

# Effect of predation threat on repeatability of individual crab behavior revealed by mark-recapture

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Received: 10 April 2013 / Revised: 20 November 2013 / Accepted: 4 December 2013 / Published online: 20 December 2013  
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**Abstract** The persistence of behavioral types *in situ* and the drivers of persistence are central to predicting the ecological effects of intraspecific behavioral variation. We surveyed individual refuge use of mud crabs (*Panopeus herbstii*), a behavior related to the strength of a trait-mediated trophic cascade in oyster reefs, in the absence and presence of toadfish (*Opsanus tau*) predation threat. We then released these crabs into the field and using mark-recapture, measured the repeatability of this behavior in the absence and presence of threat, and how behavioral change was affected by time in the field (a month on average, up to 81 days), crab size, and sex. Because crabs exhibited some evidence of a circatidal rhythm in refuge use, we also tested how tidal height during observation influenced behavioral change. Predation threat increased refuge use, and small crabs used the refuge more than large crabs, particularly under threat. In recaptured crabs, refuge use was more repeatable under threat. Neither time in the field, crab size, crab sex, nor tidal height had any effect on behavioral change. Our results support the non-mutually exclusive hypotheses that (1) prey organisms in the presence, rather than absence, of predation threat should exhibit less behavioral variability because the fear of dying (a severe fitness consequence) should take precedence over less immediately important influences on behavior (e.g., hunger) and that (2)

individual behaviors tied to fixed traits (e.g., the body size dependence of refuge use under threat in this study), rather than variable traits, should be more repeatable over time.

**Keywords** Anti-predator · Behavioral syndrome · Body size · *Crassostrea virginica* · Phenotypic plasticity · Personality

## Introduction

Consistent variation in behavior between conspecific individuals, variously referred to as behavioral types, behavioral syndromes, coping styles, animal personality, and/or temperament, is a common and taxonomically widespread phenomenon that has fundamentally changed the way ecologists view behavior (Gosling 2001; Sih et al. 2004). Here, we define consistent behavioral variants as behavioral types (BTs) and correlations between multiple behavioral traits or the same behavior across multiple contexts as behavioral syndromes following the terminology of Sih et al. (2004). Traditionally, animal behavior was considered highly plastic, shaped by both the organism's internal state as well as the external environment to maximize fitness (Emlen 1966; Stephens and Krebs 1986). Yet, increasingly, the importance of constraint imposed by individual BTs on behavioral flexibility is recognized as a common aspect of behavior. Accordingly, a new research front explores the effects of BTs, as well as other aspects of individual-level variation more broadly (Bolnick et al. 2003; Bolnick et al. 2011), on population and community dynamics (Sih et al. 2012). Individual constraints on animal behavior have recently been used to successfully predict the form and strength of intra- and interspecific interactions that ultimately determine community structure (e.g., Pruitt and Ferrari 2011; Griffen et al. 2012; Pruitt et al. 2012). For example, Pruitt and Ferrari (2011) found that the proportion of aggressive versus docile BTs in spider

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Communicated by T. Breithaupt

**Electronic supplementary material** The online version of this article (doi:10.1007/s00265-013-1666-7) contains supplementary material, which is available to authorized users.

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(*Anelosimus studiosus*) colonies determines the nature of their interactions (commensal vs. mutualistic) with other spider species that inhabit their webs.

Determining both the long-term persistence of ecologically relevant BTs and the drivers of persistence in wild animals is critical to understand the ecological and evolutionary consequences of BTs (Bell et al. 2009; Archard and Braithwaite 2010). If the behavioral traits of individuals change over time in the field, then the ecological effects of these traits will also change accordingly. At one extreme, traits that prove highly variable (i.e., non-persistent) in the field will offer little predictive power in ecology. Furthermore, measuring the persistence of BTs, particularly in natural field situations, can shed light on the internal and environmental drivers of BTs (Stamps and Groothuis 2010) and is necessary to fully understand the timing and overall effects of BTs on individual fitness (Dingemanse and Réale 2005).

Two methodologies common to BT studies currently impede our ability to assess the long-term persistence and thus ecological relevance of BTs. First, many studies of BTs assess repeatability (i.e., proportion of total phenotypic variation due to between-individual variation, a measurement of the temporal consistency of individual behavior) over relatively short time intervals of a few days or less, and second, studies commonly house animals in the lab between longitudinal behavioral measurements (Bell et al. 2009; Archard and Braithwaite 2010; but see e.g., Réale et al. 2000; Wilson and Godin 2009; Ferrari et al. 2013). These approaches exacerbate the difficulty of extrapolating to field situations. For example, repeatability can decline over time (Bell et al. 2009), and so traits that are shown to be repeatable over a few days should not be assumed repeatable over longer periods. Furthermore, short repeatability estimates can be strongly influenced by stochastic variation in environmental conditions during behavioral measurement (Dingemanse et al. 2002; Gabriel and Black 2010). Repeatability of wild versus lab-held animals may differ because the field generally provides a more dynamic environment and therefore traits with any environmental dependence should change at a faster rate (Hoffman 2000). Holding animals in the lab for long periods between behavioral measurements (e.g., weeks or months) can also impede learning and development dependent on field conditions or experiences that may drive behavioral change in the field (Archard and Braithwaite 2010). Lastly, because the lab is foreign to animals, they may become acclimated to the lab when held for long periods, distorting measurements of repeatability and potentially yielding behavioral patterns that are inconsistent with those of animals accustomed to field conditions (Butler et al. 2006; Biro 2012; but see Herborn et al. 2010).

Short duration experiments on animals housed in the lab are particularly common with invertebrates that have become important model systems for studying the ecological effects of BTs (Mather 2013; more specifically, decapods: Gherardi et al. 2012; spiders: Pruitt and Riechert 2012; anemones:

e.g., Rudin and Briffa 2012). Indeed, the majority of studies measuring repeatability of invertebrate behavior do so over a period of a few days (median = 3 days; see electronic resource 1 for durations of repeatability studies conducted using invertebrates), and few studies have measured the persistence of individual behavior for invertebrates living under natural field conditions, as opposed to being held in the lab, between behavioral measurements (but see recent studies of anemones: Briffa and Greenway 2011; Hensley et al. 2012). This dependence on the lab is understandable because the small size and cryptic nature of invertebrates make them difficult to follow or relocate in the field. Yet, the longer-term repeatability and thus the ecological relevance of BTs in this important group of model organisms remain understudied. Measuring the persistence of BTs in invertebrates is also important for evaluating the welfare of invertebrates in animal research, which has been overlooked in comparison with the welfare of vertebrate species (Horvath et al. 2013).

In the present study, we assessed the long-term (a month on average, up to 81 days) repeatability of individual refuge use behavior in mud crabs (*Panopeus herbstii*) measured in the absence and presence of toadfish (*Opsanus tau*) predation threat. Specifically, we measured crab refuge use behavior in the lab, marked and released crabs into the field, and then recaptured crabs and measured behavior once again in the lab to assess repeatability. Refuge use behavior of mud crabs has important ecological consequences for oyster (*Crassostrea virginica*) reef community dynamics (Griffen et al. 2012). Individual refuge use mediates the strength of an indirect species interaction common in reefs along the Atlantic and Gulf coasts of the United States. Mud crabs that feed on scorched mussels (*Brachidontes exustus*) and juvenile oysters respond to chemical cues from predatory toadfish by taking refuge under oyster shells, and this increased refuge use reduces their bivalve consumption rate (Grabowski 2004; Grabowski and Kimbro 2005). Yet, individual crabs differ in their response to toadfish threat along a shy-bold continuum (Griffen et al. 2012; this study). Accordingly, individual refuge use by crabs helps predict their mussel consumption in the presence of toadfish threat, mediating the strength of the indirect interaction (Griffen et al. 2012). While some variation in crab refuge use behavior can be explained by crab size, there is additional variation in refuge use not explained by size or other crab characteristics that is important in determining the consumption rate of crabs (Griffen et al. 2012). Here, in addition to measuring the repeatability of refuge use behavior in the absence and presence of threat, we tested how duration in the field between behavioral measurements and individual crab traits (body size and sex) influenced change in refuge use behavior over time.

We formulated several hypotheses regarding the persistence of individual crab refuge use behavior. First, we hypothesized that refuge use under toadfish predation threat would be

more repeatable than refuge use in the absence of predation threat. This is because the fear of dying should take precedence over an organism's current energetic state (e.g., hunger level) or other environmental conditions (e.g., social situation) that influence behavior but have less immediate fitness consequences (termed here ‘the predation hypothesis’; Fodrie et al. 2012). Therefore, behavioral variability derived from these less important behavioral influences should be reduced. Second, it is likely that large *P. herbstii* reach a size refuge from predation in the field (Hill 2011; Heinonen and Auster 2012; Toscano unpublished data) and therefore may have little reason to fear and avoid toadfish. By the opposite reasoning behind our first hypothesis, we expected that larger crabs should exhibit greater flexibility in refuge use due to a size refuge that reduces the importance of predator avoidance, thus elevating the relative importance of their current internal state and environmental conditions. Lastly, we hypothesized that repeatability of refuge use would decline with time spent in the field due to greater opportunity for environmental effects, learning, development, and conditioning: processes that have been demonstrated to drive behavioral change (Bell et al. 2009). Furthermore, individuals for whom repeatability is measured over a long time interval are more likely to change physiological state (e.g., hunger level) between measurements compared with individuals measured over shorter time intervals (Bell et al. 2009), which again should decrease repeatability over time.

## Methods

We first surveyed the individual refuge use of 247 mud crabs in the absence of a toadfish chemical cue and the refuge use of 224 separate crabs in the presence of the cue (i.e., under predation threat) from May to August 2012. All crabs were collected by hand from an oyster reef known as Oyster Landing in North Inlet estuary, Georgetown, SC, USA (33°20'N, 79°10'W). Crabs were collected within a 20 m × 20 m area at the center of the reef. Behavioral measurements were made in a screened-in wet laboratory at the adjacent Belle W. Baruch Institute for Marine and Coastal Sciences. Measuring crab refuge use behavior in the field was not possible due to the high turbidity of water in North Inlet during the summer months that limits visibility (Dame et al. 1986).

### Initial behavioral measurements

The following describes our procedure for a single observational block. Thirty-two observational blocks were run over the course of the study (May–August). Sixteen crabs were collected between 20 and 30 mm carapace width (CW) from Oyster Landing reef, and we attempted to ensure that each

collection reflected the entire crab size range (20–30 mm CW). We randomly assigned eight of these 16 crabs to the toadfish cue absent treatment and the other eight to the toadfish cue present treatment. Due to constraints on the number of crabs a single person could observe in a night, we observed eight crabs per night over two consecutive nights, generally from 2000 to 2300 h. During each night, four crabs receiving the no cue treatment and four crabs receiving the cue treatment were observed. The night measured (first or second) had no effect on refuge use behavior (ANOVA:  $p > 0.05$ ), so the blocking factor used in our analyses was the 2-day span over which 16 crabs were measured. Any crabs molting, carrying eggs or dying during their time in the lab were removed from the data set.

Refuge use was measured following the behavioral assay protocol used in Griffen et al. (2012). All crabs were starved for 24 h before their refuge use behavior was measured. Each crab was observed in a separate glass mesocosm (50 cm × 28 cm × 30 cm) containing a 3 cm layer of sand/mud substrate and 5 L of oyster shell (8–12 cm shell length) that had been dried and cleaned to remove epifauna. This amount of shell ensured that crabs had ample refuge to hide completely. Mesocosms were completely filled with a continuous supply of seawater. Eight large scorched mussels were suspended near the water surface in a mesh bag to release prey chemical cues and induce crab searching behavior while remaining out of reach of crabs. To create the toadfish cue treatment, crabs received a continuous supply of seawater that was first pumped through a holding chamber that contained a single adult oyster toadfish. Crabs assigned the no cue treatment received a continuous supply of seawater pumped through a holding chamber without a toadfish.

Crabs were observed under red light with the observer located behind a blind to minimize crab disturbance. Crabs were first given a 15-min acclimation period in the observation tanks, after which their refuge use was observed once every 6 min over 3 h (30 observations in total for each crab). Refuge use was measured as the proportion of the 30 observations where crabs were completely in the oyster shell refuge and thus invisible to the observer. The refuge was a matrix of shells, so crabs could be hiding under a single shell or multiple shells (i.e., at the bottom of the matrix). When crabs were observed out of the refuge, they were usually walking on top of the shell substrate (i.e., were active). In addition to refuge use behavior, we measured the carapace width and sex of each crab.

### Repeatability and behavioral change

After refuge use behavior was measured, each crab was marked with a unique ID number and released back into the field. To mark crabs, we glued (with super glue) a piece of laboratory labeling tape numbered with permanent marker to

the center of the crab's carapace (Stachowicz and Hay 1999). We realized during the middle of the study that these handwritten numbers were becoming illegible over time in the field, and so the last 80 crabs from the survey were marked using plastic bee tags (queen marking kit: the Bee Works, Orillia, Ontario, Canada) that did not wear over time. All marked crabs were released in a 10 m × 10 m area at the center of Oyster Landing reef. To assess the persistence of refuge use behavior for crabs released into the field, we recaptured the crabs and measured their refuge use for a second time using the same behavioral assay procedure in the lab. Recaptured crabs were observed under the same treatment (toadfish cue absent or present) that they were observed under before release. We recaptured the crabs by hand within the release area of Oyster Landing reef. We searched the reef over two separate search periods (end of July and end of August) until no more marked crabs were recovered at each search period. Because crabs were released regularly over the duration of the study (after each block) but resampled just twice, individual crabs were recaptured after different durations in the field, allowing us to test the effects of duration in the field on behavioral change over time.

#### Statistical analysis

Initial graphical exploration of refuge use behavior over the course of the study revealed persistent oscillations in the mean refuge use observed each night with approximately a 14-day period. These oscillations in behavior appeared to be negatively correlated with the mean tidal level at Oyster Landing (the collection site of crabs) at the time of observation in the lab. Crabs used the refuge most while it was low tide (when they are generally inactive in the field), indicative of a circatidal rhythm in refuge use. We tested for this influence of the tidal cycle on refuge use behavior, among other factors affecting crab refuge use behavior, in the following analysis.

To explore factors influencing crab refuge use behavior (pre-release), we tested the effects of toadfish predation threat, crab carapace width, an interaction between threat and carapace width, and mean tidal level during observation on refuge use with generalized linear mixed models (GLMM, lme4 package in the statistical software R). Female crabs were smaller than male crabs (Welch two-sample *t*-test:  $t = -8.267$ ,  $p < 0.001$ ), which confounded crab sex with crab size. Therefore, we tested the effects of these factors on refuge use separately for males and females. Observational block was modeled as a random factor in both GLMM. Because crab refuge use (the response variable) was proportional, we modeled this behavior using a binomial distribution and logit link (Bolker et al. 2009).

After recapturing a portion of these original crabs (108 crabs recaptured), we calculated the repeatability of their refuge use behavior using pre-release and post-recapture

behavioral measurements. Repeatability ( $r$ ) is defined as the proportion of the total variation that occurs within individuals as opposed to between individuals and is calculated as  $r = s_A^2 / (s^2 + s_A^2)$ , where  $s_A^2$  is the among-individual variance and  $s^2$  is the within-individual variance (Bell et al. 2009; Nakagawa and Schielzeth 2010). Thus, repeatability provides a metric of the amount of behavioral variation between relative to within individuals, where a higher repeatability value indicates a higher level of individual behavioral consistency between measurements. Again, due to the proportional behavioral measure (refuge use), we used GLMM-based repeatability estimation (rptR package in R, Nakagawa and Schielzeth 2010) with a binomial distribution and logit link. Repeatability was calculated separately for crab refuge use in the absence and presence of the toadfish cue, and confidence intervals (95 %) and statistical significance ( $p$ -values) were estimated using parametric bootstrapping with 1,000 resamplings.

Next, we explored factors driving change in refuge use behavior (i.e., deviation from perfect repeatability) of recaptured crabs after time in the field. We calculated behavioral change by subtracting the value of the first behavioral measurement (pre-release) from the second behavioral measurement (post-recapture). Behavioral change was log-transformed to meet assumptions of linear regression. To test for a predominant direction in behavioral change, we first tested whether behavioral change was significantly different than zero in the absence and presence of toadfish predation threat using one-sample *t*-tests. We then used general linear models testing the fixed effects of duration (days) in the field, crab carapace width, and crab sex on individual behavioral change of recaptured crabs. Two separate linear models were used to test the effects of these factors in the absence and presence of toadfish predation threat.

As previously mentioned, crab refuge use behavior oscillated with a circatidal rhythm over the course of the study. This means that if a recaptured crab was originally observed (pre-release) during one tidal height and observed for a second time (post-recapture) at a different tidal height, then behavioral change would be generated. We tested for this tidal influence on behavioral change as follows. We first subtracted for each individual crab the tidal height when the pre-release observation was made from the tidal height when the post-recapture observation was made. We then used the absolute value of this difference as a factor (termed 'tidal influence') in general linear models testing the effects of duration in the field, carapace width, sex, and the tidal influence on the absolute value of behavioral change in the absence and presence of predation threat. This analysis allowed us to explore the relative influences of these factors on the overall magnitude of behavioral change.

Lastly, we tested for the differential recapture of crabs with low versus high refuge use (i.e., a sampling bias). We did this

by comparing the recapture rate of crabs from the lower and upper quartiles of refuge use behavior using Fisher's exact tests. We conducted this analysis separately for crabs with refuge use measured in the absence and presence of toadfish predation threat.

## Results

The presence of toadfish predation threat caused crabs to spend more time in the oyster shell refuge (Table 1, Fig. 1). Large crabs spent less time in refuge than small crabs (Table 1, Fig. 1), and this negative effect of crab size on refuge use was enhanced in the presence of predation threat (Table 1, Fig. 1). Mean tidal level in the field during behavioral observation in the lab reduced the refuge use of male crabs but not female crabs (Table 1).

We recaptured 108 crabs out of the 484 crabs that were released over the course of the study. However, 30 of these crabs had labels where the ID number had faded beyond recognition, leaving 78 identifiable crabs. Thirty-five of these crabs had been assayed for refuge use in the absence of toadfish predation threat, while the other 43 had been assayed for refuge use in the presence of toadfish predation threat. The duration that these recaptured crabs spent in the field ranged from 11 to 81 days, with a mean of about a month (mean $\pm$ 1 SD: 32 $\pm$ 16 days).

Refuge use both in the absence ( $r=0.021$ , 95 % CI: 0–0.066,  $p=0.032$ , Fig. 2a) and presence of toadfish predation threat ( $r=0.173$ , 95 % CI: 0.084–0.304,  $p=0.001$ , Fig. 2b) was repeatable over time, though repeatability was approximately eight times higher in the presence of threat. Furthermore, the significance of repeatability in the absence of predation threat was driven by an influential data point

**Table 1** Results of generalized linear mixed models (GLMM) testing the effects of fixed factors on pre-release refuge use behavior of male and female crabs.  $P$ -values of significant model factors ( $\alpha=0.05$ ) are shown in bold. Observational block was modeled as a random factor in these models

Model factors	Estimate	SE	$z$	$P$
Response: pre-release refuge use behavior of male crabs				
Predation threat	-3.652	0.438	-8.336	<0.001
Crab size	-0.224	0.013	-17.626	<0.001
Tidal level	-0.359	0.120	-2.984	0.003
Predation threat $\times$ crab size	0.115	0.016	6.962	<0.001
Response: pre-release refuge use behavior of female crabs				
Predation threat	-3.718	0.909	-4.089	<0.001
Crab size	-0.374	0.028	-13.203	<0.001
Tidal level	0.101	0.283	0.358	0.721
Predation threat $\times$ crab size	0.106	0.037	2.898	0.004

(Fig. 2a, see figure caption); when removed, repeatability was reduced substantially ( $r=0.011$ , 95 % CI: 0–0.048) and was no longer significant ( $p=0.139$ ).

Neither behavioral change in the absence (one-sample  $t$ -test:  $t=-1.245$ ,  $p=0.222$ ) or presence (one-sample  $t$ -test:  $t=1.541$ ,  $p=0.131$ ) of toadfish predation threat was significantly different from zero, indicating no predominant direction of behavioral change. None of the factors tested including time in the field, crab size, or crab sex had any effect on directional behavioral change in recaptured crabs (Table 2). We also tested whether these same factors, as well as the 'tidal influence' factor, affected the overall magnitude of behavioral change regardless of direction. Again none of these factors (duration in the field, crab size, crab sex, or the tidal influence) had a significant effect on absolute behavioral change, either in the absence or presence of toadfish predation threat (general linear models:  $p>0.526$ ).

Lastly, the recapture rate of crabs with low refuge use versus high refuge use did not differ. This was true of crabs with refuge use measured in the absence (Fisher's exact test:  $p=0.459$ ) and presence (Fisher's exact test:  $p=0.285$ ) of toadfish predation threat.

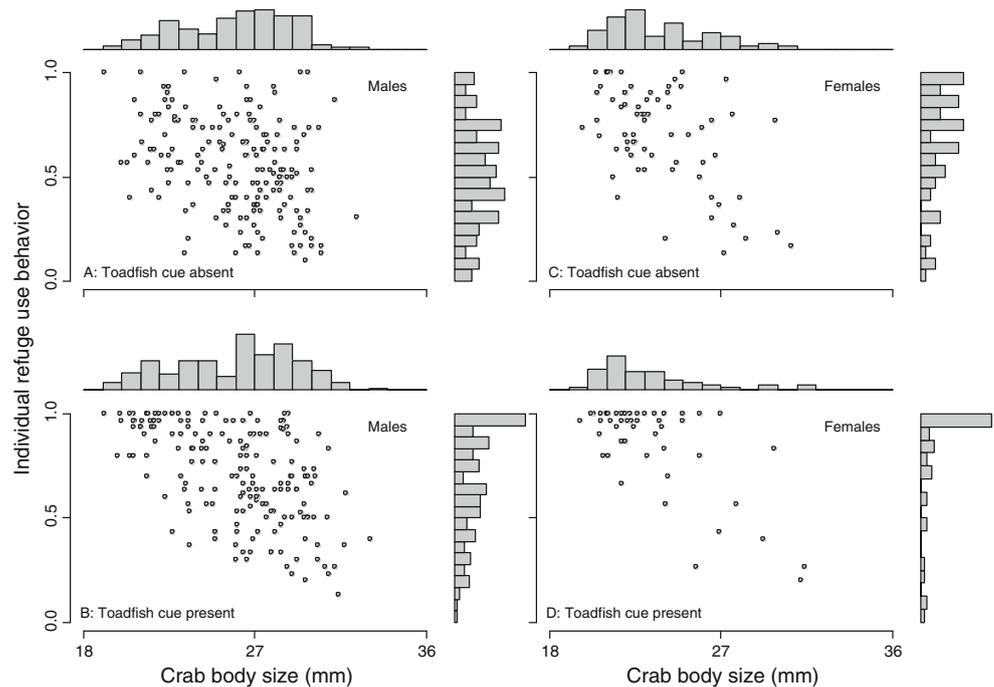
## Discussion

Our study revealed repeatability of crab refuge use behavior after substantial time in the field (a month on average, up to 81 days) and strong size scaling of this behavioral trait. Most interestingly, the presence of predation threat during behavioral observation increased the repeatability and size dependence of individual crab refuge use behavior. Furthermore, contrary to our hypotheses, we found that time in the field between behavioral measurements and crab body size did not influence change in refuge use behavior over time in wild crabs. Below, we discuss potential reasons for these effects of predation threat on the repeatability and size scaling of refuge use behavior, as well as the implications of these results for the community ecology of oyster reefs.

### Factors driving crab refuge use behavior

By measuring crab behavior daily over 3 months, we were able to detect a persistent effect of the tidal cycle on crab refuge use behavior measured in the lab. Had our study been conducted over a shorter duration (and thus over fewer oscillations of the tidal cycle), we likely would have overlooked this tidal influence on behavior. Male crabs used the refuge less (i.e., were most active) when they were observed during high tides at the Oyster Landing reef where crabs were collected. Though the influence of the tidal cycle on the behavior of other crabs species (e.g., Barnwell 1966; Saigusa 1992) and marine invertebrates in general (Palmer 1973) has been shown

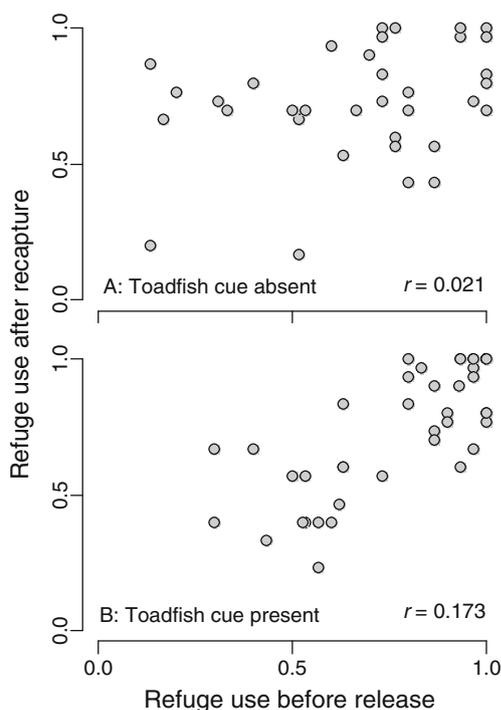
**Fig. 1** Size scaling of individual refuge use behavior in male and female mud crabs (*Panopeus herbstii*) with histograms of body size and behavior distributions: **a** Male crab refuge use in the absence of toadfish (*Opsanus tau*) predation threat, measured as the proportion of observations over 3 h, where a crab was observed taking refuge under oyster shell; **b** male crab refuge use in the presence of toadfish predation threat, measured in the same way but in the presence of chemical cues from toadfish; **c** female crab refuge use in the absence of toadfish predation threat; **d** female crab refuge use in the presence of toadfish predation threat



to dissipate with time in the lab, the crabs used in this experiment were collected from the field just 24–48 h before behavioral observations. This lack of a substantial acclimation

period could explain the remaining tidal influence on refuge use behavior shown here. We discuss the potential effects of the tidal cycle on measuring the persistence of crab behavior below in ‘repeatability and behavioral change’.

In line with previous work (Griffen et al. 2012), we found that individual refuge use in *P. herbstii* is negatively related to crab body size and positively related to toadfish predation threat. However, the study by Griffen et al. (2012) differed from ours in that they used larger crabs (>30 mm) and found that the size scaling of refuge use behavior was the same in the absence and presence of toadfish predation threat. In contrast, using smaller crabs (<30 mm), we found that the effect of body size on refuge use was more pronounced in the presence than absence of predation threat for both male and female crabs (Fig. 1); smaller crabs in particular spent more time in refuge and less time active in the presence of threat. The



**Fig. 2** Individual refuge use behavior of marked and recaptured mud crabs (*Panopeus herbstii*) measured in the absence (**a**) and presence (**b**) of toadfish (*Opsanus tau*) predation threat; repeatability ( $r$ ) of refuge use was statistically significant in both cases ( $\alpha=0.05$ ); however, significance of repeatability of refuge use in the absence of predation threat was dependent on an influential data point (bottom left corner of panel **a**)

**Table 2** Results of general linear models testing the effects of fixed factors on directional behavioral change measured in the absence and presence of toadfish predation threat

Model factors	$\beta$	SE	$t$	$P$
Response: directional behavioral change (toadfish cue absent)				
Duration (days) in the field	0.003	0.003	0.907	0.371
Crab size	-0.006	0.016	-0.344	0.733
Crab sex	0.128	0.113	1.131	0.267
Response: directional behavioral change (toadfish cue present)				
Duration (days) in the field	-0.003	0.002	-1.141	0.261
Crab size	-0.013	0.008	-1.522	0.136
Crab sex	0.004	0.060	0.063	0.950

enhanced size scaling of refuge use under predation threat shown here is most likely the result of the heightened vulnerability of small crabs to predation. Toadfish, a major consumer of mud crabs in South Carolina (Wilson et al. 1982), are gape-limited predators (Gudger 1910), and the resident toadfish that inhabit North Inlet's reefs are generally small (<15 cm, Toscano personal observations). Though not confirmed, it is likely that larger crabs gain a size refuge from predation by resident toadfish in the field, as has been observed in other systems (Hill 2011; Heinonen and Auster 2012). This is supported by feeding trials in the lab (Toscano unpublished), where toadfish (mean total length $\pm$ 1 SD: 14.73 $\pm$ 2.58 cm) not limited by their gape size were averse to eating larger crabs (>22 mm carapace width), perhaps due to the damage these crabs can inflict with their claws. Thus, smaller crabs below this size refuge have reason to be more responsive to predation threat, while large crabs, safe from toadfish predation, would gain no benefit and instead lose foraging opportunities by modifying their behavior in the presence of a toadfish risk cue, particularly when foraging opportunities are limited (these intertidal crabs can only forage during high tides).

The mesocosms where behavior was measured contained live mussels that released prey cues, stimulating crab foraging behavior. Thus, refuge use in this study was a measure of the risk that a crab is willing to take (i.e., its boldness) in order to gain energy through foraging. Numerous studies have detected such a link between individual body size and boldness, but both positive and negative relationships have been reported (Dowling and Godin 2002; Brown and Braithwaite 2004). In situations where a prey reaches a size refuge from predation (e.g., the present study), it should be expected that boldness is positively related to body size. Alternatively, small individuals may be less averse to risk when foraging under predation threat due to their proportionally higher metabolic rates and thus greater energy requirements (Dowling and Godin 2002; Brown and Braithwaite 2004). This indicates that the relationship between body size and boldness can depend on both individual-level (i.e., prey metabolic rate) and community-level processes such as predator prey dynamics.

#### Repeatability and behavioral change

Our recapture rate of marked *P. herbstii* was fairly high: about 22 % of crabs released were recaptured within the 10 m $\times$ 10 m release area. This rate of recapture is in general accord with a previous study (Stachowicz and Hay 1999), in which 20 *P. herbstii* were marked and released into an intertidal oyster reef, and six individuals were recovered within a 5 m<sup>2</sup> search area after 48 h. While our recovery rate was similar, the duration of time that crabs spent in the field here was much greater (a month on average but up to 81 days). This suggests that *P. herbstii* movement rates within and between North Inlet's reefs are fairly low, considering that we failed to

recapture crabs that molted or died during the course of the study yet still recaptured a substantial portion of crabs released.

We hypothesized that crab refuge use would be more repeatable in the presence versus absence of predation threat because the fear of dying, a severe fitness consequence, should take precedence over other influences on behavior (e.g., current energetic state or social situation) that can introduce behavioral variability (termed here ‘the predation hypothesis’). A related explanation is that higher repeatability of refuge use under predation threat is driven by the stronger size dependence of refuge use in the presence versus absence of threat (Fig. 1). Crab body size was fixed over the duration of this study (none of the recaptured crabs had molted), and behaviors that are dependent on some fixed property of the individual should be more consistent over time than behaviors that depend on shorter-term changes in an individual's internal state or environmental conditions (termed here ‘the fixed-trait hypothesis’) (Bell et al. 2009). Either or both of these non-mutually exclusive drivers of behavioral trait persistence could be operating in the present study. The predation hypothesis could be tested by manipulating crab energetic state (e.g., hunger level) and testing repeatability in the presence and absence of predation threat, with the expectation that repeatability should decrease faster in the absence of predation threat with increasing hunger level (Dowling and Godin 2002). The fixed-trait hypothesis could be tested by measuring the behavioral change of crabs over successive molts, with the expectation that behavior will change with increasing body size.

In a meta-analysis of repeatability estimates from a wide range of both invertebrate and vertebrate species, Bell et al. (2009) found that repeatability generally decreased with time between behavioral measurements. Contrary to our hypothesis, we found no evidence of behavioral change dependent on time. This is despite crabs spending up to 81 days in the field, and for reference, the lifespan of *P. herbstii* has been estimated at 2.3 years (McDonald 1982). However, we cannot discount the possibility that we tended to recapture crabs that were more consistent in their behavior over time, though we detected no bias regarding the differential recapture of crabs with low versus high refuge use behavior. Clearly, crabs change their refuge use behavior over ontogeny (Fig. 1), but the lack of behavioral change with time in the present study suggests that most behavioral change happens during molting when crabs grow or at other times of the year. This consistency of behavior over time while crab body size was fixed again suggests high repeatability of individual behavior when that behavioral trait is dependent on a fixed trait of the individual.

As previously mentioned, we detected an influence of the tidal cycle on crab refuge use behavior over the course of the study. Measuring an individual crab at two different tidal levels (pre-release, post-recapture) in this tidally influenced behavioral cycle should reduce behavioral consistency, even if

the individual's actual BT relative to other crabs is not changing. Our analysis however did not detect any affect of the tidal cycle on behavioral change. This could be due to individual variation in responsiveness to the tidal rhythm, as has been shown in other marine invertebrates (Palmer 1973). Still, such persistent circatidal rhythms in activity have been observed in a variety of intertidal marine invertebrates (Palmer 1973), and biological temporal rhythms are taxonomically widespread (Dunlap et al. 2004). Thus, behavioral ecologists should be cognizant of such endogenous temporal rhythms in behavior, particularly when taking repeated longitudinal behavioral measurements to assess behavioral consistency (Koski 2011).

#### Implications for oyster reef community ecology

Individual crab refuge use behavior and body size, as studied here, have previously been shown to mediate the consumption rate of crabs foraging on mussels in the presence of toadfish predation threat (Griffen et al. 2012), thereby determining the strength of indirect interactions that are a major community-structuring force in oyster reefs (Grabowski 2004; Grabowski and Kimbro 2005). The repeatability of refuge use revealed here suggests that this crab behavioral trait is relatively stable over time in the field. Thus, any ecological effects of crab refuge use are not just a transient feature of the reef food web. Rather, if individual crabs remain on the same reef or within the same location within a reef for long periods, then their individual refuge use behavior could drive spatial variation in ecological interactions (Griffen et al. 2012). Such testable predictions can only be made by measuring the persistence of BTs and the drivers of persistence in natural field situations, thus advancing mechanistic study of the ecology of individual behavior (Sih et al. 2012).

**Acknowledgments** Funding was provided by the National Science Foundation Graduate Research Fellowship under Grant No. DGE-0929297 and by the National Science Foundation Grant No. OCE-1129166. We thank D.S. Wethey, J.L. Dudyca and the anonymous reviewers for the helpful discussions and suggestions that improved the manuscript.

**Ethical standards** This research complies with the current laws of the United States.

**Conflict of interest** We declare no conflict of interest.

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