333	1.000