Spontaneous alternation in marine crabs: Invasive versus native species

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1. Introduction

Spontaneous alternation is the behavioral pattern emerging from an organism’s tendency to explore places that have been least recently visited without any reinforcement mediation. This means that the organism has not been trained through reinforcement (e.g., it is not escaping an aversive state nor is it receiving a reward) to exhibit this behavioral pattern (Richman et al., 1986). Spontaneous alternation has been demonstrated in several organisms ranging from paramecia and flatworms (Aderman and Dawson, 1970; Harvey and Bovell, 2006) to rats and humans (Still, 1966; Schultz, 1964). Different processes have been proposed to underlie this pattern, including reactive inhibition (Hull, 1951), curiosity and response to novelty (Dember, 1956), and spatial working memory (e.g., Stefani and Gold, 2001). It is thought that different phyla rely on different cues in exhibiting this pattern. Some invertebrates have been shown to rely on body turn whereas vertebrates employ directional and/or odor cues (for a review see Richman et al., 1986). Irrespective of the possible underlying mechanism(s), spontaneous alternation appears to be innate and the behavior may be beneficial during exploration and foraging (Estates and Schoeffler, 1955; Dember and Earl, 1957). For example, this pattern might increase the likelihood of discovering an unexploited resource, especially in heterogeneous environments with patchy prey distributions. Based on this rationale, we compared the spontaneous alternation performance of two Portunid (F. Portunidae) crabs: the invasive European green crab (Carcinus maenas [Linnaeus, 1758]) and the native, swimming blue crab (Callinectes sapidus Rathbun, 1896).

Native to the Atlantic coast of Europe, the green crab has proven to be a highly successful invader worldwide with established populations on both coasts of North America (e.g., Ropes, 1967; Cohen et al., 1995; Carlton and Cohen, 2003). Its success has in part, been attributed to its ability to tolerate a wide range of temperatures and salinities (Eriksson et al., 1975), as well as exposure to air (Crothers, 1968). Moreover, it is a highly effective predator on a wide variety of benthic invertebrates (e.g., Ropes, 1967; Taylor, 2005), and life history traits such as high fecundity, long planktonic larval stage, and fast growth have also facilitated its global expansion (Roman and Palumbi, 2004). Once established, the green crab has become the dominant intertidal crab in some areas, affecting the abundance, size structure, and defense response of native species (Ropes, 1967; Cohen et al., 1995; Tyrell and Harris, 1999; Yamada et al., 2005). In bays and estuaries on the east coast of the United States green crabs may be a major competitor with the ecologically and commercially important blue crab (Roudez et al., 2007) as they broadly overlap in habitat utilization and diet (Williams, 1984). Thus, recent research has focused on examining potential competitive
interactions between these two species (e.g., DeRivera et al., 2005; Henry, 2005; MacDonald et al., 2007). This led us to compare these species in a continuous spontaneous alternation paradigm using a plus-maze submerged in seawater.

The objectives of the present research were to determine (1) if spontaneous alternation behavior is exhibited by two species of portunid crabs; (2) whether there are interspecific differences (i.e., invasive versus native species) in their spontaneous alternation performance; and (3) if spontaneous alternation performance for individual subjects improved over the course of an experimental session, by which we aimed to address the innateness of this behavioral policy (not learned via recent experience).

2. Materials and methods

2.1. Subjects

A total of 19 C. maenas (carapace width = 5.50, ±0.15 cm SE) and 18 C. sapidus (carapace width = 11.70 ±0.73 cm SE) were obtained from Tuckerton, New Jersey in August/September 2008 and transported in coolers to the Institute of Marine and Coastal Sciences (IMCS) in New Brunswick for experiments. Carapace length was measured from tip to tip of the longest lateral spines. Green crabs were all adult females (catches at this time consisted of only females) and blue crabs were an even mixture of adult males and females. Blue crabs did not exhibit significant differences in spontaneous alternation performance between the sexes, t(14) = 0.06, p = 0.95 (female mean = 0.30; male mean = 0.30). Crabs were housed individually in minnow traps (to prevent agonistic interactions) partly submerged, in temperature controlled running seawater tables (salinity = ~32; temperature = ~20 °C) at a 12 h light: 12 h dark photoperiod. They were fed with local mussels (Geukensia demissa) every 2 days. Testing for spontaneous alternation performance was conducted in a separate light controlled room, fed with seawater that was maintained at the same temperature and salinity as the seawater tables where crabs were maintained. Crabs were kept for 5–7 days prior to conducting experiments to give them time to adjust to their new surroundings. The carapace of each crab was also marked with a small amount of “white out” to aid in viewing crabs under the dimly lit experimental conditions.

2.2. Apparatus

A glass plus-maze was built in house (arm length: 31.0 cm, arm width: 13.0 cm, arm height: 25.5 cm) and placed at one end of a fiberglass seawater table (length: 141.0 cm, width: 91.0 cm, height: 14.0 cm) (Fig. 1). Five cylindrical objects of different size (diameters ~4–12 cm) and color (i.e., white, grey, and black) were placed around the plus-maze (Fig. 1) to potentially aid crabs in spatial navigation. Identical pieces of wood (3 cm × 1 cm cube) were secured at the end of each maze arm, and a white waterproof flooring was placed in the last 1/3 of each arm (10 cm from the end of each arm). The wood provided some structure within the maze, whereas the white flooring indicated, under dim lit conditions, the “threshold” individual crabs had to cross in order for us to score each of the arm entries as an arm choice (described below). The seawater table and thus the plus-maze were filled with water to a depth of 13 cm for each experimental session.

2.3. Procedure

For each session, the subject was gently placed in the center, open area of the plus-maze (Fig. 1) and its movements within the maze were recorded for 63 min (to ensure 60 min of observation in the absence of the experimenter) under dimly lit conditions using a digital video camera (Sony DCR-SR42) and a PC. Following placement of the subject in the maze, the experimenter promptly left the room. After each session, the water was flushed and renewed to a large extent for the next subject. Crabs were released back to their natural habitat upon completion of the study.

2.4. Data analysis

2.4.1. Scoring

The subject was considered to have made an arm choice (an arm entry) when at least half of its body passed the threshold (1/3 of the arm length—see apparatus). Each choice was noted and time stamped. Consistent with the scoring regimes used in studies of continuous spontaneous alternation and because of the difficulty in defining an immediate repeated arm choice, repeated entries of the same arm were not included in the data analysis (also see Anisman, 1975; Kokkinidis and Anisman, 1976; McNay and Gold, 2001; Lennartz, 2008). Alternation performance was assessed within overlapping runs of five choices. If the subject chose four different arms within five consecutive choices, that sequence was scored as a successful alternation (1) else it was scored as an unsuccessful one (0). The overall alternation performance of each subject was the ratio of successful alternations to the number of all possible alternations (n – 4, where n is the total number of arm choices). For instance, assume that a crab made the following scored choices: A-C-B-A-D-C-D-C-A-B, where it had 4 out of 6 possible alternations (111001), thus scored (4/6 = 0.67). Subjects making fewer than 10 arm choices were not included in the data analysis, so three green crabs and two blue crabs were excluded (green crabs n = 16; blue crabs n = 16).

Alternation performance scores were compared with chance level which was calculated by the following method: a subject’s first arm choice was always novel (4/4). Since we did not consider immediate repeated choices, its second arm choice was also always novel (3/3). On the third arm choice a subject had a 2/3 probability of choosing an arm that was not its first choice (note that repeated choices were not considered). If the subject had a novel third choice, on the fourth choice it had 1/3 probability of choosing an arm that was novel/not chosen as its first or second choice. At this point, the subject did not need another choice to have chosen four different arms. Thus, partial probability of choosing four different arms here is (4/4)(3/3)(2/3)(1/3)(1) = 0.22. The subject however, could have made a mistake on its third choice but could still choose all four arms within five choices, where
the probability is \((4/4)(3/3)(1/3)(2/3)(1/3) = 0.07\). The subject could also have made a mistake on its fourth choice but could still choose all four arms within five choices, where the probability is \((4/4)(3/3)(2/3)(2/3)(1/3) = 0.15\). Summing the partial probabilities (0.22 + 0.07 + 0.15) gives the chance level performance of 0.44 (also see Lennartz, 2008). Interspecific comparisons were analyzed with independent sample t-tests and comparisons of group performance to the chance level (0.44) were done with one-sample t-tests. Pearson’s correlation coefficients were also calculated to determine if relationships existed between some parameters (i.e., number of arm entries versus alternation performance). An alpha level of 0.05 was selected for all inferential statistics. For statistical analyses we used Matlab (Mathworks, Natick, MA, USA) and SPSS 10.0.

2.4.2. Analysis of acquisition patterns

In order to determine whether individual subjects acquired the alternation pattern over the course of the experimental session or performed at their best from the beginning of the session, we applied a relative-likelihood change-point algorithm (originally developed by Gallistel et al., 2004) on the sequence of scored choices (0 and 1 s). This algorithm assumes the sequence was drawn from a Bernoulli distribution, which has a single parameter (i.e., the probability of drawing 1 \(p\)). The algorithm proceeds datum-by-datum (0 s or 1 s derived from the overlapping sequences of 5 choices), checking at each point, whether the binary sequence up to and including that point is better represented by a model in which there has been no change in \(p\) or by a model in which there has been a change in \(p\) (at some earlier point in the sequence). Briefly, the change model assumes that the alternation performance improved or worsened during testing. The no-change model assumes that the alternation performance neither improved nor worsened during testing. We used two decision criteria for testing these models: 10 (liberal) and 100 (conservative). For a criterion of 100 for instance the odds had to favor the change hypothesis by 100:1 in order for the algorithm to determine that there had been a change in the expectation of the distribution from which the measures were drawn (also see Balci et al., 2009). These two criteria were used to ensure the generalizability of our conclusions. Three blue crabs that exhibited alternation performance of 0 were not included in this analysis because there cannot be any change in a sequence of 0 s. Briefly, with this analysis we determined if there were “significant” improvement or worsening of the alternation performance of individual subjects in the course of testing (see Gallistel et al., 2004 for the Matlab code for a variant algorithm). Here, “significant” means that the odds in favor of a change in the alternation performance exceeded the decision criterion used, which is slightly different from but analogous to its traditional use in statistical hypothesis testing.

3. Results

Spontaneous alternation performance was significantly higher in green crabs (mean = 0.62) compared with blue crabs (mean = 0.30), \(t(30) = 3.89, p < 0.001\) (see Fig. 2A). A clear difference between species was also evident in the cumulative distribution of individual performances. The interquartile range of alternation performances was 0.55–0.77 for green crabs and 0.07–0.48 for blue crabs (Fig. 2B).

Species comparisons to chance level alternation performance indicated that green crabs alternated at a level that was significantly greater than the chance level of 0.44, \(t(15) = 3.94, p = 0.01\) whereas, blue crab performance was not significantly different from the chance level, with a statistical trend for lower than chance level performance (\(p < 0.06\)). Although green crabs had a higher number of arm entries (30.25 ± 3.33 SE) than the blue crabs (21.88 ± 2.61 SE), this difference was not statistically significant (\(p = 0.06\)). Moreover, there was no significant correlation between the number of arm entries and alternation performance in either green (\(r = –0.1\)) or blue crabs (\(r = –0.1\)). Alternation performance was also not significantly correlated with size in either species (blue crabs: \(r = –0.1\); green crabs: \(r = 0.1\)).

Based on the more conservative decision criterion (100) for the relative-likelihood change-point algorithm, 69% (11/16) of green crabs and 92% (12/13) of blue crabs did not improve over the course of their experimental session (Fig. 2C). In other words, an improvement in performance whether it was followed by a decline or not,

**Fig. 2.** (A) Average spontaneous alternation performance (proportion correct) with ± SE for green and blue crabs. Dashed line = level of chance alternation. (B) Cumulative distribution of individual performances for each species. Dashed line = level of chance alternation. (C) Depiction of performance patterns detected with relative-likelihood change-point algorithm. Pair of numbers below each depiction indicates number of green crabs, blue crabs, and total number of individuals (both species combined) exhibiting each corresponding pattern based on liberal and conservative decision criteria (values outside and in parentheses, respectively). From left to right, depictions represent: no change in performance, an abrupt improvement; an abrupt improvement followed by an abrupt decline; an abrupt improvement followed by an abrupt decline and subsequent improvement; an abrupt decline; and an abrupt decline followed by an abrupt improvement. Locations of abrupt changes in performance are depicted arbitrarily.
was only observed in 31% (5/16) and 8% (1/13) of green and blue crabs, respectively. Where crabs demonstrated an improvement in alternation performance, it was abrupt; namely crabs reached their asymptotic performance in a single step rather than in multiple steps. No declines from the initial levels of alternation performance (last two panels of Fig. 1C) were observed for either species using the more conservative decision criterion. Finally, we examined if “successfully” alternating crabs (performance >0) were moving consistently clockwise or counter-clockwise. The arm choices for both species showed that they did not move in one direction without frequent interruptions, where interruptions were choices in the opposite direction (from the previous one) or to the opposite arm. In a separate analysis for both species, 53% (±2 SE) were in the clockwise direction, 44% (±2 SE) were in the counter-clockwise direction and around 3% (±1 SE) were to the opposite arm within a session.

4. Discussion

Recent research investigating physiological (Henry, 2005), predatory (DeRivera et al., 2005; MacDonald et al., 2007) and behavioral (e.g., agonistic interactions, MacDonald et al., 2007) differences between the invasive European green crab, C. maenas and native blue crab, C. sapidus have revealed potential competitive interactions. An advantage of C. maenas over C. sapidus (juveniles and/or adults) has been revealed during agonistic interactions for food (MacDonald et al., 2007) and in their learning abilities during foraging, where their performance was assessed in terms of their speed in locating a hidden food source over successive trials (Roudez et al., 2007).

In the present study, we investigated spontaneous alternation as an additional behavioral mechanism that might result in the competitive success of green crabs over blue crabs, in areas where they co-occur. For the first time, to our knowledge, spontaneous alternation behavior was demonstrated in Crustacea and significant interspecific differences in alternation performance were observed between an invasive versus a native species. Specifically, C. maenas had a higher alternation performance than C. sapidus which exhibited chance level performance in a plus-maze where the crabs were able to explore their environment freely.

Spontaneous alternation behavior may be beneficial to organisms during exploration; whether it be to search for new habitats, food, or mates to avoid predators. Exploring novel localities rather than those most recently explored may increase the likelihood of discovering an unexploited resource or locating a chemical/visual cue which can facilitate a more directed search in locating its source (e.g., food, potential mate) (Chiussi et al., 2001; Weissburg and Dusenbery, 2002). Even after the organism discovers a food patch, spontaneous alternation might reduce environmental uncertainty which would be beneficial in guiding its foraging behavior when local changes occur in resource abundance (also see Inglis et al., 2001). This may be especially useful in heterogeneous environments with patchy prey distributions and complex habitats such as the rocky intertidal, where green crabs primarily, but not exclusively, occur. Thus, our results suggest that this relatively fundamental behavior may contribute to the success of the green crab in expanding its range especially at relatively small spatial scales, as well as augmenting its competitive success in areas where these two crabs co-occur.

Green crabs on average alternated at levels higher than chance, whereas blue crabs on average exhibited chance level performance. The canonical interpretation of this finding is that spontaneous alternation exists in the behavioral repertoire of the green but not the blue crabs. It is possible, however, that the level of task/exploratory complexity imposed by the plus-maze alternation task might have simply prevented the successful manifestation of this behavioral pattern in blue crabs. In a plus-maze subjects have to remember which one of the last three arms was least recently visited. On the other hand, in the t-maze or y-maze paradigms they only have two arm choices. This is analogous to two different systems both having memories, but with one being able to function with more items in store than the other one. Under this rationale the consistent high levels of alternation performances observed for green crabs compared to the lower levels observed in the blue crabs in our plus-maze task, rather than the absence of spontaneous alternation in blue crabs itself, might be advantageous for green crabs. In other words, green crabs may be able to exert this behavioral pattern over a larger area than the blue crabs. This observation is especially relevant since marine crabs live in structurally complex environments. Assessment of blue crabs in simpler alternation paradigms might contribute to our understanding of the chance level performance of this species, which might further emphasize the distinction between the alternation policy itself and its behavioral manifestation under highly complex exploratory scenarios.

It is also possible that green crabs might have differentially adopted strategies that could bolster their spontaneous alternation performance in more complex settings (e.g., plus-maze versus y-maze). Moving along the maze walls unidirectionally for instance could result in high alternation performance without any mediation of other information. Our observations, however, did not support this unidirectional scenario. Both green and blue crabs exhibited equal degrees of preference for movement in different directions within a session (i.e., the percentages of clockwise, counter-clockwise, and opposite arm movements were identical in blue and green crabs). Moreover, both species tended to move sideways along the maze walls rather than across the center area of the maze to oppose arms.

A spontaneous alternation pattern may be mediated by multiple sources of information such as spatial, odor, directional, or body turn cues (for a review see Richman et al., 1986). Reliance on one of these cues might minimize or eliminate the role of others. Although spontaneous alternation has been reported to be a behavior shared by invertebrates and vertebrates, different phyla have indeed been shown to rely on different cues in exhibiting this behavioral pattern. In the present study several cues were available for use by the crabs in their alternation task including body turn cues (inherent to the task), odor trails, and visual/spatial cues provided by objects placed outside the maze. Multiple sources of information were utilized in an effort to reduce possible biases favoring one crab species over the other as we were interested in the spontaneous alternation performance itself rather than the form of information mediating this process.

To address the question of innateness of spontaneous alternation we investigated how individual alternation performance changed over the course of an experimental session. For most of the subjects, their alternation performance did not improve over the course of testing, which as expected suggests the innate nature of this behavior. In a few individuals, there was an abrupt improvement in performance. These changes were, however, usually followed by another abrupt decrease in performance later in the session. This observation might in itself also point to asymptotic performance, but a noisier one from the onset.

The implications of the present study are two-fold adding to both the general areas of experimental psychology and behavioral ecology. To our knowledge, this study constituted the first investigation of spontaneous alternation behavior in Crustacea and the first assessment of continuous spontaneous alternation performance in invertebrates. Invertebrates have traditionally been tested in discrete spontaneous alternation paradigms, where they were not allowed to explore their environment freely (e.g., Harvey and Bovell, 2006). It also constitutes one of the few comparisons that have...
explicitly set out to examine differences in spontaneous alternation performance at the species level (for exception see Harvey and Bovell, 2006). Invasive green crabs had a higher alternation performance than native blue crabs, which exhibited chance level performance. Our results suggest that this fundamental behavioral pattern may contribute to the success of the green crab in expanding its range especially at relatively small spatial scales as well as augmenting its competitive success(s) in areas where the blue and green crabs co-occur.

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