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A comparison of two methods for sampling the Gulf of California mud shrimp, *Neotrypaea uncinata* (Crustacea: Thalassinidea)

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Methods for collecting mud shrimp are highly variable, and the efficiency of these techniques is poorly known. In October 1999, on a mud flat near Puerto Peñasco, Sonora, México, we compared the 'kiwi' method for extracting mud shrimp from their burrows, with a core sampling method designed to accomplish the same goal. The coring method involved pressing a 10 cm × 90 cm PVC pipe into the substratum, creating suction in the pipe, and withdrawing a core of mud and the shrimp within. The kiwi method required 8–10 researchers to march in a circle on the mud flat to gradually liquefy the mud, thereby forcing shrimp to the surface as the circle closed inward. Two transects of 30 cores conducted at high and at low tide yielded similar numbers of adult animals of both sexes. Previous results show that mud shrimp tend to aggregate within burrows when breeding. In this collection, the distribution of mud shrimp in cores did not differ from a Poisson distribution, suggesting that these shrimp were non-reproductive. The kiwi method provided no such information on mud shrimp distributions. Moreover, this method generated significantly fewer intact mud shrimp than were obtained in cores. Our results indicate that compared to the kiwi method, the coring method generates more animals, is less destructive of mud shrimp habitat, and provides more detailed information on mud shrimp populations.

KEYWORDS: *Neotrypaea*, mud shrimp, collection methods, spatial distribution.

Introduction

Burrowing mud shrimp of the family Callianassidae are among the most abundant crustaceans inhabiting mud flats in temperate and tropical estuaries (MacGinitie, 1934). These small crustaceans are responsible for considerable disturbance of marine sediments and are thus similar to earthworms in their ability to recycle nutrients and disrupt sediment stratification (Branch and Pringle, 1987). Their distribution is world-wide and most studies of this family concern the biology and ecology of the numerous species. Hailstone and Stephenson (1961) examined the distribution and

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life cycle of *Callianassa australiensis* Dana found on the eastern Australian coast. This study included a description of the 'yabby pump' used for collection and sampling animals. Breeding and growth studies were conducted for *Callianassa kraussi* Stebbing, a species found in many South African estuaries, as well as studies of their effect on sediment turnover (Forbes, 1976; Branch and Pringle, 1987). Respiration studies by Torres *et al.* (1977) of *Callianassa californiensis* Dana detailed pleopod function and physiology. Witbaard and Duineveld (1989) and Rowden and Jones (1994) reported general biology and ecology for *Callianassa subterranea* Montagu found in the North Sea. *Callianassa subterranea* has also been the focus of studies on thallassinidean feeding (Nickell *et al.*, 1998; Pinn *et al.*, 1999a, 1999b) and burrow morphology (Nickell and Atkinson, 1995). Tamaki *et al.* (1996) provided similar information regarding general biology and ecology for *Callianassa japonica* Ortmann inhabiting western Japan.

Studies of mud shrimp require removal of individuals from their burrows in mud flats. However, regular population samples are often difficult to obtain because shrimp burrow deep within the substratum, are small in size and do not normally tend to aggregate (Forbes, 1976). In addition, the methods for collecting mud shrimp are highly variable. These range from digging shrimp up with a shovel or spade (Pohl, 1946; Hailstone and Stephenson, 1961), to the 'kiwi' method (Torres *et al.*, 1977) or *Callianassa* Stomp (Sitarek *et al.*, 1998; Montgomery and Shuster, unpublished data), a method which involves manually liquifying the mud to capture shrimp swimming to the surface, to using a boxcorer (Tamaki and Ingole, 1993), to a number of variations on a cylindrical core or cylindrical pump (Hailstone and Stephenson, 1961; Devine, 1966; Dumbauld *et al.*, 1996; Tamaki *et al.*, 1997). The latter method seems to be the most common in the literature, however, the efficiency and effectiveness for this or any other method of collecting mud shrimp has never been quantified (but see Manning, 1975). The purpose of this paper is to compare and quantify the effectiveness of two of these methods for sampling populations of *Neotrypaea uncinata* Milne Edwards, formerly *Callianassa uncinata* Milne Edwards (Manning and Felder, 1991), a mud shrimp inhabiting the northern Gulf of California.

Materials and methods

We collected our samples at Estero Morua, a negative estuary, or *estero*, located approximately 10 km south-east of Puerto Peñasco, Sonora, México. We used two methods to remove shrimp from the substratum. The first method involved collection at low tide, of 30 mud substratum cores. For each core we pressed a 10 cm diameter × 90 cm long PVC pipe into the substratum, corked a small hole in the end of the core to pressurize its contents, and withdrew the mud and mud shrimp contained within the core. Hailstone and Stephenson (1961) used a similar technique. Cores were collected along a stretch of mud flat orientated parallel to the shore and were approximately 1 m apart. We separated animals from cores by hand and placed them into 225 ml cups containing seawater for transport to the laboratory. We repeated this procedure approximately 100 m away from the first sample in a second set of cores collected 3 h after low tide as the tide had begun to cover the transect. The total mud flat area sampled in each transect of cores was $[\pi(0.05\,\mathrm{m})^2]30$ cores $= 0.24\,\mathrm{m}^2$.

Our second sampling technique was the 'kiwi' method (Torres *et al.*, 1977) also known as the '*Callianassa* Stomp' (Sitarek *et al.*, 1998; Montgomery and Shuster, unpublished data). This method involved 8–10 researchers marching in a 5 m diameter circle on a section of mud flat containing mud shrimp burrows. The total mud flat area sampled in each circle using this method was $\pi(2.5\,\mathrm{m})^2 = 19.63\,\mathrm{m}^2$. As the mud liquefied, the researchers closed in their circle and mud shrimp were collected by hand when they swam to the surface. Each animal was placed in a 225 ml plastic cup containing seawater for transport to the laboratory. We conducted two stomps at similar stretches of mud flat separated by approximately 25 m at the same height from shore. The first stomp was performed at low tide; the second was performed 3 h later as the tide had begun to cover the mud flat. Both stomps and cores were conducted on the mud flat at identical distances from the shore.

In the laboratory, we identified each animal by sex, and estimated body size by measuring the length of the carapace to the nearest 0.125 mm. Females were identified by their possession of pleopods modified for carrying eggs on the abdominal segment of the body. Males were identified by their lack of ovigerous pleopods as well as by their possession of an enlarged dominant chela relative to the dominant chela of females. We recorded any damage found on each mud shrimp's body.

To identify the spatial distribution of shrimp within the mud flat, we used a *G*-test to compare the number of mud shrimp within cores for each transect, with the number of mud shrimp per core predicted by a Poisson distribution. No such comparison was possible within or between stomp samples. A random distribution of shrimp within cores could suggest that mud shrimp construct burrows where they settle and remain; a clumped distribution could suggest that mud shrimp tend to aggregate within burrows or prefer to construct burrows close to each other. An even distribution could suggest that mud shrimp aggressively defend regularly spaced territories. Previous research suggests that mud shrimp may aggregate to breed (Wildey and Vuturo, 1999; Wildey and Shuster, 2000), thus the results of this test could also indicate an approaching breeding season for this population. Mud shrimp may also aggregate according to settlement cues or sediment type (Griffis and Chavez, 1988).

We used 2×2 *G*-tests to examine the total number of mud shrimp collected between replicates within each sampling method as well as between each method. This test provides an overall test of independence between replicates and sampling methods as well as a means for isolating the source of differences within the contingency table. These comparisons were accomplished by (1) pooling across row cells within columns and comparing column totals, (2) pooling across column cells within rows and comparing row totals and (3) pooling the diagonal cells within the table and comparing the cross-totals (table 1). All three comparisons involved a *G*-test with df = 1.

Table 1. Number of mud shrimp sampled in cores.

	Transect 1	Transect 2	Totals
Adults	34	53	87
Juveniles	5	2	7
Totals	39	55	94

The mud shrimp sampled with the coring technique was not significant in numbers of animals sampled between the two transects. However, it is significant in age demographics, representing a primarily adult population. $G = 75.6$, df = 1, $P < 0.001$, $N = 94$).

We used this method to identify differences in (a) the number of juvenile and adult individuals between each replicate sample and between each sampling method, (b) differences in the number of males and females collected within each replicate sample and between each sampling method, as well as (c) differences in the number of intact and damaged mud shrimp within each replicate sample and between each sampling method. In all *G*-tests, we used William's Correction to reduce the probability of a type I error (Sokal and Rohlf, 1995).

We calculated the sex ratio of individuals collected within each class of cores ($N_{\text{females}}/N_{\text{males}} = R$), and then examined the relationship between R and the number of shrimp per core. A significant positive slope would suggest that few males tend aggregations of females in burrows. A significant negative slope would suggest that aggregations of mud shrimp in our samples consisted mainly of males. No slope would suggest that at the time of our sample, no relationship existed between sex ratio and the distribution of mud shrimp in cores. Lastly, we used a two-way ANOVA to compare the body sizes of males and females within each sample type as well as the overall sizes of individuals between samples.

Results

Although there was no significant difference in the total number of juveniles and adults between the stomp and core samples ($G=2.90$, $P>0.05$), the number of animals collected in cores was significantly larger than the number of animals collected in stomps (figure 1) ($G=5.73$, $P<0.05$, $N=158$). There was also a significant difference in the total number of males and females between the stomp and core samples (figure 2) ($G_{\text{adj}}=27.69$, $P<0.01$). Male and females were equally abundant in core samples yet male abundance exceeded female abundance in stomp samples by nearly 10-fold. The number of damaged individuals was somewhat higher in stomp samples compared to core samples, although this difference was not significant ($G_{\text{adj}}=1.56$, $P>0.05$).

Although the relationship between the number of shrimp per core and sex ratio (R) was non-significant ($r_s=0.872$, $P=0.08$), the negative slope of this relationship

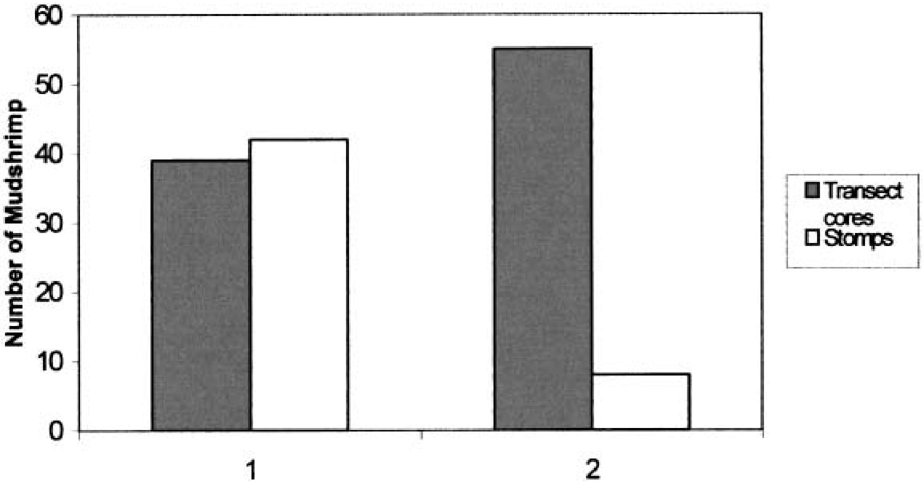

FIG. 1. The total number of animals collected in cores (average = 47.0, SE = 8.0) and stomps (average = 25.0, SE = 17.0).

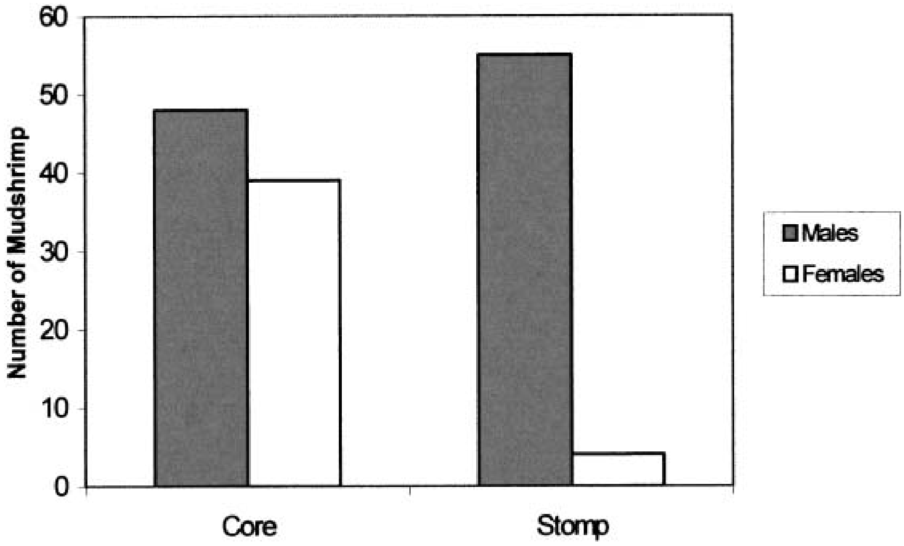

FIG. 2. The proportions of males and females collected in cores and stomps.

suggests that at the time of our samples, males were not tending aggregations of females in burrows.

The results of the two-way ANOVA showed significant main effects of collection method and sex on carapace length ($F_{[2,128]} = 3.32$, $P < 0.039$), but no significant interaction ($F_{[1,129]} = 0.0089$, $P > 0.925$). One-way ANOVA of carapace length and sex was significant ($F_{[1,129]} = 4.42$, $P < 0.03$), and a one-way ANOVA of carapace length and collection method was also significant ($F_{[1,129]} = 4.71$, $P < 0.03$). Thus, females were larger than males in both samples and larger individuals were collected in cores (figure 3).

There was no significant difference in the spatial distributions of mud shrimp within each transect of core samples ($\chi^2_{[0.05,\ df=4]} = 3.13$, $P > 0.50$, $N = 60$). Thus,

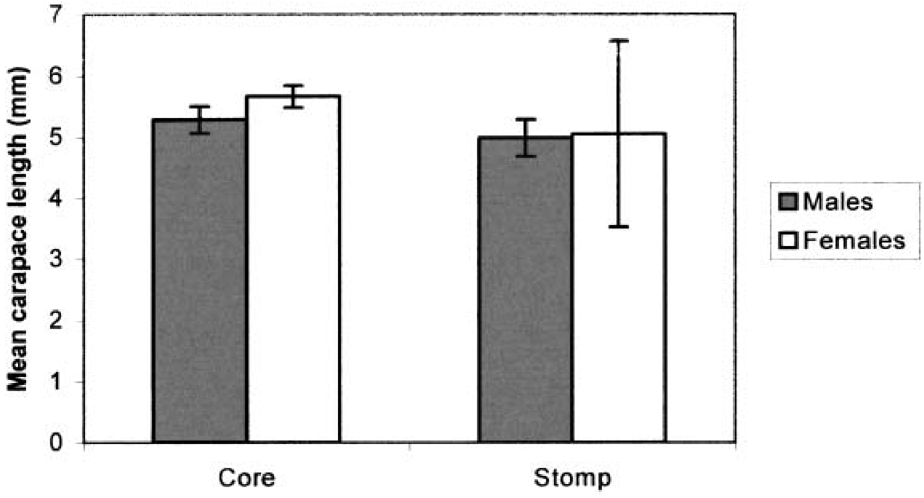

FIG. 3. The mean carapace length of males and females collected in cores and stomps.

we pooled these samples and compared the aggregate sample with that predicted by a Poisson distribution. These distributions were also not significantly different from each other ($\chi^2_{[0.05,\ df=6]}=5.78$, $P>0.50$, $N=60$), indicating that the spatial distribution of mud shrimp burrows was not significantly different from random. There was no significant difference in the proportions of adults and juveniles contained in each transect ($G_{\text{adj}}=2.39$, $P>0.50$, $N=94$), thus we pooled these samples. There were, however, significantly more adults than juveniles in the aggregate collection (figure 4) ($G_{\text{adj}}=80.08$, $P<0.001$). There was also no significant difference in the numbers of males and females between the two transects ($G_{\text{adj}}=1.41$, $P>0.50$, $N=87$).

Discussion

The random spatial distribution of mud shrimp observed in the two coring transects at high and low tide indicate that these animals were not aggregated, and their spatial distribution was not affected by tidal fluctuations. However, this was only observed for one tidal cycle. Observation over several tidal cycles would be required to address this question fully. We have found few references concerning the effects of tidal height on distributions of mud shrimp, however, it is logical to assume there are some vertical migrations that mud shrimp do make in response to the rising and receding tide. In addition, the substratum during flood tides does become more saturated. This saturated substratum is more difficult to sample, as the mud is more viscous and falls out of the coring device more easily, making it harder to pull a true full core out of the substratum. Shrimp are also more mobile in saturated mud, perhaps allowing them to escape collection more often. The coring method yielded equal numbers of males and females, suggesting that the horizontal distribution of the sexes within the mud flat is similar. Stomp samples did not allow investigation of these spatial patterns.

The two sampling methods were similar in their tendency to collect more adults than juveniles. However, core samples yielded significantly more animals and significantly more females than stomps. Although we collected animals from just two stomps and from two transects of cores, our results suggest not only that cores are

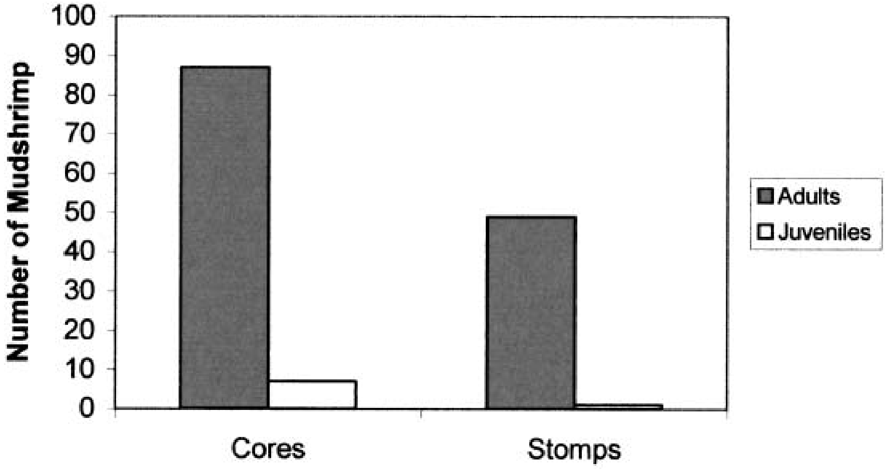

FIG. 4. The proportions of adults and juveniles collected in the core samples.

a more efficient sampling method but also that cores provide a more representative picture of the adult mud shrimp population than stomps.

The tendency for fewer females to be collected in stomps suggests either that females are located deeper in the mud flat than males and therefore less able to reach the surface, or that females are physically less able to reach the surface after stomp sampling. This is an important distinction because sex ratios for this population (Sitarek *et al.*, 1998) and others (Kevrekidis *et al.*, 1997) have been reported as skewed towards males, when in fact the skewed sex ratio may be a by-product of sampling technique and not an actual characteristic of the population. Tamaki and Ingole (1993) found that all adults and 55% of juveniles were found 10 cm below the surface for *Callianassa japonica* Ortmann. This difference in distributions of adults and juveniles on a vertical scale suggests that sampling techniques that do not sample deep enough in the substratum may lead to incorrect inferences about shrimp distributions. We did not note the position of males and females and juveniles individually within cores and therefore are unable to distinguish between these hypotheses.

The number of damaged or dead individuals between the two collection methods was not significantly different. However, we were only able to collect individuals from stomp samples that were carried to the surface by the feet of the stompers or were able to swim to the surface themselves. Any incapacitated mud shrimp are likely to have remained underground, perhaps explaining the difference in sample size between cores and stomps.

Manning (1975) commented on two common methods for collecting decapods in shallow water in the Mediterranean, the pushnet and the yabby pump. He qualitatively described the usefulness and range of habitats where the yabby pump could be used, but made no quantitative comparisons for these or any other methods for collecting mud shrimp. The current study differs from Manning (1975) in this regard. We provide data on numbers and types of shrimp collected for two collection methods, coring and stomping, as well as information on the numbers of damaged shrimp as a result of the collection methods. We conclude that the coring method of sampling is likely to produce a more accurate representation of the mud shrimp population than the 'kiwi' method. The cores produced more animals, a more even distribution between the sexes and fewer damaged individuals. Overall, the coring method appeared to be a more efficient and less destructive means for sampling mud shrimp populations.

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