Influence of the commensal gastropod *Crepidula plana* on shell choice by the marine hermit crab

Pagurus longicarpus, with an assessment of the degree of stress caused by different eviction

techniques

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ABSTRACT

Hermit crabs are notoriously choosy about the gastropod shells they live in. Many periwinkle shells at Nahant, Massachusetts, U.S.A. contain the gastropod *Crepidula plana*, which forms a thin flat shell inside the periwinkle shell. This study examined how the presence of *C. plana* affected shell selection behavior by the hermit crab *Pagurus longicarpus*: naked hermit crabs were offered different combinations of shells, including some containing *C. plana* and some that had been drilled by naticid gastropods, and their choices were recorded. Because we had to evict hermit crabs from their shells before conducting our choice tests, we also determined rates of recovery from five commonly used shell eviction techniques. No hermit crabs were successfully evicted using low salinity seawater. For the other techniques used, hermit crabs took significantly longer to recover after being removed by gentle but continuous pulling on the carapace and appendages than after being removed by immersion in heated seawater, cracking the shell using a vise, or prodding the hermit crab in the abdomen through a hole drilled in the shell. Even so, most hermit crabs recovered from all of the stresses tested within 20 h, and from at least some stresses within 2-6 h. If shells are to be re-used for experiments, heating seawater is the best technique for evicting *P. longicarpus*; otherwise, cracking the shell using a vise is recommended. When provided with intact shells and similarly-sized shells containing adult *C. plana*, all of the hermit crabs chose shells without *C. plana*, and did so within only about 30 min. However, given a choice between shells housing *C. plana* and those bearing drill holes—shells which they normally avoid—the hermit crabs were about evenly divided in their choice of shells, and many of the hermit crabs never made a final decision even after 18 or 19 h, suggesting that the presence of *C. plana* greatly reduces shell attractiveness and suitability. The presence of *C. plana* increased total shell weight and reduced internal shell volume considerably, and also weakened the shell substantially, likely making the hermit crabs more vulnerable to predators; all

of these factors may play a role in selecting for the intense avoidance behavior exhibited by this species for periwinkle shells bearing *C. plana*.

1. Introduction

Marine hermit crabs tend to be very particular about the shells they occupy: given options, they carefully evaluate empty shells for size, weight, weight distribution, geometry, internal volume, and various types of damage (e.g., Arce and Alcaraz, 2012; Bertness, 1980; Conover, 1978; Elwood, 1995; Fotheringham, 1976a; Hazlett, 1987, 1989; Kaiser et al., 2005; Reese, 1962; Turra and Leite, 1992; Wilbur, 1990). Shell appeal can also be affected by the presence of various organisms living in or on the shells (Briffa and Elwood, 2005; reviewed by Williams and McDermott, 2004). For example, at Nahant, Massachusetts the slipper snail *Crepidula convexa* is often found living on the outer surface of empty periwinkle shells (*Littorina littorea*) (Li and Pechenik, 2004). The hermit crab *Pagurus longicarpus* strongly avoided such shells when the wet weight of the symbiont was about 10% or more of the empty shell weight (Li and Pechenik 2004), either because of the slipper snail's impact on total shell weight or weight distribution, or both (Arce and Alcarez, 2012; Conover, 1976; Li and Pechenik, 2004).

Many periwinkle shells at Nahant also provide habitat for the related gastropod *C. plana*, which lives inside the shells; hermit crabs are often found occupying such shells (Coe, 1948; Gould, 1952; McDermott, 2001; McGee and Targett, 1989; Scully, 1979; Shenk and Karlson, 1986; Williams and McDermott, 2004). The hermit crab *Pagurus longicarpus* ranges from Nova Scotia to Texas and Florida (Kuhlmann, 1992; Wilber Jr., 1989), while the gastropod *Crepidula plana* ranges at least as far south as Georgia (Collin 2000), and possibly as far north as Prince Edward Island in Canada (Collin, 2000; Hoagland, 1977); thus these two species are likely to have been interacting routinely throughout much of their range.

The shells of *C. plana* are quite thin and flat, with the forward edge placed near the periwinkle shell's aperture and the shell itself taking up much of the inner surface of the host shell's outer body whorl (Hoagland, 1974; JP, CD, RB personal observation). We have found more than 15% of the hermit crabs sampled at our study site to be living in shells harboring *C. plana* internally in some years (JP, unpublished data). However, when Conver (1976) gave individuals of *P. longicarpus* a choice between shells with and without *C. plana*, most individuals (40 out of 54) avoided shells bearing *C. plana*. Very few methodological details were provided in that study, and the influence of *C. plana* on total shell weight, internal shell volume, and shell integrity were not reported. The degree to which such shells are avoided when hermit crabs are given a variety of shell choices has also not been examined previously.

In this study, we offered naked hermit crabs (*P. longicarpus*) a choice of shells with and without *C. plana* in a series of shell choice experiments. We also tried to determine how much these hermit crabs avoid occupying shells that harbor *C. plana* by offering naked hermit crabs two bad choices: shells that either had *C. plana* living within them or that were of similar size but that had been drilled by moonsnails (Pechenik and Lewis, 2000). *Pagurus longicarpus* is

extremely reluctant to occupy drilled shells: in previous studies, Pechenik and Lewis (2000) found that hermit crabs of this species would occupy drilled shells only when the intact shell offered as an alternative was dramatically smaller than the preferred size—suitable in fact for hermit crabs only ¼ the weight of those being tested. In contrast, given the choice between a drilled shell of appropriate size for that crab and a smaller intact shell suitable for a hermit crab ½ the weight of the crab being tested, about 95% of the hermit crabs chose the too-small but intact shell over the appropriately-sized drilled shell, and made their decision within only about 30 min (Pechenik and Lewis, 2000). These hermit crabs are clearly averse to occupying drilled shells.

Because symbionts may alter shell strength, in either direction (Buckley and Ebersole, 1994; Statchowitsch, 1980)--secretions from *C. plana*, for example, might thin and weaken the shells, or the symbiont might instead strengthen the shells by increasing functional shell thickness--we quantified the impact of *C. plana* on the force required to break the periwinkle shells housing them. We also quantified the influence of the potential symbiont *Crepidula plana* on the internal volume and total weight of *Littorina littorea* shells.

Finally, because we had to evict hermit crabs from their shells before conducting our shell-choice experiments, we also examined the impact of several shell removal techniques on hermit crab behavior. Hermit crabs have been removed from their gastropod shell homes in a variety of different ways in different studies (* indicates studies that focused on or included *P. longicarpus*): crushing or cracking the shells with a vise or pliers (e.g., Bach et al., 2006*; Briffa and Dallaway, 2007; Briffa and Elwood, 2000; Briffa and Twyman 2011; Briffa et al., 2013; de la Hay et al., 2011; Hazlett, 1993; McGuire and Williams, 2010*; Turra and Gorman, 2014; White et al., 2013; Wilber Jr., 1990*); heating in fresh water (e.g., Vance, 1972; Scully, 1979*;

Whitman et al., 2001*); heating the shell apex with a soldering iron, flame, or other means (e.g., Arce and Alcarez, 2012; Bertness, 1980, 1981; Orians and King, 1964; Kellogg, 1976, 1977; Reese, 1962; Straughn and Gosselin, 2014); anesthetizing the hermit crab with xylocain or warmed seawater (e.g., Blackstone and Joslyn, 1984; Gravel et al., 2004; Osorno et al., 1998); gentle but prolonged pulling on the head and claws (e.g., Angel, 2000; Gravel et al., 2004; Straughan and Gosselin, 2014); drilling a hole in the shell and poking the abdomen with monofilament line or a flexible plastic rod (e.g., Conover 1976*; Hazlett et al., 2005; McClintock, 1985); inserting a plastic strip into the shell aperture and poking the abdomen (e.g., Barnes, 1999; Barnes and de Grave, 2002); or burying the hermit crab in sediment (Turra and Gorman, 2014). Some of those techniques may be more stressful than others, and might influence the outcome of subsequent shell selection studies. We therefore sought to determine whether hermit crabs recover more quickly following some eviction procedures than others, and in particular whether they recovered more quickly following techniques that removed the hermit crabs without destroying the shells. The eviction techniques tested included subjecting the hermit crabs to reduced ambient salinity or to mild heat stress in seawater (34°C), drilling a hole near the apex of the shell and prodding the animal's abdomen with monofilament line through the hole, manually removing the hermit crab by pulling gently but continuously on the claws, and gently cracking the shell with a vise.

2. Materials and methods

2.1. Collection and maintenance of animals

All hermit crabs (*Pagurus longicarpus*) used in this study were collected intertidally at low tide from Nahant, Massachusetts, U.S.A. Only hermit crabs inhabiting periwinkle (*Littorina littorea*) shells were collected; approximately 95% of hermit crabs in shells with a total length

larger than about 3 mm are found in shells of that species at this site (JP, unpubl. data).

Approximately 80 hermit crabs living in shells with the commensal gastropod *C. plana* were

collected at low tide in October 2011 along with another group of good quality hermit crab-

occupied shells that did not contain *C. plana*, to determine the impact of *C. plana* on shell weight

and internal volume. Approximately 200 hermit crabs were collected in June 2012 for

experiments on the degree of stress caused by different eviction techniques and another 150 were

collected in August 2012 and again in August 2013 for shell selection experiments. Additionally,

to investigate the impact of *C. plana* on shell strength, approximately 50 intact *L. littorea* shells

without *C. plana* were collected in October 2013, along with another 50 shells containing adult

C. plana. All shells contained hermit crabs when collected. In the laboratory, the hermit crabs

were maintained in two 10-gallon glass aquaria containing 50% artificial seawater (Instant

Ocean, salinity 30 ppt) and 50% unfiltered natural seawater from Nahant (~30 ppt) at

approximately 23°C with constant aeration. Cylinders (made using cable ties) of 8 mm diameter

mesh stiff plastic nylon screen were placed in the tanks to divide the space into sections and to

allow individuals to also isolate themselves by climbing. The hermit crabs were periodically fed

a mixture of fish (artificial crab meat) and shrimp pellets *ad libitum* and were tested within 30

days of being collected.   All animals were returned to the collection site when the studies were

completed.

2.2. Extraction procedures used

In evaluating recovery from extraction, we considered five of the most widely used

techniques: manual extraction, removal by cracking the shell with a vise, heating the animal,

subjecting the animal to low salinity stress, and drilling-poking. Manual extractions were

accomplished by gently holding the hermit crab's thorax behind the eyes and gently pulling forward until the hermit crab released its grip on the internal shell columella. The vise extraction was accomplished by placing the shell (aperture facing upwards) of each hermit crab into a table-mounted vise and gently increasing the pressure applied until the shell cracked sufficiently; we then removed all shell fragments. For the thermal stress extraction, we heated the artificial/natural seawater mixture to 34°C in 200 ml of seawater in a glass dish on a hotplate. The hermit crab was completely submerged within the warmed seawater for up to several minutes. Once the hermit crab had partially emerged from its shell, gone limp, and released its hold on the shell columella, it was gently pulled from the shell by hand while it was still submerged and then removed to well-oxygenated seawater at room temperature. In the final successful eviction treatment tested, a small hole was drilled (Dremel drill model 260, 1 mm bit) into the periwinkle shell near the hermit crab's abdomen. Most hermit crabs vacated the shell while it was being drilled, but if they did not leave the shell voluntarily the hermit crab's abdomen was then prodded with monofilament fishing line through the hole until the shell was abandoned.

Low salinity stress was also explored as a potential shell eviction technique. Treatment boxes were set up to contain 30 ppt, 25 ppt, 20 ppt, and 15 ppt seawater solutions, with dilution achieved using deionized water. Five hermit crabs in periwinkle shells were placed into each salinity treatment and monitored for activity, with the intention of pulling them gently from the shell when they became limp.

The procedures used for extracting hermit crabs are summarized in Table 1.

2.3. Evaluating the immediate effects of extraction

Shell choice experiments were conducted following previously used protocols (Pechenik and Lewis, 2000). Fifteen hermit crabs were chosen haphazardly for each shell eviction treatment. Each hermit crab's shell aperture diameter was measured with calipers to the nearest 0.1mm. Two empty periwinkle shells similar in aperture size to that of the shell originally occupied by the hermit crab were placed in an 8 cm x 12 cm plastic box containing approximately 175 ml of oxygenated seawater, which was enough to completely submerge the hermit crab. One shell in each box was intact while the other shell had previously been drilled by a moonsnail (*Lunatia heros*). Both shells were without symbionts.

Each hermit crab was evicted from its shell using one of the 4 treatment methods described in Section 2.2, and immediately placed in a treatment box at a point equidistant from both empty shells. The hermit crabs were not allowed to investigate the empty shells prior to their eviction. The crabs were then monitored continuously for 120 min, and the time taken for each hermit crab to occupy either shell was measured to the nearest second. Because hermit crabs much prefer to inhabit intact shells over drilled shells of comparable size (Pechenik and Lewis, 2000), the time taken for each hermit crab that had initially chosen a drilled shell to finally choose an intact shell was also measured. All assays were run under fluorescent laboratory lighting at room temperature, 20-22°C. Each hermit crab was used in only one experiment.

2.4 Evaluating recovery from different eviction techniques

To determine if the hermit crabs could eventually recover from eviction stress, the above experiment was repeated using a different group of hermit crabs (N = 10 individuals per treatment), except that the naked hermit crabs were now given 20 h to recover from the eviction

procedures before being presented with the two new shells. We also ran one additional experiment using our two favored eviction techniques—heated seawater and the vise—this time giving the evicted hermit crabs (N = 15 per treatment) only 6 h to recover before being tested. Note again that no hermit crabs were used in more than one experiment.

2.5   Discrimination between shells with and without Crepidula plana

Fifteen hermit crabs were evicted by placing them in warmed seawater (34ºC) for up to several minutes, as described previously. Each naked hermit crab was then weighed to the nearest 0.01g and held individually in room-temperature seawater for 6 h. Each naked hermit crab was then placed in the center of a rectangular plastic box containing a mixture of artificial and filtered natural seawater at room temperature and two *L. littorea* shells were placed adjacent to each other at one end of the box. One shell was intact and without symbionts and the other shell was intact but contained an adult of the gastropod *C. plana*. Both shells were the appropriate size for each hermit crab, based on its wet weight (Angel 2000). After placing each hermit crab into a box, we monitored them continuously, recording when each hermit crab moved into a shell and whether it had moved into the shell with or without the associated *C. plana*. After the hermit crabs had made their initial choice of shells, we checked the shell choice of each hermit crab after an additional 15 min, 30 min, 1 h, and 3 h to see whether it had left its originally chosen shell or not. The final shell chosen was recorded after 18 h.

2.6. The hermit crab's dilemma: responses when faced with 2 bad choices

Two experiments were undertaken to determine the degree to which hermit crabs of this species would avoid living in shells harboring *C. plana*. Fifteen hermit crabs were evicted from

their shells by placing them for several minutes in warm seawater (34°C). Each naked hermit

crab was weighed to the nearest 0.01 g and placed in a separate container at room temperature

for later use. Six h after eviction, each hermit crab was placed in the center of a rectangular

plastic box containing 175 ml of artificial seawater as described earlier. At one end of each box

were two *L. littorea* shells. In one experiment, the two shells were the appropriate size for each

hermit crab, based on the animal's wet weight (Angel 2000), but one shell contained a large *C.*

plana and the other was empty but had a hole that had been drilled by a moon snail (Pechenik

and Lewis, 2000).

In a second experiment, naked hermit crabs were again offered one shell that contained

C. plana and one shell with a drill hole. However, in this second experiment the drilled shells

were of the appropriate size for each hermit crab, whereas the shells containing *C. plana* were

chosen to have the same internal volume as the drilled shells, but were necessarily larger since *C.*

plana reduces the internal volume of periwinkle shells (see Section 2.7). Hermit crabs were then

monitored as described above, noting the first shell chosen by each hermit crab and then the shell

chosen after 15 min, 30 min, 1 h, 3 h, and 18 h. In this second experiment, the hermit crabs were

monitored for one additional hour after the first 18 h to see if they had all made their shell final

selections.

2.7. *Effect of* Crepidula plana *on periwinkle shell characteristics*

In order to determine the effect of *Crepidula plana* on the weight of *Littorina littorea*

shells, 79 *L. littorea* shells housing *C. plana* and 81 *L. littorea* shells without *C. plana* ("empty")

were examined, covering a range of aperture lengths between 10.2 and 18.4 mm. All shells had

originally contained hermit crabs. Aperture lengths were measured with calipers to the nearest

0.1 mm. All shells were shaken in air to remove most of the seawater from inside the shell. A flexible plastic pipet was then used to remove any seawater remaining near the shell apex. The outside of each shell was then blotted with a paper towel and shells were weighed to the nearest 0.1 mg using a Mettler Toledo AL104 balance.

To determine the effect of *C. plana* on the internal volume of *L. littorea* shells, 28 of the *L. littorea* shells housing *C. plana* and 24 of the *L. littorea* shells without *C. plana* (empty shells) were examined, covering a range of aperture lengths between 8.8 and 18.4 mm. Internal volume was determined by first wetting the shells, blotting them dry, and weighing them empty to the nearest 0.1 mg; shells were then agitated in a beaker of seawater to dislodge all air. We then removed the shells from the water with the aperture facing upwards and, keeping the shells in the same orientation, added seawater using a pipet until the water was level with the plane of the aperture. Shells filled with seawater were then re-weighed and the internal volume was determined by converting the weight of the seawater to volume.

We also examined the relationship between the presence of *C. plana* and resistance of the periwinkle shells to crushing. Following the approach used in several previous studies (e.g., Bach et al., 2006; Barnes, 1999; Buckley and Ebersole, 1994; Gravel et al., 2004; LaBarbera and Merz, 1992; Pechenik et al., 2001), we measured the force required to crack shells as a way of measuring shell integrity. Thirty-one empty *L. littorea* shells in perfect condition (i.e, no holes, no apex or aperture damage, and no symbionts) with aperture lengths ranging from 11.1 to 17.2 mm and 31 intact *L. littorea* shells harboring *C. plana* covering a range of aperture lengths between 11.2 to 17.2 mm were selected to determine the ultimate failure load. The mean sizes (determined by aperture length) of the shells chosen for each group were nearly identical (t = 0.0395, d.f. = 60, p = 0.969). All shells used had previously housed hermit crabs.

The tests were performed using an Instron Model 3366 uniaxial compression-testing machine with displacement and force accuracies of, respectively, 0.057 micron and 0.001 N. The machine was equipped with standard compressive loading platens and with a dedicated load cell of 1 kN. Experiments were performed at room temperature with a constant displacement rate of 1 mm/min. All recorded data (time, actuator position, and load cell readings) were captured at a sampling rate of 20 Hz.

All shells were kept hydrated before tests were initiated. At that point, one shell at a time was placed on the flat metal plate with the aperture facing downwards (Fig. 1), as in several previous studies (Buckley and Ebersole, 1994; Pechenik et al., 2001), although aperture orientation apparently has no measurable effect on the force required to crack the shells (Pechenik et al., 2001). Loading, with constant displacement rate, was continued until complete shell failure occurred.

2.8.   *Data analysis*

All statistical tests were conducted using Prism 4.0 (Graphpad, Software Inc). A one-way ANOVA was used to determine if there were any significant differences in mean shell length for shells used in the eviction studies. For the time to enter a shell after eviction and time to enter an intact shell after eviction, each treatment was tested for normality using a D'Agostino and Pearson omnibus normality test. Since almost none of the treatments produced data that were normally distributed, the times to enter a shell and the times to enter an intact shell after each eviction technique were compared using the non-parametric Kruskall-Wallis test. Dunn's multiple comparisons post tests were subsequently used to test for significant differences between specific treatments. For the experiment testing the stress of eviction techniques after a 6

hour recovery period, the non-parametric Mann-Whitney test was used to compare the results from the two treatments (removal by vise vs. heating). Values for aperture diameter, shell weight, and internal volume were all log transformed before conducting regression analyses. After regressing shell weight and internal volume separately against aperture diameter, regression lines of shells containing or lacking *C. plana* were compared by analysis of covariance (ANCOVA).

The data from hermit crab shell selection studies (intact and empty shells vs. intact shells of the same size but bearing *C. plana*; drilled and empty shells vs. intact shells of the same size bearing *C. plana*; drilled and empty shells vs. intact shells of the same internal volume bearing *C. plana*) were analyzed using χ^2 goodness of fit tests.

A one-factor ANCOVA was performed to determine whether a different amount of force was required to break shells with and without *C. plana*, while controlling for differences in shell aperture size; the required force has previously been shown to vary with shell size (Pechenk et al., 2001). The factor was shells with or without *C. plana*, the co-variate was aperture length, and the dependent variable was the force required to break the shell.

3. Results

3.1 *General observations on hermit crab shell selection behavior*

As in previous studies (e.g., Reese, 1962; Wilber, 1990; Elwood, 1995; Côté et al., 1998), naked hermit crabs usually entered the first shell that they encountered and then began examining other options. Hermit crabs displayed several behaviors during these experiments when investigating shell quality. Initially, the hermit crabs investigated shells with their chelipeds to determine shell quality, as reported in previous studies (e.g., Côté et al., 1998; Elwood, 1995; Pechenik and Lewis, 2000; Rees, 1963). The hermit crabs also sometimes investigated shells by

briefly inserting their abdomen into the shell and then withdrawing it. On occasion, hermit crabs switched shells several times before making their final choice.

3.2. Evaluation of eviction techniques

No hermit crabs were successfully evicted using low salinity; all individuals withdrew far into their shells and remained there. The other techniques were all successful in removing hermit crabs from their shells. When hermit crabs were presented with empty shells immediately after being extracted from the original shell, they took significantly less time (usually less than 2 min) to occupy any shell if they had been extracted by drilling or by using the vise (Kruskal-Wallis = 19.35, n = 4, p = 0.0002; Dunn's tests, p<0.05; Fig. 2A). Hermit crabs extracted manually or by heat stress moved sluggishly for some time after extraction compared to those extracted by other means, or not at all. Hermit crabs extracted manually took about twice as long (more than 3 min longer), on average, to enter a shell as did those extracted by using heated seawater, although the difference was not statistically significant (Dunn's tests, p > 0.05)(Fig. 2A).

When evicted hermit crabs were allowed to recover for 20 h before being presented with empty shells, shell-removal technique had no significant effect on the amount of time (a little under 2 min, on average) that hermit crabs took to enter a shell (Kruskal-Wallis = 0.21, n = 4, p = 0.98).   Although a few of the animals that had been removed by drilling took an unusually long time to select a shell (Fig. 2B), those individuals did not otherwise appear to behave any differently than did hermit crabs from other treatments.

For hermit crabs that initially chose a drilled shell soon after being evicted, the time it took them to switch to an intact shell was significantly shorter if they had been removed by using the vise than if they had been removed manually or by heating (Kruskal-Wallis = 10.76, n = 4, p

= 0.013; Dunn's tests, p < 0.05; Fig. 3A); these behavioral differences were not seen if the crabs were given 20 h to recover before being tested (Kruskal-Wallis = 2.14, n = 4, p = 0.55; Fig. 3B).

When given only 6 h rather than 20 h to recover after eviction by vise or by bathing in warm seawater, hermit crabs in both treatments typically entered a shell in only about one minute (average 57 sec for heating, average 49 sec for vise; Mann-Whitney test, U = 99, p = 0.589), and any hermit crabs that initially chose drilled shells quickly switched to intact shells (average 78 sec for the heated seawater treatment, average 172 sec for vise removal treatment; Mann-Whitney test, U = 11, p = 0.165)(data not shown).

The method used to evict hermit crabs had no effect on their ability to eventually choose an adequate shell, as nearly all hermit crabs regardless of treatment chose intact shells over drilled shells and did so within 2 h (Fig. 4; Contingency table analysis, χ^2 = 3.86, df = 3, p = 0.277).

The results of experiments on eviction techniques are summarized in Table 1.

3.3. Effect of Crepidula plana *on periwinkle shell characteristics*

The relationships between log shell aperture length and log shell weight or log shell volume were linear, with or without *C. plana* present, with high r^2 values (Fig. 5). Presence of *C. plana* within the shell had a pronounced effect on both shell weight and shell volume. The *L. littorea* shells that were internally occupied by adult *C. plana* were on average 49.3% heavier (ANCOVA,F = 15.92, d.f. = 1, 156, p = 0.0001 for slopes; Fig. 5A) and had an internal volume that was 36.9% less than that of shells of the same aperture size that lacked *C. plana* (ANCOVA, F = 0.92, d.f. = 1, 48, p = 0.34 for slopes, p <0.0001 for intercepts; Fig. 5B).

3.4. Effect of shell size and C. plana *on shell strength*

Shells housing *C. plana* broke with less applied force than did empty shells (Fig. 6A). Although

the slopes of the two lines relating required compression force and shell aperture length were not

significantly different (ANCOVA, $F_{1,58}$=0.61, P = 0.44), the intercepts did differ significantly

($F_{1,59}$ = 5.49, P = 0.023)(Fig. 6B). Within each treatment, larger shells tended to require more

force to break than smaller shells, as found previously (Fig. 6B)(Pechenik et al., 2001).

3.5. Discrimination between shells with and without Crepidula plana

When naked hermit crabs were presented with both an empty, intact shell and an intact

shell of the same size but with *C. plana* inside, all of the hermit crabs chose to occupy the empty,

intact shell, and all made their final decisions within 30 min; indeed, nearly 90% of the hermit

crabs were occupying the empty, intact shell within 15 min (χ^2 = 8.07, df = 1, p = 0.004, Fig. 7).

Once they occupied an empty, intact shell, the hermit crabs never left that shell.

3.6. The hermit crab's dilemma: action when faced with 2 bad choices

When presented with drilled shells or shells containing *C. plana*, both of the same ideal

aperture diameter for crabs of the determined wet weight (Angel, 2000), hermit crabs did not

show a significant preference for either type of shell (χ^2 = 0.60, df = 1, p = 0.44, Fig. 8A).

Unlike the previous experiment (Fig 7), substantial shell switching behavior was still being

observed one h after the experiment began, although all hermit crabs had made their final shell

choice by 3 h.

When given the same choice as above (drilled shell vs shell bearing *C. plana*) except that

now both the drilled and symbiont-bearing shells had the same internal volume, hermit crabs did

not show a significant preference for either type of shell for at least the first 3 h ($\chi^2 = 0.60$, df = 1, p = 0.44, Fig. 8B). Some hermit crabs in this experiment never made a final decision, as some shell switching behavior was still observed after 18 h. At some time points beyond 18 h, the hermit crabs showed a significant preference for drilled shells over those bearing *C. plana* ($\chi^2 = 5.40$, df = 1, p = 0.02), but frequent switching by some crabs often abolished this effect (Fig. 8B).

4. Discussion

The time taken for *P. longicarpus* to re-enter shells soon after eviction depended on the eviction technique used, suggesting that some eviction techniques used in this study were more physiologically stressful than others. In particular, manual eviction seemed the most stressful (Fig. 2). Surprisingly, using a vise to crack the shell, which was the easiest of all the techniques to execute, was apparently the least stressful (Figs. 2A, 3A). Low salinity stress proved to be an especially poor technique, as all of the hermit crabs withdrew well within their shells and could not be observed or removed.   Several previous studies with *P. longicarpus* employed a combination of low salinity and heat in evicting hermit crabs from their shells (e.g., Scully, 1979; Whitman et al., 2001); successful eviction in those studies was apparently more a function of thermal stress than the stress of low salinity.

Eviction technique had no statistical impact on hermit crab behavior after a 20 h recovery period in our studies, although removal of the crabs by drilling may have been somewhat more stressful than the other techniques used, for at least some individuals (Fig. 3B).   If there is no need to retain the intact shell, then removing the shell using a vise would be the preferred

technique for *P. longicarpus*, as eviction is accomplished quickly and the animals were able to enter new shells within minutes of being evicted. If shells need to be maintained intact after eviction, anaesthetizing the hermit crabs with heated seawater (34 °C) would be the preferred technique, as hermit crabs removed in this way behaved normally in our study within 2-6 h of their eviction. Whether hermit crabs of other species respond to different shell-removal techniques in the same way that individuals of *P. longicarpus* were found to respond remains to be determined. Such information would be particularly important in experiments that examine hermit crab behaviors shortly after their removal from shells (e.g., Coté et al., 1998; Osorno et al., 1998; Reese, 1962), and perhaps less important in studies in which hermit crabs are given at least 6-24 h to recover before being tested (e.g., Arce and Alcaraz, 2012; Briffa and Dallaway, 2007; Briffa and Twyman, 2011; Li and Pechenik 2004).

As previously reported by Conover (1976), individuals of *P. longicarpus* clearly avoided shells housing *C. plana* when offered an empty shell of similar size as an alternative; in our study, final decisions were made within about 30 min (Fig. 7). As oceans are continuing to absorb excess CO_2 and becoming more acidic (e.g., Coleman et al., 2014), future studies might investigate the impact of reduced seawater pH on the ability of *P. longicarpus* to make this distinction between shells that do or do not harbor *C. plana*; shell assessment ability and behavior by the related species *P. bernhardus* has already been shown to be affected by reduced seawater pH (de la Hay et al., 2011).

The responses made by hermit crabs in our study when presented with shells containing *C. plana* depended on the alternatives that were provided. When we gave hermit crabs a choice between two shells of the same size, one harboring *C. plana* and one that had been drilled, the hermit crabs never made a clear decision; even after 18 h in our study, about half the hermit

crabs were in the drilled shells (Fig. 8A), and many were still switching back and forth between shells. This is quite a striking result, as hermit crabs of this species occupying drilled periwinkle shells are far more vulnerable to predatory green crabs (*Carcinus maenus*), the impact of reductions in ambient salinity, and eviction by conspecifics than those in intact shells (Pechenik et al., 2001). Indeed, previous studies have found that these hermit crabs strongly avoided occupying drilled shells of perfect size, even when the only alternative was an intact but smaller shell appropriate for a hermit crab only one-half the weight of the one that was tested (Pechenik and Lewis, 2000), or a shell bearing a large individual of *C. convexa* on the outside (Li and Pechenik, 2004). The full consequences of occupying a shell harboring *C. plana* remain to be determined. Finding hermit crabs occupying such shells in the field (e.g., Pechenik et al., in review) certainly suggests very strong competition for an inadequate supply of high quality shells at the sampled site in some years.

Why do these hermit crabs avoid living in shells housing *C. plana*? The presence of large *C. plana* within shells increased total shell wet weight and decreased internal volume substantially (Fig. 5). However, when confronted with the dilemma of choosing between a drilled shell of ideal size (and volume) without *C. plana* living inside and an intact but larger shell containing *C. plana* but of equivalent internal volume, 80-90% of the hermit crabs eventually chose the drilled shells over the larger shells, although it took them many hours to make that choice (Fig. 8B). This suggests that their reluctance to occupy shells bearing *C. plana* (Fig. 7A; and Conover, 1976) was not primarily because of the reduced internal shell volume, and that available internal shell volume is a less important factor than total shell weight for this species when deciding which shells to occupy, as suggested previously for several hermit crab species (Reese, 1963; Wilber Jr., 1990; Elwood and Neil, 1992; Osorno et al., 1998). Carrying

heavier shells increases energy costs (Briffa and Elwood, 2005; Côté et al., 1998; Elwood and Neil, 1992; Elwood, 1995; Hazlett et al., 2005; Osorno et al., 1998). Even so, it is again remarkable that the hermit crabs preferred drilled shells in this situation, considering the several downsides of living in such shells (Pechenik et al, 2001), as already mentioned.

Pagurus longicarpus may recognize the presence of *C. plana*, perhaps by chemosensory means, and avoid occupying shells containing that snail for reasons other than effects on shell weight or internal shell volume, perhaps having to do with shell integrity. Indeed, we found in this study that shells housing adult *C. plana* were considerably easier to crush than comparably-sized empty shells, probably making resident hermit crabs more vulnerable to predators, as previously reported for hermit crabs occupying drilled shells (Pechenik et al., 2001). The presence of *C. plana* might also hasten the destruction of such shells by crushing predators or by abrasion, thereby decreasing future shell availability to other members of the hermit crab population. Further studies will be needed to determine whether *C. plana* actually weakens the periwinkle shells, perhaps through secretions that affect shell architecture or thickness, or whether *C. plana* is just naturally found in weaker shells—shells housing large *C. plana* may simply be older than most other available shells, for example. Alternatively, the hermit crabs may be responding to a change in internal shell geometery or texture (Arnott and Elwood, 2007) caused by the presence of *C. plana* rather than to its impact on shell weight, internal volume, or strength.

The presence of *C. plana* within shells may also affect the growth rates, reproductive biology, or fecundity of occupying hermit crabs, or their vulnerability to predators, consequences that have been shown to result from some other associations and types of shell damage for various hermit crab species (e.g., Angel, 2000; Bach et al., 2006; Bertness 1981, 1982; Elwood et

al., 1995; Fotheringham, 1976a, b, c; Hazlett et al., 2005; Markham, 1968; Pechenik et al., 2001; Wilber, Jr. 1989, 1990; Vance, 1972). These aspects of the association with *C. plana* could be examined in future studies.

In conclusion, most of the approaches used in previous studies for evicting *P. longicarpus* from their shells seem valid, provided that the hermit crabs are allowed to recover for at least 2-20 h before being used in behavioral assays. In addition, we show that *P. longicarpus* avoids shells containing large individuals of *C. plana*, possibly because those gastropods decrease shell volume and increase shell weight substantially, and increase the risk of damage by shell-crushing predators.

Acknowledgements

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Table 1: Summary of techniques used to evict hermit crabs (*P. longicarpus*) from their shells for experiments on shell selection behavior.

Eviction technique	Equipment required	Were crabs evicted?	Shell damage?	Quickness of eviction	Crab recovery rate	Recommended if crab is to be immediately used in experiments?
Manual extraction	None	Yes	No	Slow	Slow	No
Vise	Bench Vise	Yes	Yes	Fast	Fast	**Yes**
Thermal stress	Heating Device	Yes	No	Moderate	Moderate	No
Drilling shell	Dremel Drill	Yes	Yes	Fast	Fast	**Yes**
Low salinity	None	No	NA	NA	NA	No

Figures:
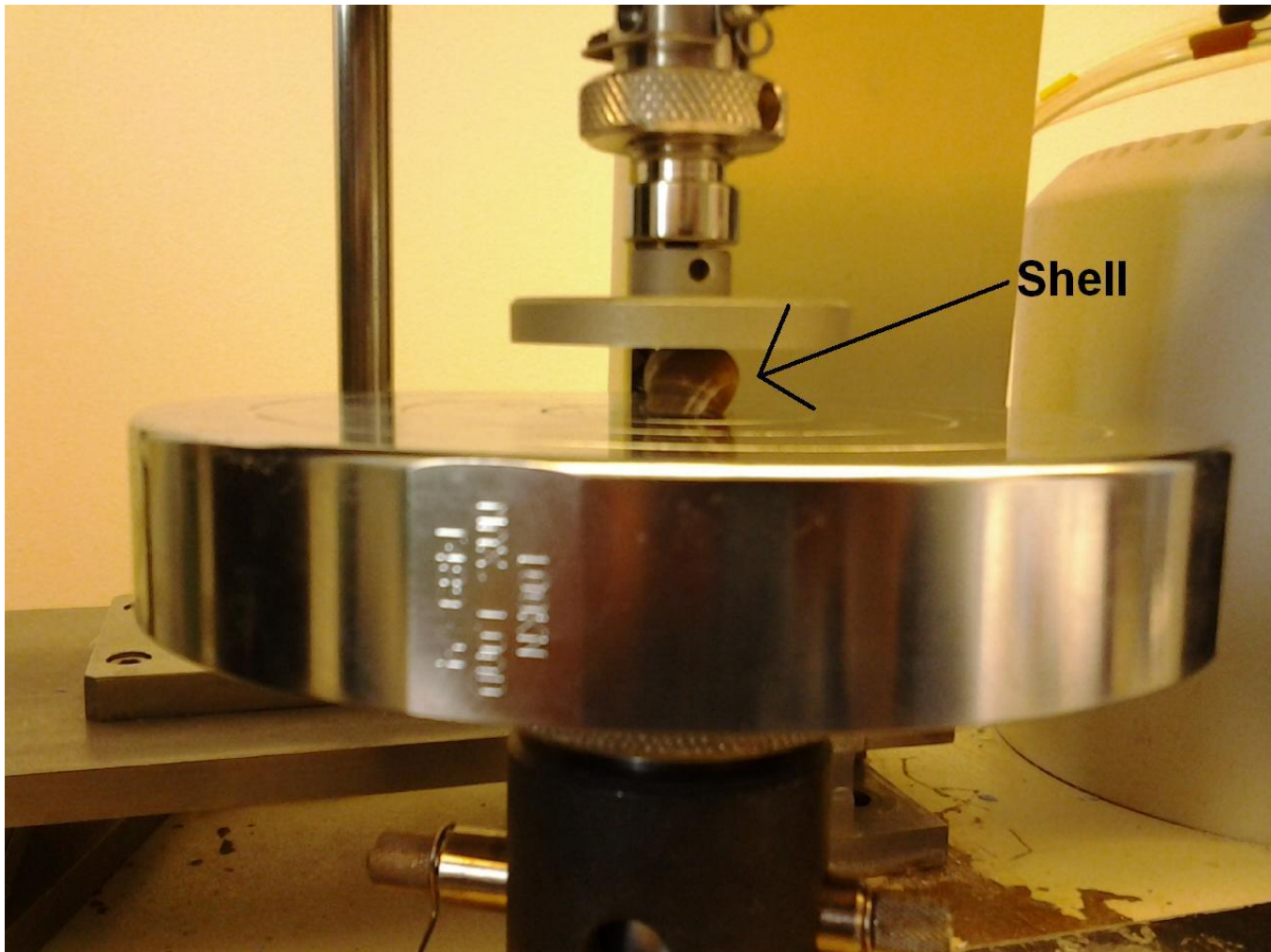
Figure 1.

Fig. 1. Instron setup for determining breaking force of periwinkle (*Littorina littorea*) shells with and without *Crepidula plana* on the inside. Shells were oriented with the aperture facing downwards, and the crushing surface was projected downward at 1 mm min^{-1}.
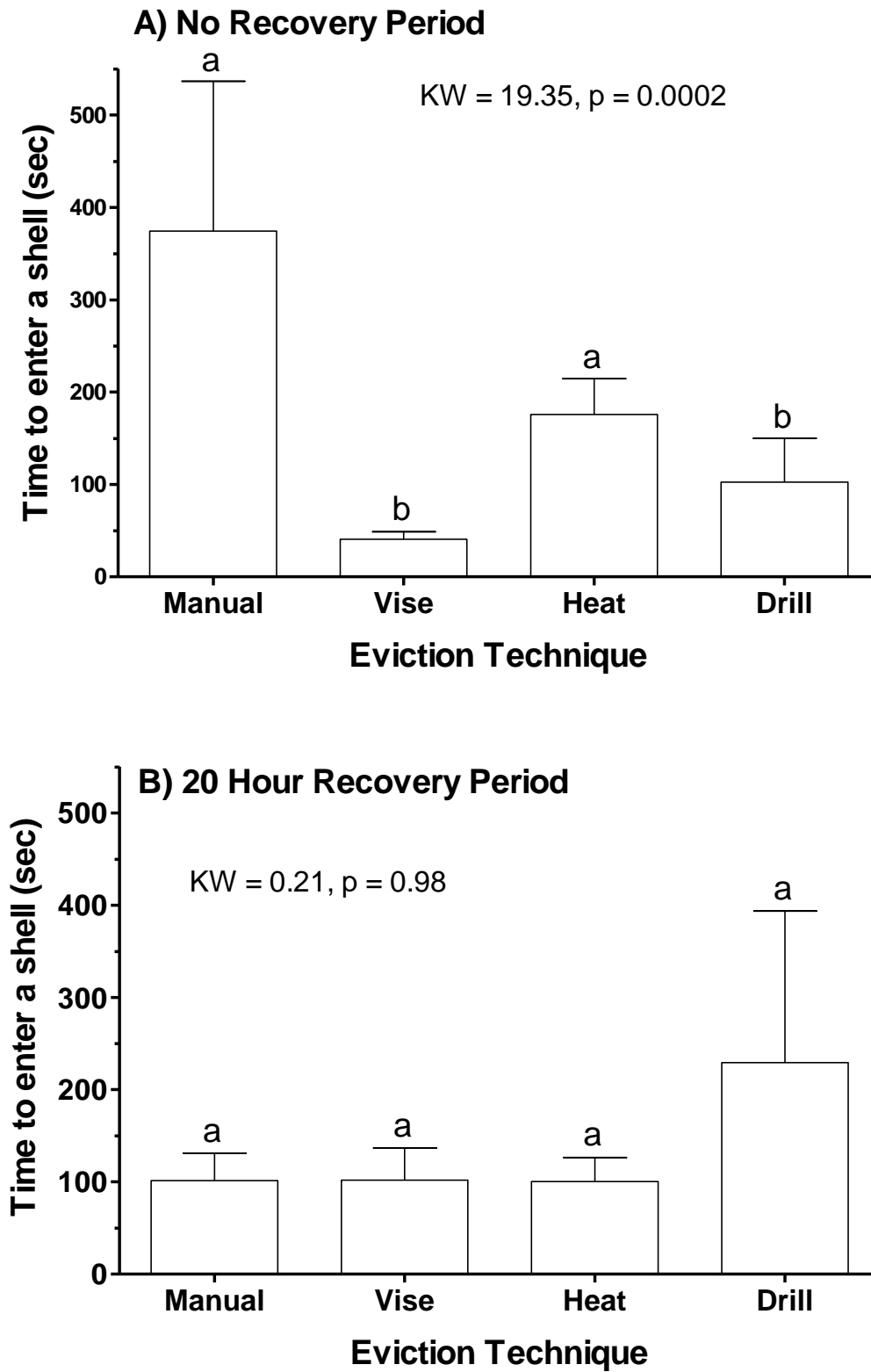
Figure 2

Fig. 2. Effect of eviction technique on the time taken for naked hermit crabs (*Pagurus longicarpus)* to enter any shell.   n = 15 crabs per treatment in (A) and n = 10 crabs per treatment in (B); mean + S.E.M shown for all treatments. Hermit crabs were evicted from their *Littorina littorea* shells by slowly pulling the crab out of its shell (Manual), crushing the shell with a vise (Vise), warming the seawater to approximately 34ºC (Heat), or drilling a small hole in the shell and poking the crab with monofilament line (Drill). Naked hermit crabs were offered a new, intact shell and a new, drilled shell of the same aperture size immediately after eviction (A) or after a 20 hour recovery period (B), and the time it took each crab to enter either shell was measured. Within each experiment, means that do not have the same letter are significantly different ($p < 0.05$, Dunn's multiple comparisons test).
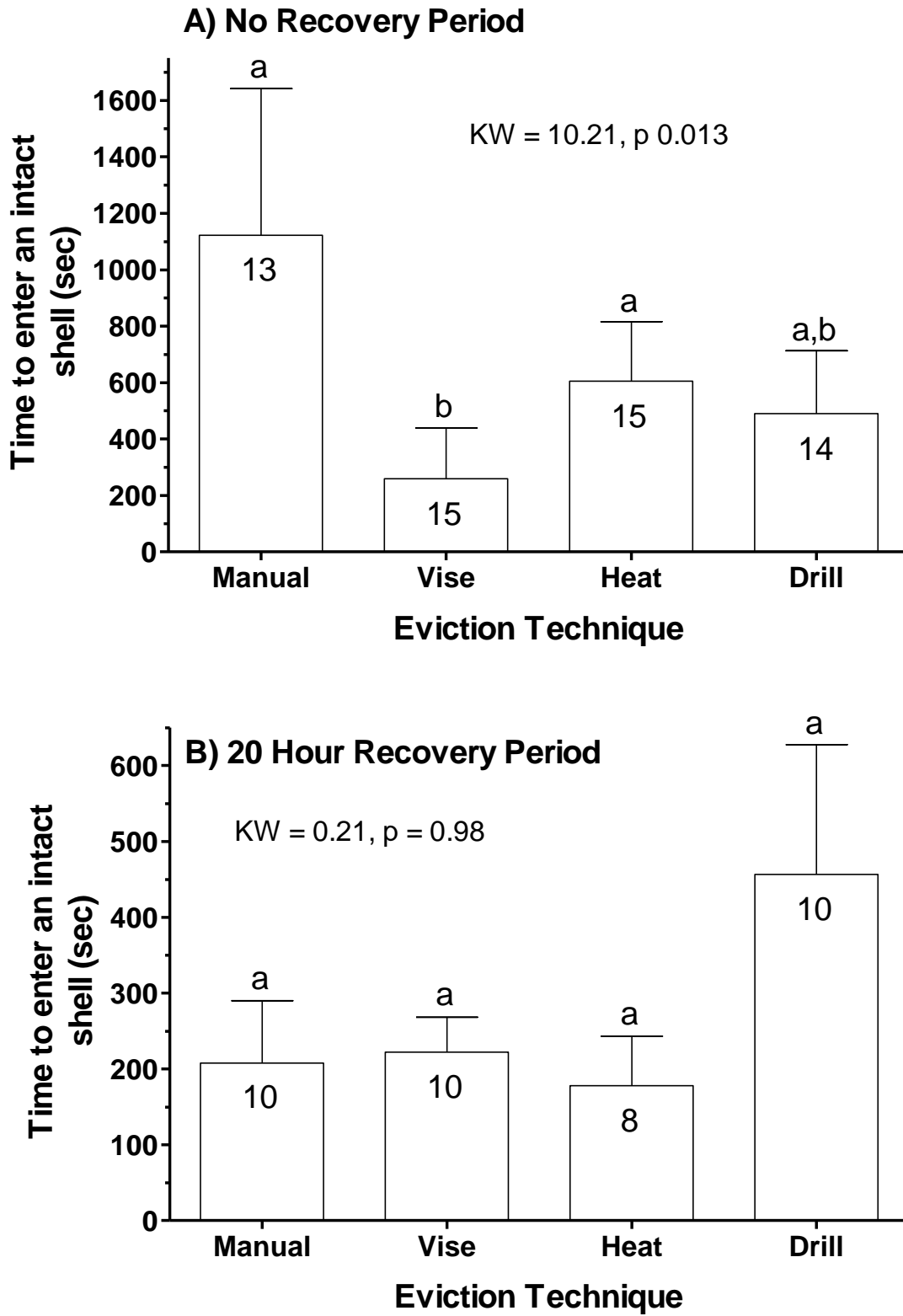
A) No Recovery Period

KW = 10.21, p 0.013

Time to enter an intact shell (sec)

a — 13 (Manual)
b — 15 (Vise)
a — 15 (Heat)
a,b — 14 (Drill)

Eviction Technique

B) 20 Hour Recovery Period

KW = 0.21, p = 0.98

Time to enter an intact shell (sec)

a — 10 (Manual)
a — 10 (Vise)
a — 8 (Heat)
a — 10 (Drill)

Eviction Technique

Figure 3

Fig. 3. Effect of eviction technique had on time taken for naked hermit crabs (*P. longicarpus)* to enter an intact shell. Some of the hermit crabs entered an intact shell at the outset, whereas others entered a drilled shell initially, and then switched shells later. Naked hermit crabs were offered a new, intact shell and a new, drilled shell immediately after eviction (A) or after a 20 hour recovery period (B) and the time it took each crab to enter the intact shell (even if had first entered a drilled shell) was measured. See Fig. 2 for additional details. Within each experiment, means that do not have the same letter are significantly different ($p < 0.05$, Dunn's multiple comparisons test).
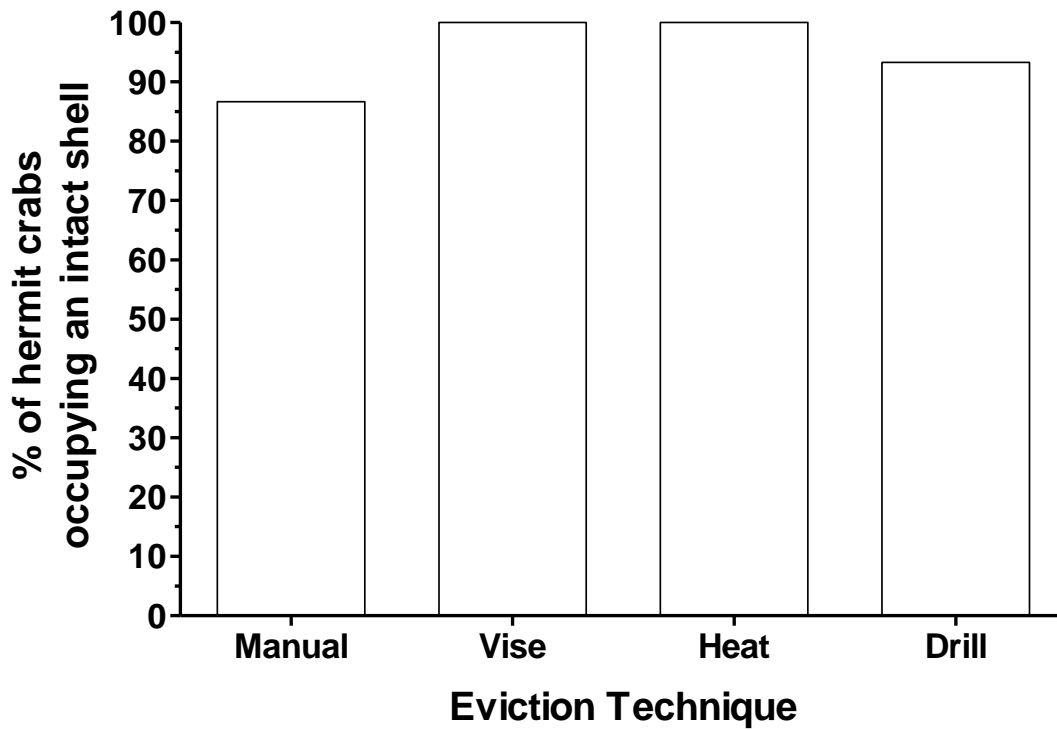
Figure 4

Fig. 4. Impact of eviction technique on shell selection behavior within the first 2 h after eviction. Naked hermit crabs (*P. longicarpus*, n = 15 crabs per treatment) were offered shells immediately following their eviction: one intact shell one drilled shell of the same size. See Fig. 2 for details of the eviction techniques used. Final shell choice was recorded after 2 h. The actual time it took for these hermit crabs to enter the shells is presented in Figures 2 and 3.
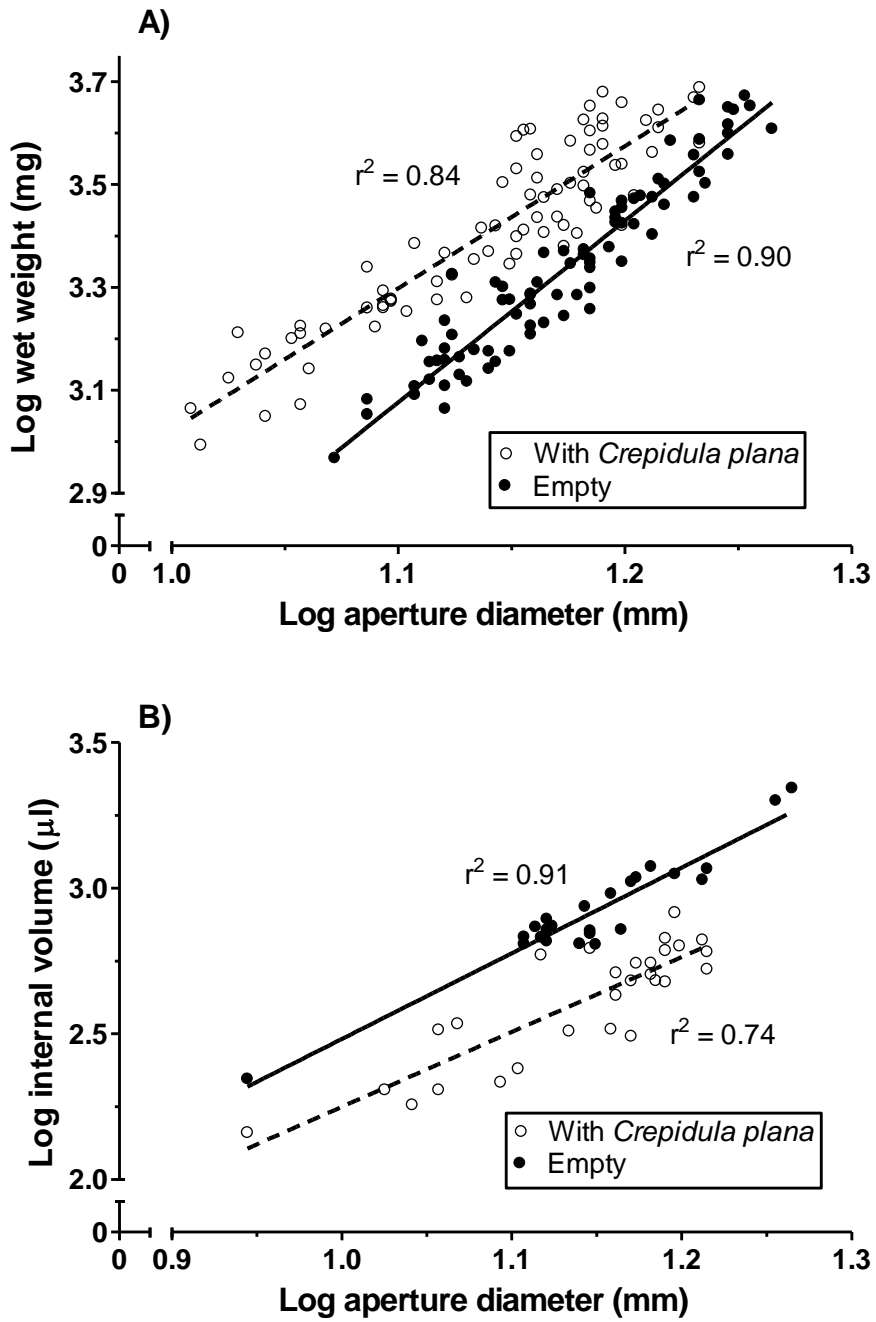
A)

B)

Figure 5

Fig. 5. Effect of the commensal gastropod *Crepidula plana* on the weight (A) and internal

volume (B) of the periwinkle shells (*Littorina littorea)* they inhabit. Intact periwinkle shells

containing hermit crabs (*P. longicarpus*) were collected from Nahant, MA, USA in October,

2011. Shells were emptied of water, blotted, and weighed (A), and water was added to the inside

of each shell until it was filled (B). Linear regressions diverge significantly in (A) (n = 81

without *C. plana*, n = 79 with *C. plana*; ANCOVA, p = 0.0001 for slope) and in (B) (n = 24

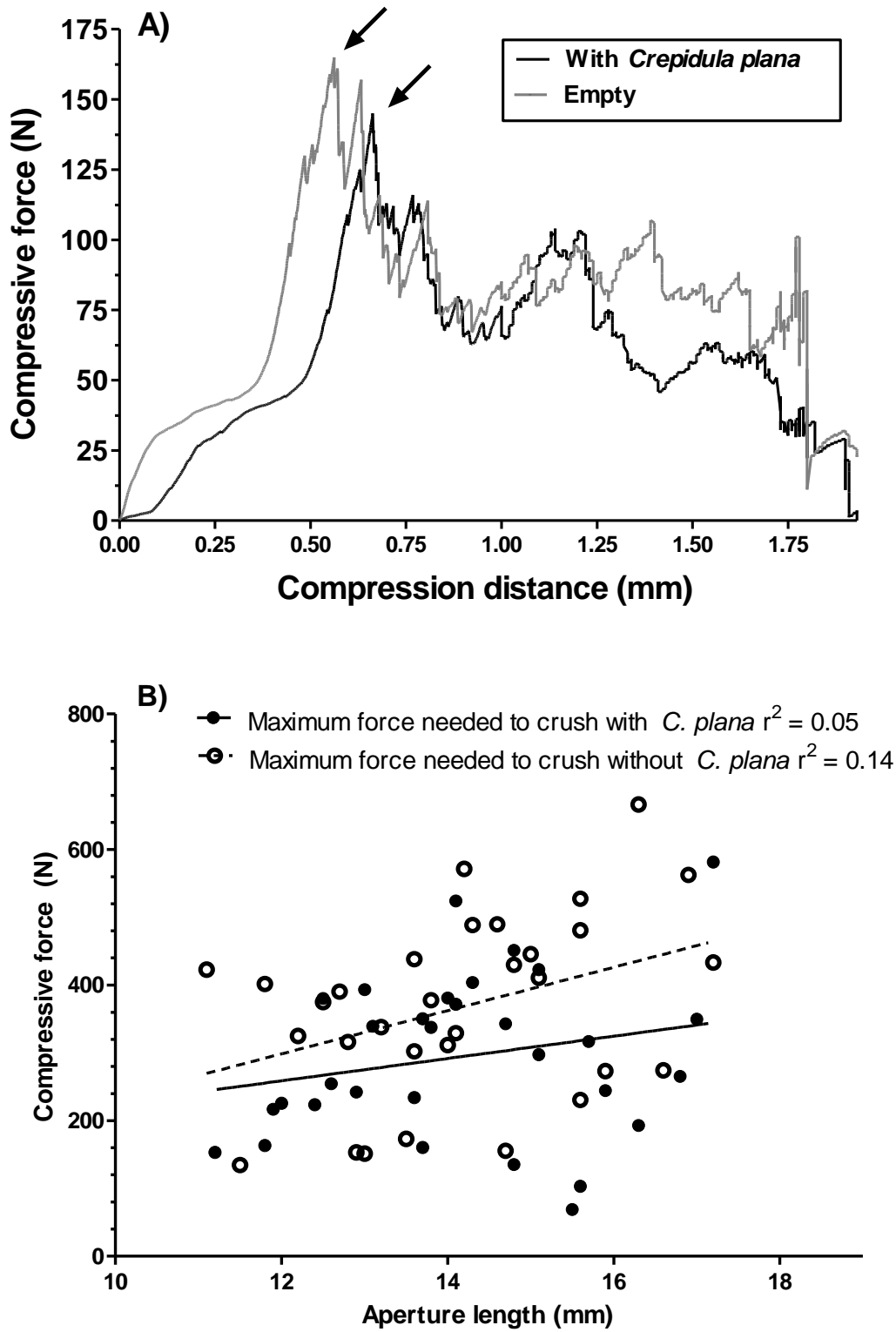without *C. plana*, n = 28 with *C. plana*; ANCOVA, p = 0.34 for slope, p < 0.0001 for intercept).

Figure 6

Fig. 6. Effect of the commensal gastropod *Crepidula plana* on the force required to break periwinkle (*Littorina littorea)* shells. Each experiment lasted about 60 sec. (A) Average force displacement responses for the 31 empty intact shells and the 31 intact shells bearing internal *C. plana*. Arrows indicate the force at which the shells broke. These forces are displayed as single points (n = 31 for each treatment) in (B); shells that did not contain *C. plana* took significantly more force to break than those housing the symbiont (ANCOVA, $F_{1,59} = 5.49$, $p = 0.023$).
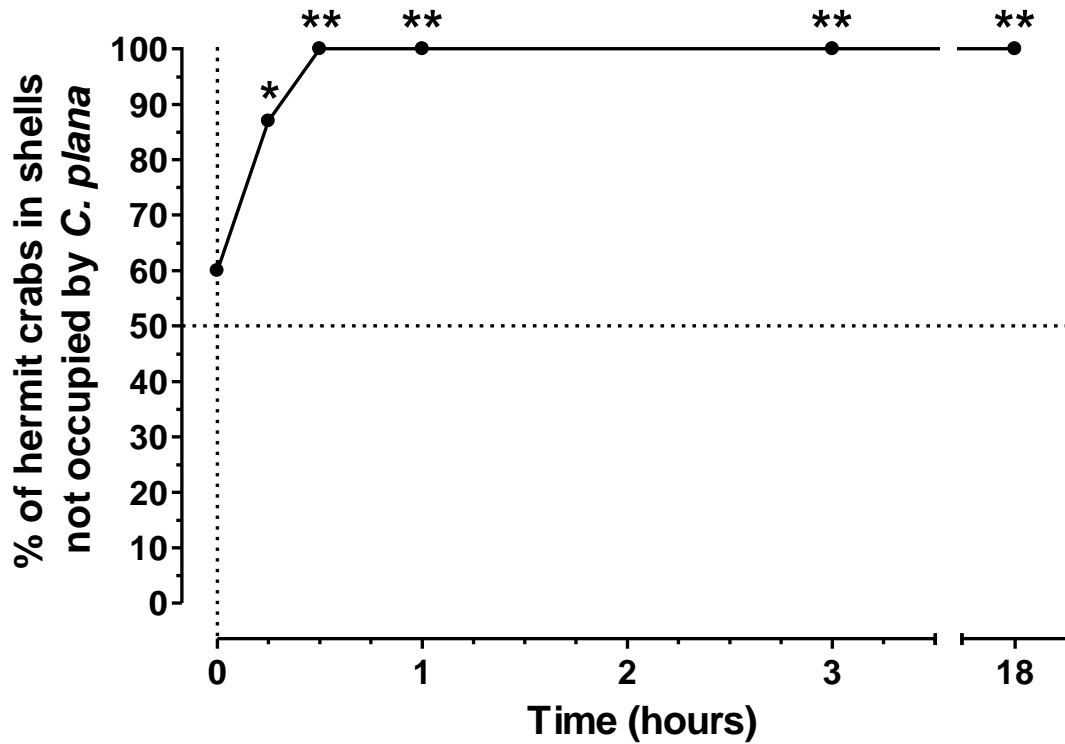
Figure 7

Fig. 7. Extent to which *Pagurus longicarpus* hermit crabs preferred shells that did not contain the gastropod *Crepidula plana*. Fifteen hermit crabs were individually offered a choice between an empty, intact shell of the appropriate size (Angel, 2000) and an intact shell of the same size but containing *C. plana*. Asterisks indicate a significant tendency to choose shells without *C. plana* at a particular time point (*, $p < 0.05$; **, $p < 0.01$; χ^2 test).
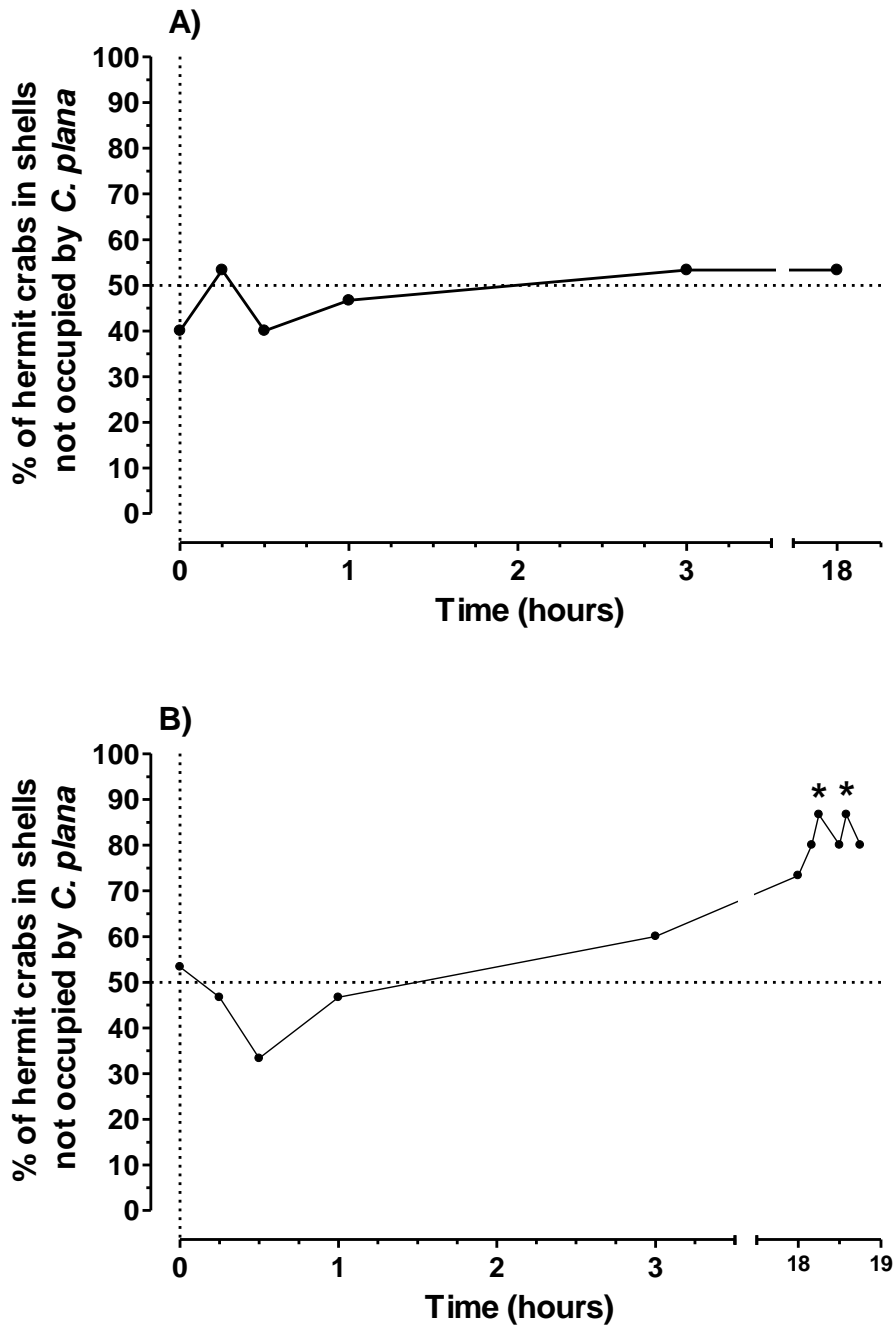
Figure 8

Fig. 8. Shells selected by *Pagurus longicarpus* when faced with two bad choices. Fifteen naked

hermit crabs were individually offered the choice of (A) an empty, drilled shell of the appropriate

size and an intact shell of the same aperture length containing *C. plana*, or (B) an empty, drilled

shell of the appropriate size and an intact shell of the same volume (and thus a larger aperture

size, Figure 4) but containing *C. plana*. Asterisks indicate a significant tendency to choose shells

without *C. plana* at a particular time point (*, $p < 0.05$; χ^2 test).