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Research Article

Aliens eating aliens: an introduced amphipod as a potential prey of an invasive rocky shore crab in laboratory experiments

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Abstract

Behavioural interactions between introduced predators and introduced prey are still largely underestimated. The present work takes advantage of the co-occurrence of two introduced species, the Asian shore crab *Hemigrapsus sanguineus* and the amphipod *Ptilohyale littoralis*, respectively first recorded on rocky shores along the French coast of the eastern English Channel in 2005 and 2016. In this context, the predation by male and female *H. sanguineus* on *P. littoralis* was examined under controlled laboratory conditions, by presenting either juveniles of the blue mussel *Mytilus edulis* or adult *P. littoralis* to *H. sanguineus*. We subsequently assessed the potential prey preference of the Asian shore crab for *P. littoralis* and *M. edulis* by presenting the two prey items simultaneously in the same proportion. In the absence of choice, male *H. sanguineus* preyed significantly more on *M. edulis* than *P. littoralis*. In contrast, females preyed significantly less on *M. edulis* than *P. littoralis*; however, male and female *H. sanguineus* consumed similar numbers of *P. littoralis*. When choice was possible between *P. littoralis* and *M. edulis*, the crab did not exhibit preference *stricto sensu* for any type of prey. These results suggest that the Asian shore crab cannot be considered as a naive predator when confronted to a newly introduced prey. Our results also suggest that the amphipod *P. littoralis* did not exhibit any effective antipredator response towards the crab. These observations nevertheless warrant further work on the effects of abiotic factors (e.g. temperature) as well as other biotic interactions (e.g. presence of other prey or predators for *H. sanguineus*) may have on the observed prey-predator interactions between *H. sanguineus* and *M. edulis* and *P. littoralis*.

Key words: Asian shore crab, *Hemigrapsus sanguineus*, amphipod, *Ptilohyale littoralis*, predation, behaviour

Introduction

Behaviour is one of the most critical components in the success of introduced species to become invasive (Holway and Suarez 1999; Chapple et al. 2012). More specifically, behavioural flexibility is thought to facilitate the invasion process, particularly during its early stages (Wright et al. 2010). In the last two decades,

numerous studies have documented the importance of behavioural traits in the invasion success of both invertebrates and vertebrates in both terrestrial and aquatic ecosystems; see Chapple et al. (2012) and Chapple and Wong (2016) for reviews. In aquatic ecosystems, crustaceans are considered as one of the most successful groups of invasive species (Hänfling et al. 2011), and both their feeding behaviour and the nature of the subsequent predator-prey interactions appear particularly relevant to their success (Weis 2010, 2016). The predatory behaviour of invasive predators is well documented (Sih et al. 2010a; Grosholz and Wells 2016), and this holds for crustaceans (Weis 2010, 2016). In contrast, the role behaviour may have in mediating the success of invasive prey species is still a relatively untapped area of research, despite evidence that the role of behaviour in the success of invasive prey may be more important than the behaviour of native predators (Pintor and Byers 2015; Mennen and Lakowski 2018; Papacostas and Freestone 2019).

Based on the lack of co-evolution history between introduced species (either predator or prey) and their native counterparts, a range of evolutionary hypotheses has been introduced to describe novel predator-prey interactions; see e.g. Grosholz and Wells (2016) for a review. These hypotheses are essentially based on the dichotomy between the preference of both native and non-native predators towards evolutionary familiar or unfamiliar prey. Native predators may consume more of a native prey species than an invasive one, because they fail to recognize an invader (Enemy Release Hypothesis; Torchin and Mitchell 2004) and/or they are unable to overcome their defense (Novel Defense Hypothesis; Elton 1958) or because of ineffective antipredator behaviour by naive prey (Naive Prey Hypothesis; Sih et al. 2010a). Invasive predators may also favour evolutionarily familiar invasive prey species over native or unfamiliar invasive prey species (Facilitation Between Invaders Hypothesis; Grosholz and Wells 2016). Alternatively, native predators may prefer evolutionary novel non-native prey over familiar ones (Biotic Resistance Hypothesis; Elton 1958), and invasive predators may consume more of an evolutionarily novel prey (either native of from another non-native region) than evolutionary familiar ones, hence be at competitive advantage (Novel Weapons Hypothesis; Callaway and Ridenour 2004). Other hypotheses related to the Optimal Foraging Theory (Pyke 1984) and Optimal Diet Theory (Sih and Christensen 2001) respectively state that a predator consumes the prey that is the easiest to capture and the most profitable, regardless of evolutionary or historical familiarity.

The nature of the abovementioned predator-prey interactions is, however, noticeably both species- and location-dependent, and may also vary along the invasion process due to a potential loss of naiveté (Grosholz and Wells 2016). It is, however, essential to the understanding of the impact of invasions on ecosystem structure and function to examine the behaviour of invasive species in their different introduction areas, since behavioural flexibility may lead a given species to express different behavioural variants in distinct ecological conditions (Wright et al. 2010). In this context, the present work takes advantage of the recently documented (Spilmont et al. 2018) co-occurrence of the Asian shore crab *Hemigrapsus sanguineus* and the North American amphipod *Ptilohyale littoralis* along the French coast of the eastern English Channel. Studies on predation and prey selection by the Asian shore crab showed that this species is omnivorous, though it exhibits a preference towards animal prey (Brousseau and Baglivo 2005). In particular, juveniles of the blue mussel *Mytilus edulis* are actively selected (Bouwmeester et al. 2020), including over other invertebrates both in field and laboratory experiments (Lohrer and Whitlatch 2002). In addition, amphipods are known to be a recurrent component of the diet of *H. sanguineus* (McDermott 1999; Lohrer et

al. 2000; Blasi and O'Connor 2016). The present study assesses, based on experiments run under controlled laboratory conditions, (i) if *Hemigrapsus sanguineus* is actually able to prey on *Ptilohyale littoralis* from the same introduced area, and (ii) if *H. sanguineus* exhibits a preference between *P. littoralis* and the blue mussel *Mytilus edulis*, known as one of its preferred prey.

Methods

Study species

Hemigrapsus sanguineus (De Haan, 1835) is a native species of the North-West Pacific coast which has been reported along the North Atlantic European coast in the late 1990's (Breton et al. 2002) and up north on the Opal Coast circa 2005 (Dauvin et al. 2009). Populations now extend from Normandy up to the coast of Sweden (Gothland et al. 2013; Jungblut et al. 2017) and the species has been recorded in Great Britain (Seeley et al. 2015). The biology of *H. sanguineus* outside its native range is well documented, especially in North America (see Epifanio 2013 for a review) where it has first been recorded in New Jersey in the late 1980's (Williams and McDermott 1990). In contrast, far less is known about European *H. sanguineus*, especially regarding its behaviour (Spilmont et al. 2015).

The North American amphipod *Ptilohyale littoralis* (Stimpson, 1853) was first reported in Europe in the Port of Rotterdam in 2009, where it is believed to have been introduced through ballast water and/or hull fouling, and subsequently at the mouth of the Westerschelde estuary and in Yerseke (Faasse 2014). *P. littoralis* has recently been recorded at a site colonized by *H. sanguineus* in Wimereux, France (Spilmont et al. 2018), where it was consistently found within beds of the common blue mussel *Mytilus edulis*.

Animal collection and maintenance

Animals were collected during early spring (March-April 2016) in the mid-intertidal zone of the rocky reef "Fort de Croy" located in Wimereux, France (50°45.766'N, 1°35.962'E). Adults of *H. sanguineus* were collected manually, brought back to the nearby laboratory (LOG, Station Marine de Wimereux), sex determined, and males and non-ovigerous females were kept in separate tanks (52 cm × 46 cm × 35 cm) containing rocks from the sampling site, with running natural seawater at *in situ* temperature (11 °C) under a natural day/night cycle. Food (commercial fish pellets) was provided *ad libitum* but, to standardize hunger levels, crabs were starved for 24 h prior to experiments.

During the low tide preceding each experiment, sediment was collected at the same sampling site. Following Blasi & O'Connor (2016), sediment containing amphipods was placed into a bucket filled with seawater and swirled by hand to cause amphipods to swim up into the water. The water was then sieved (1 mm mesh-size) and visually inspected for the presence of *Ptilohyale littoralis*. Living individuals were carefully collected with forceps and checked under binoculars. Undamaged large individuals of similar sizes (10–12 mm length) were then kept in the laboratory in PVC cylinders (10 cm in diameter) with mesh bottom, suspended in aquaria with running natural seawater. Juvenile mussels (*Mytilus edulis*; length in the range 5–10 mm) recovered from the same sediment samples were kept in separate aquaria (same conditions as *H. sanguineus*). All living animals were returned to the sampling location after completion of the experiments.

Experimental design

For all predation trials, non-moulting adult *H. sanguineus* (carapace width CW = 21.02 ± 2.24 mm, mean ± SD, $N = 100$) with intact chelae were individually isolated in small plastic aquaria (16.0 cm × 9.5 cm × 10.5 cm) without sediment (to easily retrieve non-consumed amphipods), closed with plastic lids and immersed in larger glass aquaria (90 cm × 50 cm × 30 cm) with running natural seawater. A shelter was offered in each experimental aquarium in the form of a dark gray PVC pipe (6.5 cm long, 4 cm in diameter) longitudinally cut in half. Since *H. sanguineus* preferentially feeds at night due to its photophobic behaviour (Spilmont et al. 2015), all experiments were conducted for 12 h during nighttime (i.e. the experiments started at dusk, were run overnight, and stopped early in the morning). At the end of each experiment, crabs were removed and the number of remaining prey (amphipods and/or mussels) in each aquarium was counted. No significant differences in individuals CW were found (Wilcoxon-Mann-Whitney *U*-test, $P > 0.05$) between males and females within each treatment and between treatments for each sex; as such, potential differences observed between sexes and treatments could not be attributed to a size effect. To ensure the independence of the trials, each experimental individual was used only once and, between each experiment, aquaria and PVC shelter were washed with seawater, rinsed with 95% ethanol, then with distilled water (3 times) and allowed to dry until the next experiment.

Predation trials and prey preference

Attempts to provide a definition to food/prey 'preference' have a long-lasting history (see e.g. Rapport and Turner 1970; Johnson 1980). The related definition, associated experimental setups and data analysis have been more recently debated (Underwood et al. 2004; Underwood and Clarke 2005, 2006, 2007; Manly 2006; Taplin 2007). However, to date, no consensus has arisen which critically stresses the need to be very specific about the definition of 'preference' and the choice of experimental setups (Underwood and Clarke 2006).

Here, we specifically considered preference *stricto sensu* as a behaviour implying an active choice between distinct food items. To be objectively and quantitatively inferred, this definition requires the comparison of food consumption when only one prey species is available with consumption when several prey species are available, as initially defined by Rapport and Turner (1970) and Johnson (1980). Specifically, our experimental design was setup following Underwood et al. (2004) and Underwood and Clarke (2005). This approach has previously been used to determine food preference in crabs (Laitano et al. 2013) and is typically applied in food preference experiments (Astudillo et al. 2018). The design is based on a two-stage experiment. First, prey items are presented one type at a time to the predator; the results of these 'no-choice experiments' (or in Stage 1) provide the consumption rate of each type of prey (number of prey eaten per predator). In turn, in 'choice experiments' (Stage 2), two distinct prey items are presented simultaneously in the exact same proportion. Note that differences in consumption rates do not constitute preference *stricto sensu* but are rather the result of differences in detectability, catchability, handling time and satiety power in prey. As a consequence, preference by the predator towards a specific type of prey can only be tested by comparing the predicted proportions of prey items consumed in the choice experiments under the hypothesis of no preference with the actual proportions observed in Stage 2. This also implies that, in the framework of this definition of preference, the preferred prey is not necessarily the most consumed.

It has also been suggested that, in choice experiments, not only the proportions but also the order in which prey are consumed is important to determine preference (Taplin 2007). Since our experimental setup did not allow such a monitoring, we conducted a supplementary experiment, hereafter called Stage 2bis, to confirm the results obtained in Stage 2. In the Stage 2bis experiment, prey were presented in unbalanced proportions, i.e. each crab was offered one individual of a type of prey and 5 individuals of the other type. If one type of prey is actually preferred over the other one, it would be consumed first, irrespectively of its relative abundance (Taplin 2007), i.e. it would always be consumed when offered alone vs. 5 items of the other prey.

The prey tested in the present study were either *P. littoralis* or *M. edulis* (5 individuals offered to each crab: no-choice experiments) or a mixture of both (5 individuals of each prey offered to each crab: choice experiments); in Stage 2bis, 1 individual of one type and 5 individuals of the other were offered to each crab. A batch of 10 *H. sanguineus* individuals was tested during each experimental night; sexes were randomly selected for each batch to attain a total of 15 males and 15 females for each type of predation trial (Table 1). After each experiment, we counted the number of prey that were eaten. Furthermore, to assess the natural mortality of *P. littoralis*, control aquaria were run with only 5 *P. littoralis* present, with the same setup as described above. No mortality was recorded and all 5 amphipods were always recovered at the end of the 10 controls run.

Table 1. Experimental treatments used to test for predation of female and male *H. sanguineus* on *Mytilus edulis* and *Ptilohyale littoralis* in no-choice experiments (Stage 1) and choice experiments (Stage 2 and Stage 2bis). Control experiments were run to test on the mortality of *P. littoralis*.

Experiment	Prey		Predator		replicates (*N*)
	M. edulis	*P. littoralis*	female *H. sanguineus*	male *H. sanguineus*	
Control	0	5	0	0	10
Stage 1	5	0	0	1	15
	5	0	1	0	15
	0	5	0	1	15
	0	5	1	0	15
Stage 2	5	5	0	1	15
	5	5	1	0	15
Stage 2bis	1	5	0	1	15
	1	5	1	0	15
	5	1	0	1	15
	5	1	1	0	15

Statistical analysis

To compare results obtained from the no-choice experiments, we used the non-parametric Wilcoxon-Mann-Whitney U test (WMW test hereafter).

For preference tests, we used the procedure proposed by Underwood and Clarke (2005). This procedure, that takes into account potential sampling errors when samples are small, uses a Chi-square test (χ^2) where the expected numbers on the basis of no preference are obtained through maximum likelihood equations calculated after results obtained from no-choice (Stage 1) and choice experiments (Stage 2; section 2.4 and Appendix A.3 in Underwood and Clarke 2005). Since we considered that preference *per se* can only be determined by comparing predicted

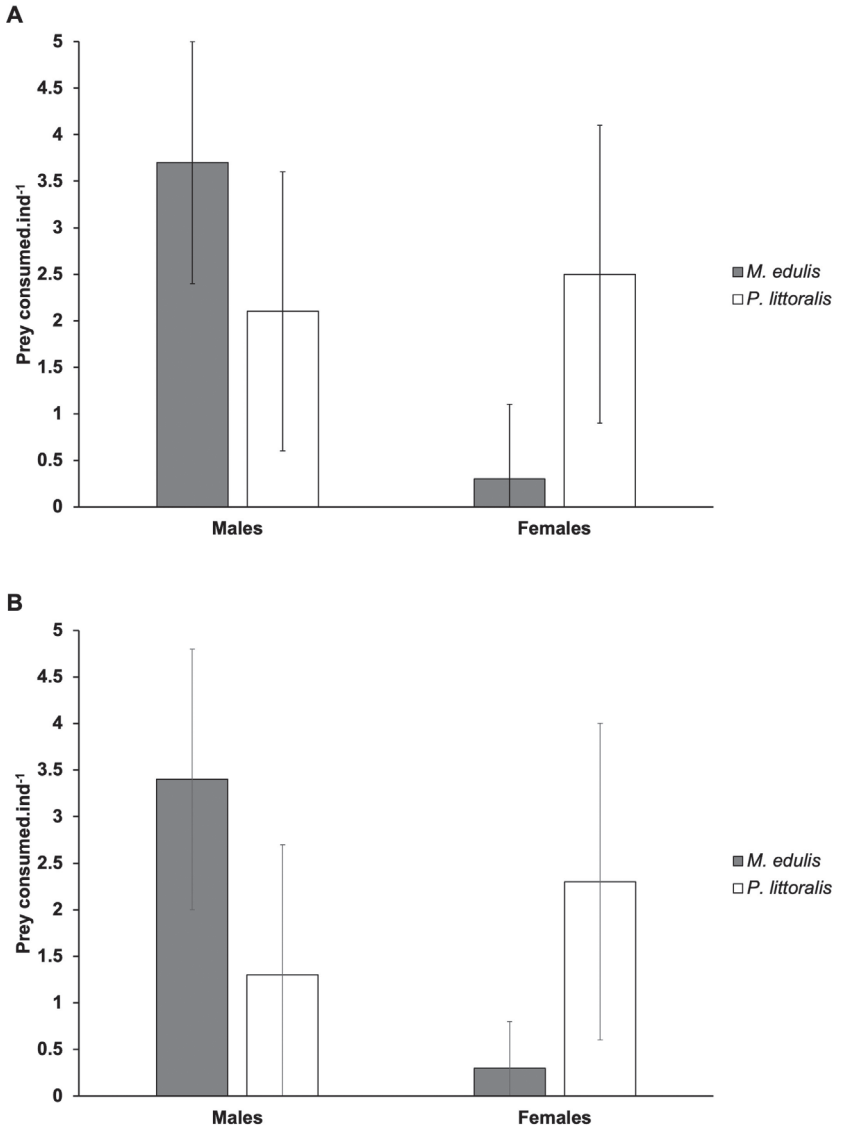

Figure 1. Number (mean ± S.D., $N = 15$ for each bar) of prey (*Mytilus edulis*, grey bars; *Ptilohyale littoralis*, white bars) consumed by male and female *Hemigrapsus sanguineus* over 12 h (nighttime) during no-choice experiments (**A:** Stage 1, prey presented one type at a time) and choice experiments (**B:** Stage, two types of prey presented simultaneously).

values for choice experiments under the hypothesis of no preference with observed values in these experiments, χ^2 tests were computed comparing observed numbers with expected numbers only for Stage 2. A global χ^2 test was therefore computed, by pooling data obtained from all replicates, as implemented by Laitano et al. (2013). To examine the potential inter-individual variation (Manly 2006; Taplin 2007), random pairs of experimental units of predation on Stage 1 and Stage 2 were chosen for comparison (Hall-Scharf and Stallings 2014; Astudillo et al. 2018); 15 of these random pairs were examined for male and female *H. sanguineus* by calculating individual χ^2 tests.

Results

In no-choice experiments (Stage 1; Fig. 1A), male *H. sanguineus* exhibited a higher consumption rate on *M. edulis* (prey consumed per individual: 3.7 ± 1.3 prey ind^{-1}; mean ± standard deviation) than on *P. littoralis* (2.1 ± 1.5 prey ind^{-1}; WMW test: $P = 0.004$). The reverse situation was observed for females (WMW test:

$P = 2.1\ 10^{-4}$) with a higher consumption of amphipods (2.5 ± 1.6 prey ind^{-1}) than mussels (0.3 ± 0.8 prey ind^{-1}).

The consumption rate of *M. edulis* significantly differed between male and female *H. sanguineus* (WMW test: $P = 4.4\ 10^{-6}$). Specifically, twelve females did not prey on *M. edulis* and the maximum number of mussels eaten by a single individual was 3. In sharp contrast, all males consumed at least one mussel and five of them did prey on the 5 individuals presented. The consumption rate of *P. littoralis* was non-significantly different between males and females (WMW test: $P = 0.445$).

The results of the choice experiments (Stage 2; Fig. 1B) showed that the consumption rate for each type of prey did not significantly differ from the no-choice experiment (WMW tests, $P > 0.148$). In addition, the lack of significant difference between the expected and observed consumption rates indicates the absence of preference *stricto sensu* for any type of prey (Table 2), either for males (Chi-square test: $\chi^2_1 = 0.76$, $P = 0.385$) or for females (Chi-square test: $\chi^2_1 = 0.01$, $P = 0.904$). Thirteen out of the 15 individual χ^2 tests comparisons for males showed no significant differences, and all 15 tests were not significant for females (Table 2); this confirmed that the observed consumption rates were not influenced by inter-individual variability. These results were further supported by those from the complementary choice experiment (Stage 2bis, i.e. prey presented in unbalanced proportions, Fig. 2) which showed than neither *M. edulis* nor *P. littoralis* was systematically consumed when a single individual was offered (none of the prey was systematically consumed first), i.e. none of the prey is preferred *stricto sensu* following Taplin (2007).

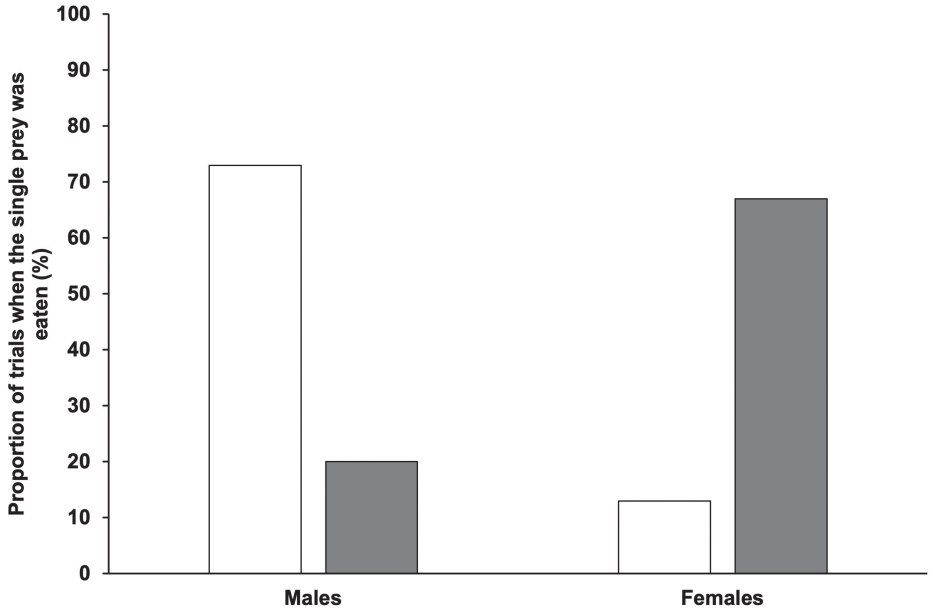

Figure 2. Proportion of trials when the single prey was eaten (%) during the choice experiments with unbalanced proportions of prey (Stage 2 bis; white bars: 1 mussel and 5 amphipods presented simultaneously; grey bars: 5 mussels and 1 amphipod presented simultaneously).

Discussion

The present study provides evidence, under laboratory-controlled conditions, for the existence of predator-prey interactions between two introduced crustaceans, the Asian shore crab *Hemigrapsus sanguineus* and the North American amphipod *Ptilohyale littoralis*, co-occurring outside their distinct native ranges in the intertidal zone of the eastern English Channel. Specifically, in no-choice experiments,

Table 2. Consumed individuals of each type of prey (*Mytilus edulis*, *M.e.* and *Ptilohyale littoralis*, *P.l.*) by adult female and male *Hemigrapsus sanguineus* observed (Obs) during no-choice experiments (Stage 1) and choice experiments (Stage 2). Expected values (Exp) were obtained from the equations of maximal likelihood given by Underwood and Clarke (2005). Chi-square tests were used to evaluate differences between observed and expected values in Stage 2. For the global χ^2 test, the total number of each type of prey offered equals 75 both in Stage 1 and Stage 2 (5 prey × 15 replicates). For individual χ^2 tests, the total number of each prey offered was 5, both in Stage 1 and Stage 2.

Males	**Stage 1**		**Stage 2**				**χ^2**	***P* value**
	***M.e.* Obs**	***P.l.* Obs**	***M.e.* Obs**	***P.l.* Obs**	***M.e.* Exp**	***P.l.* Exp**		
Global	55	32	47	20	43.6	23.4	0.76	0.385
1	3	4	2	3	2.1	2.8	0.01	0.916
2	5	3	5	2	4.5	2.5	0.16	0.693
3	4	4	3	2	2.6	2.4	0.16	0.691
4	3	2	5	3	4.9	3.1	0.01	0.937
5	3	2	3	0	2.1	0.9	1.24	0.266
6	4	3	5	1	3.8	1	0.99	0.318
7	5	0	3	0	3	0	0	1
8	5	1	1	3	2.8	1.2	4.03	**0.04**
9	5	1	1	4	3.4	1.6	5.43	**0.02**
10	2	5	3	2	3.3	1.7	1.46	0.226
11	1	0	3	0	3	0	0	1
12	4	3	5	0	3.4	1.6	2.40	0.121
13	4	1	3	0	2.6	0.4	0.47	0.491
14	2	2	5	0	3.7	1.3	1.78	0.182
15	5	1	4	0	3.6	0.4	0.49	0.483
Females	**Stage 1**		**Stage 2**				**χ^2**	***P* value**
	***M.e.* Obs**	***P.l.* Obs**	***M.e.* Obs**	***P.l.* Obs**	***M.e.* Exp**	***P.l.* Exp**		
Global	5	38	4	34	0.2	33.8	0.01	0.904
1	0	1	0	5	0	5	0	1
2	1	3	1	4	1	4	0	1
3	0	3	0	3	0	3	0	1
4	0	3	0	1	0	1	0	1
5	0	3	0	1	0	1	0	1
6	3	4	0	2	1	1	2.2	0.136
7	0	3	0	2	0	2	0	1
8	0	5	0	2	0	2	0	1
9	0	4	0	4	0	4	0	1
10	0	3	0	5	0	5	0	1
11	1	2	0	0	0	0	0	1
12	0	4	1	2	0.4	2.6	1.2	0.277
13	0	3	1	0	0.2	0.8	3.8	0.05
14	1	4	1	1	0.5	1.5	0.6	0.434
15	1	2	0	2	0.4	1.6	0.6	0.457

both male and female *H. sanguineus* actively selected both *M. edulis* and *P. littoralis*, with females exhibiting a significantly lower consumption rate (i) on *M. edulis* than males, but not on *P. littoralis*, and (ii) on the native *M. edulis* than on the non-native *P. littoralis*. These results are consistent with previous studies showing a stronger predation pressure of males *H. sanguineus* on *M. edulis* than females due to differences in claw morphology and strength (Brousseau et al. 2001; Bourdeau and

O'Connor 2003), and similar predation pressure on the amphipod *Hyale plumulosa* from male and female *H. sanguineus* (Blasi and O'Connor, 2016). Our observations are consistent with studies demonstrating the ability of crabs of the genus *Hemigrapsus* to feed on amphipods in laboratory experiments (McDermott 1999; Griffen and Byers 2006; Cornelius et al. 2021; Bleile and Thieltges 2021), with males exerting a higher predation pressure on mussels than amphipods (Cornelius et al. 2021; Bleile and Thieltges 2021), contrary to females (Cornelius et al. 2021).

In two-prey choice experiments, *H. sanguineus* consistently did not exhibit any preference *stricto sensu* for either native or non-native prey though significant differences in the consumption rates of both types of prey were observed. This result seems to diverge from previous evidence that the Asian shore crab preferred mussels over other animal prey (Tyrrell and Harris 2000; Brousseau and Baglivo 2005), including in experiments with amphipods (Bleile and Thieltges 2021). However, the outcome from Bleile and Thieltges (2021) regarding preference are not directly comparable to the present study due to experiments restricted to males, choice experiments conducted with 4 types of prey and differences in the definition of "preference" itself and the subsequent data analysis. We, however, showed that male *H. sanguineus* actually consumed more *M. edulis* individuals than *P. littoralis*, which is consistent with most studies defining preference as a higher consumption rate. Terminological considerations aside, this recurrent observation in laboratory experiments on *H. sanguineus* is of prime importance regarding its ecological impact. Though not always preferred *stricto sensu*, juvenile mussels may constitute a large part of the crab's diet which may ultimately have a critical influence on the composition of natural benthic intertidal communities relying on *M. edulis* as engineer species.

We, however, unambiguously showed that *H. sanguineus* is able to detect and consume *P. littoralis* although the latter was putatively introduced a decade later (i.e. circa 2016; Spilmont et al. 2018) than the former (i.e. circa 2005; Dauvin et al. 2009) at our study site. This suggests that the Asian shore crab cannot be considered as a naive predator when confronted to a newly introduced amphipod prey. This apparent lack of naiveté is consistent with the ability of *H. sanguineus* to rapidly sense, pursue and consume different amphipod preys (McDermott 1999). Despite a lack of co-evolutionary history and historical familiarity between *H. sanguineus* and *P. littoralis* in their introduced eastern English Channel range, handling skills learned by *H. sanguineus* either in their native or non-native ranges in the presence of similar prey (e.g. *Apohyale prevostii*; Spilmont et al. 2018) could have been transferred to novel prey, as shown in the shore crab *Carcinus maenas* (Hughes and O'Brien 2001). Note that the indigenous *M. edulis* was not considered as a naive prey towards *H. sanguineus*; indeed, the present experiments were conducted about 10 years after the introduction of *H. sanguineus* at our study site and it can be considered that *M. edulis* is now able to detect the presence of the Asian shore crab's cues, as recently demonstrated by Uguen et al. (2022) for mussels and *H. sanguineus* from the same study site.

Since *H. sanguineus* did not exhibit any preference *stricto sensu* for either the indigenous (*M. edulis*) or the introduced (*P. littoralis*) prey, none of the evolutionary hypotheses of novel prey-predator interactions (see Grosholz and Wells 2016) applies to *H. sanguineus* in its introduced range along the French coast of the eastern English Channel. Instead, following Grosholz and Wells (2016), *H. sanguineus* would use a combination of optimal foraging strategy and optimal diet strategy and thus consume the prey that is the easiest to catch and the most profitable. However, the optimal diet theory is usually not supported when predators attack motile prey (Sih and Christensen 2001). Indeed, consuming a motile small amphipod would unlikely maximize the net energy gain per unit time foraging compared

to the consumption of the larger non- or slow-moving mussel. It is actually much more probable that *H. sanguineus* is an opportunistic predator and that its consumption of *P. littoralis* mainly depends on their encounter rate. This hypothesis in consistent with the fact that invasive prey usually exhibit various degrees of activity, sheltering and exploratory behaviours (Chapple and Wong 2016) which, when the species is considered as a prey, would either mitigate or enhance the predation pressure. This was demonstrated in studies with the invasive freshwater amphipod *Dikerogammarus villosus* exhibiting less exploratory behaviour and greater sheltering than native species, which made the former less vulnerable to fish predation (Kobak et al. 2014; Mennen and Laskowski 2018). In our case, the degree of activity of *P. littoralis* could be estimated only by complementary experiments with continuous monitoring.

The fact that a motile prey such as *P. littoralis* was consumed in high proportion, especially by female *H. sanguineus*, implicitly suggests, that *P. littoralis* either did not exhibit any, or expressed inappropriate/uneffective, antipredator response, which would support the 'naive prey' hypothesis (Banks and Dickman 2007; Sih et al. 2010b). Though, to our knowledge, anti-predator responses have never been investigated in Hyalid amphipods, this has been demonstrated in other families (e.g. Mennen and Laskowski 2018). The consumption rates we recorded on *P. littoralis* are consistent with those observed by Blasi and O'Connor (2016) – note that *Hyale plumulosa*'s accepted name is actually *Ptilohyale plumulosa* (WoRMS 2020), which has been categorized as *P. littoralis* for the Atlantic ; see pp. 102–103 in Bousfield and Hendrycks 2002 and p. 51 in Lo Brutto and Iaciofano 2018 – in experimental enclosures without any shelter (consumption of ca. 60% of prey available). Our estimates are, however, clearly higher than the consumption rates they recorded with a refuge (sediment and rocks; consumption of ca. 15%). Despite differences in the experimental setup, these discrepancies likely reflect different behaviours and/or antipredator responses of *P. littoralis* between native and invaded ranges. For the eastern English Channel area, similar laboratory observations using native amphipods, such as the sibling species *Apohyale prevostii* or the Melitidae *Melita palmata* would help to more thoroughly elucidate interactions between *H. sanguineus* and amphipods by comparing native and introduced prey.

Our results are relevant in an experimental context and, though controlled experiments remain a stepping stone in the understanding of prey-predator interactions, the extrapolation of laboratory observations to field events remains uncertain (Brousseau and Baglivo 2005). Our experiments represented idealized foraging conditions due to the small size of the experimental container that minimized search time of the predatory crab and overestimate ingestion under natural conditions (Lohrer and Whitlach 2002; Epifanio 2013). They were also conducted at night when *H. sanguineus* is known to be more actively feeding (Spilmont et al. 2015) and without sediment in the containers which added to the optimal foraging conditions (Blasi and O'Connor 2016). Feeding patterns of the Asian shore crab on mussels and amphipods have been shown to be different in the field and in the laboratory (Brousseau et al. 2014; Blasi and O'Connor 2016); possible explanations include limited foraging opportunities due to abiotic factors (diurnal cycle, wave action, etc), increased prey choice and variable densities.

Though being part of the diet of the Asian shore crab in both its native and north-American invaded ranges, amphipods usually constitute only a small proportion of the overall food use (Lohrer et al. 2000; Griffen et al. 2008). However, a potential impact of the sibling *H. takanoi* on natural amphipod populations has recently been underlined (Cornelius et al. 2021) and our results suggest that the predatory pressure on *P. littoralis* would mainly be exerted by female *H. sanguineus*

when several prey are available. At our study site, *H. sanguineus* was found concomitantly with *P. littoralis*, but also with a range of potential other prey (e.g. the amphipods *Apohyale prevostii* and *Melita palmata*, the annelid *Eulalia clavigera* and the gastropod *Littorina littorea*), as well as the potentially competitive green crab *Carcinus maenas* (Spilmont et al. 2018). Furthermore, the Asian shore crab is itself a potential prey for fish (Heinonen and Auster 2012; Savaria and O'Connor 2013), birds (Dumoulin and van Outryve 2009) and other crab species (Papacostas and Freestone 2019), against which *H. sanguineus* could also behave as a naive prey. The potential benefit for the crab to forage on naive prey could therefore be dampened by its own naiveté (Papacostas and Freestone 2019). As long as these abiotic factors and consumptive and non-consumptive biotic interactions are not taken into account, neither the ecological role of *H. sanguineus*, which as a large benthic decapod could play a role in structuring communities (Boudreau and Worm 2012), nor the potential synergistic interaction between the two introduce species, which could accelerate impacts on native communities ('invasional meltdown': Simberloff and Von Holle 1999), can be elucidated.

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Auhthors' Contribution

N.S.: research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, original draft; writing

L.S.: research conceptualization, sample design and methodology, data interpretation, original draft; review & editing

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