Sampling technique affects the population structure assessments of fiddler crab *Minuca vocator* (Herbst, 1804) (Ocypodidae: Gelasiminae)

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**ABSTRACT**

We examined how the sampling technique can affect the evaluation of *Minuca vocator* (Herbst, 1804) population structure. We used two sampling procedures: catch-per-unit-effort (CPUE) and quadrat technique (QT). Body size, size–frequency distribution, population density, juvenile recruitment rates, proportion of ovigerous females and sex ratio were compared between the sampling procedures. QT allowed us to estimate and compare crab and burrow densities. CPUE sampled both adult crabs and the largest crabs more frequently, whereas QT recorded a greater proportion of smaller crabs. CPUE underestimated the size of *M. vocator* populations, while density estimates obtained with QT were accurate. The proportion of juveniles was higher with QT than with CPUE, suggesting that recruitment rates estimated by QT were more suitable. The sampling effort provided by CPUE was more efficient for obtaining ovigerous-dependent information than QT. Both sampling techniques showed a predominance of males in all three *M. vocator* populations. The population density estimation based on burrows overestimated the natural density of *M. vocator* in all mangroves.
Our results suggest that neither CPUE nor QT individually were accurate sampling techniques, but together provided reliable assessments of fiddler crab populations.

**KEY WORDS**

Body size, catch-per-unit-effort, quadrat, recruitment, sex-ratio.

**INTRODUCTION**

It is important to assess the abundance of individuals in the field for ecological studies, environmental impact evaluation and conservation biology (Schlacher et al., 2016). Obtaining accurate field information can be difficult, especially in estuarine sediments, where many species are burrowers and sampling time is limited to periods of low tide (Conde and Diaz, 1985; Vermeiren and Sheaves, 2014). Fiddler crabs are usually abundant macrofauna in sediments of tropical and subtropical estuarine environments and play a remarkable role in habitat functioning, as they are ecosystem engineers (Kristensen, 2008). It is therefore essential to obtain precise data on fiddler crab populations in order to learn about their dynamics and effects on the environment.

Excavated quadrats, transect sampling, burrow count, visual observation, hand digging, pitfall trapping, mark-release-recapture estimates, and photography are techniques traditionally used in fiddler crab studies (Johnson, 2003; Vermeiren and Sheaves, 2014), and each has its pros and cons. Excavated quadrat and transect sampling provide accurate density estimates of burrowing crabs (Colby and Fonseca, 1984; Skov and Hartnoll, 2001; Costa and Negreiros-Fransozo, 2003), but take time, and the movement large volumes of sediment may result in a considerable environmental impact on sensitive estuarine habitats (Schlacher et al., 2016). Density estimation by burrow counting tends to overestimate the actual density of crabs, since they can dig more than one burrow (Montague, 1980; Macintosh, 1988; Macia et al., 2001; Skov and Hartnoll, 2001). Quantifying density by visual surface observation may lead to underestimation because some crabs remain inside their burrows (Macintosh, 1988). Hand catch and pitfall trapping require knowledge of crab activity patterns, and are laborious techniques, which could limit replication over a short time (Vermeiren and Sheaves, 2014). Mark-release-recapture techniques are only suitable when captures can be carried out quickly and efficiently (Hockett and Kritzler, 1972). Photography provides accurate spatial distribution patterns on large spatial scales, but taxonomic differentiation is difficult when sympatric species are morphologically similar (Goshima and Murai, 1998; Vermeiren and Sheaves, 2014). Considering the diversity of sampling protocols, any comparisons should be made with caution (Nobbs and McGuinness, 1999).

Population studies often evaluate density, age/size composition, sex ratio, juvenile recruitment rate, size at onset of sexual maturity, and reproductive period (Colpo and Negreiros-Fransozo, 2004; Lima et al., 2014; Andrade et al., 2015; Hirose et al., 2015). The assessment of some of these population parameters can be affected by the sampling procedure chosen for the study (Macia et al., 2001; Johnson, 2003; Costa and Negreiros-Fransozo, 2003; Hirose et al., 2015). Small crabs can be overlooked by visual observation or hand digging techniques, which would affect the age/size composition and juvenile recruitment estimates (Costa and Negreiros-Fransozo, 2003). Frequency of ovigerous females and hence, the reproductive period, could be underestimated by quadrat, transect or visual observation procedures, since these females usually stay underground and finding them requires a greater capture effort (Macia et al., 2001). Fiddler crab males are more conspicuous than females because of their large cheliped; thus, hand digging and visual observation can promote bias in sex ratio estimation (Johnson, 2003).

Recently, Shih et al. (2016) reorganized the subfamily rankings of Ocypodidae and reviewed the taxonomic status of the genus *Uca* Leach, 1814 (*sensu lato*) and its subgenera based on phylogenetic relationships and a large-scale multigene study. Thus, we adopted here such new classification, in which only *Uca maracoani* (Latreille, 1803) in Brazil remained in the former genus and belongs to the Ocypodinae Rafinesque, 1815. Other fiddler crabs, from Brazilian coast, are attributed to *Leptuca* Bott, 1973 or *Minuca*
Bott, 1954 genus, which belong to the Gelasiminae Miers, 1886. Thus, as proposed by Shih et al. (2016), the fiddler crabs belonging to Gelasiminae, with occurrence in the Brazilian coast, are the following ones: 

- *Leptuca cumulanta* (Crane, 1943);
- *Leptuca leptodactyla* (Rathbun, 1898);
- *Leptuca thayeri* (Rathbun, 1900);
- *Leptuca uruguayensis* (Nobili, 1901);
- *Minuca burgesi* (Holthuis, 1967);
- *Minuca mordax* (Smith, 1870);
- *Minuca rapax* (Smith, 1870);

Considering the importance of obtaining reliable assessments of fiddler crabs, we examined how the sampling technique can affect the evaluation of population structure. To do so, we sampled three *M. vocator* populations in subtropical mangroves using two different sampling procedures: catch-per-unit-effort (CPUE) and quadrat technique (QT). For the three populations, we compared body size, size-frequency distribution, population density, juvenile recruitment rates, proportion of ovigerous females and sex ratio between sampling procedures. QT allowed us to estimate and compare crab and burrow density.

**Material and Methods**

The fiddler crab *M. vocator* inhabits shaded mud flats in tropical mangroves (Crane, 1975; Colpo and Negreiros-Fransozo, 2004). We sampled this fiddler crab species in three mangroves in São Paulo State, southeastern Brazil: mangrove of the Itapanhau River (23°49′14″S 46°09′14″W), mangrove of the Indaíra River (23°24′51″S 45°03′14″W), and mangrove of the Itamambuca River (23°24′43″S 45°01′13″W). The three populations were found in similar habitat conditions. Sampling was carried out monthly from August 1999 to July 2000, during spring low tide.

The sampling procedures used in this study were catch-per-unit-effort (CPUE) and quadrat technique (QT). In each mangrove, an area of 40 m² with dominance of *M. vocator* was selected. Half of this area was sampled using CPUE and the other half using QT. Both procedures were carried out on the same sampling day. In the CPUE procedure, an experienced collector moved randomly over the sampling area for 15 minutes, extracting crabs from their burrows using a diving knife. In the QT procedure, a 0.80 m² wooden frame was used [quadrat size follows Green (1979)]. Three random replications of the quadrat were performed in the sampling area every month. First, the number of burrows was recorded, after which the crabs were sampled by excavation. The collector spent about 15 minutes capturing fiddler crabs in QT; thus, both sampling procedures took similar times.

Samples obtained by each sampling procedure were treated separately in the laboratory. All fiddler crabs were identified, measured (carapace width – CW), and their sex was determined. We also checked females for eggs.

In order to evaluate differences in the population structure assessments, we compared body size, size-frequency distribution, population density, juvenile’s recruitment rate, proportion of ovigerous females and sex ratio of *M. vocator* collected with both sampling procedures. For each population, crab size (CW) was compared between sampling procedures by a Mann-Whitney test (Sokal and Rohlf, 1979). Size frequency distributions of crabs collected using CPUE and QT were compared within populations by Kolmogorov-Smirnov test (Sokal and Rohlf, 1979). Monthly estimates were made of the approximate density of *M. vocator* (m²) obtained by each procedure (CPUE and QT). The number of crabs collected in the sampling area destined to each (20 m²). The densities provided by each sampling procedure were compared within each of the three populations by Mann-Whitney test (Sokal and Rohlf, 1979).

The proportion of juveniles was calculated monthly from the total number of crabs sampled with each technique. These proportions were compared each month by calculating the z statistic for a paired comparison using the normal approximation of the binomial distribution (Sokal and Rohlf, 1979). We considered as juveniles any crabs with CW smaller than the smallest ovigerous female in each population (Colpo and Negreiros-Fransozo, 2003). The proportion of ovigerous crabs was calculated monthly from the total adult females captured with each sampling procedure. The frequency of ovigerous females with relation to time suggests the breeding season, so we evaluated the number of months during which they were found using CPUE and QT. A chi-square test was used to evaluate deviations in sex ratio (1 male:1 female) in each population, and the male ratio obtained using each sampling technique was compared by a z test, as described for the proportion of juveniles.
Using the data obtained with QT, we compared the density of burrows with the density of *M. vocator* monthly using Student’s test (Sokal and Rohlf, 1979). A statistical significance level of 5% was used in all analyses.

**RESULTS**

The fiddler crabs sampled by CPUE were larger than those sampled by QT in all populations (Mann-Whitney test, p<0.001) (Fig. 1). However, both sampling procedures showed the same size trend among populations, since *M. vocator* reaches larger sizes in Itamambuca than in Indaia and Itapanhau. The size–frequency distributions observed with CPUE differed from the size distribution pattern recorded with QT for all study populations (Kolmogorov-Smirnov test, p<0.01) (Fig. 2). A greater number of smaller crabs was sampled with QT, while the larger crabs were obtained with CPUE (Fig. 2). Although CPUE provided more crabs than QT (Fig. 2), the density measures obtained by QT were higher than those obtained by CPUE in Indaia and Itamambuca (Fig. 3). In Itapanhau, we observed the same trend, although there was no statistic difference between procedures. Fig. 3 shows that data obtained by QT had greater dispersion than data obtained by CPUE.

The comparison of juvenile recruitment also showed that smaller crabs were more efficiently sampled by QT than CPUE in most months, in all three mangroves (z test, p<0.05) (Fig. 4). Nevertheless, both sampling procedures showed similar year-round recruitment pattern for all three populations, since CPUE and QT suggested continuous recruitment for all study populations (Fig. 4). CPUE was more effective than QT for recording ovigerous females (Fig. 5). CPUE found that the percentage of adult females that were ovigerous was 22.4% in Itapanhau, 9.3% in Indaia, and 10.3% in Itamambuca, while QT only found ovigerous females in Itamambuca (8.9% of adult females). Furthermore, CPUE suggested a continuous breeding season at Itapanhau and Itamambuca, since we recorded ovigerous females in nine months of the sampling year. Both sampling procedures indicated prevalence of males, except for the sample obtained by QT in Itamambuca, in which the sex ratio did not...

![Figure 1. *Minuca vocator* (Herbst, 1804). Body size comparisons (CW mm) between sampling procedures, within each population (U – Mann-Whitney test). CPUE, catch-per-unit-effort. QT, quadrat technique. Mean, Median, 25–75%, Min–Max.](image-url)
Figure 2. *Minuca vocator* (Herbst, 1804). Size–frequency distribution obtained by CPUE (catch-per-unit-effort – gray bars) and QT (quadrat technique – white bars) in each population. N= total number of sampled fiddler crabs. Mean and standard deviation of population (carapace width – mm).
Figure 3. *Minuca vocator* (Herbst, 1804). Density comparisons (fiddler crab .m²) between sampling procedures, within each population (U – Mann-Whitney test). CPUE, catch-per-unit-effort. QT, quadrat technique. Mean, Median, 25–75%, Min–Max.

Figure 4. *Minuca vocator* (Herbst, 1804). Comparisons between proportions of juveniles obtained by CPUE (catch-per-unit-effort – gray columns) and QT (quadrat technique – white columns) throughout the year, in the studied mangroves. *, statistical significance. ns, no significant (z test).
show any difference (Tab. 1). Male rates did not differ between the sampling procedures in all populations (z test; p > 0.05).

The QT procedure allowed us to compare the density of burrows with the density of *M. vocator*. We recorded a mean 45.1 burrows.m$^{-2}$ at Itapanhau (burrows: individuals ratio, 4.8:1), 24 burrows.m$^{-2}$ at Indaia (3.7:1), and 34.9 burrows.m$^{-2}$ at Itamambuca (7:1). Burrow density was higher than *M. vocator* density in most months (Student’s t test p < 0.05) (Fig. 6).

**Table 1.** *Minuca vocator* (Herbst, 1804). Sex ratio (males: females) in each mangrove and sampling procedure. CPUE: catch-per-unit-effort. QT: quadrat technique.*, Chi-Square test significance (p<0.05).

<table>
<thead>
<tr>
<th>Mangroves</th>
<th>CPUE</th>
<th>Quadrat</th>
</tr>
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<tbody>
<tr>
<td>Itapanhau</td>
<td>1.25 : 1$^{*}$</td>
<td>1.91 : 1$^{*}$</td>
</tr>
<tr>
<td>Indaia</td>
<td>1.67 : 1$^{*}$</td>
<td>1.29 : 1$^{*}$</td>
</tr>
<tr>
<td>Itamambuca</td>
<td>1.28 : 1$^{*}$</td>
<td>0.9 : 1</td>
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**Figure 5.** *Minuca vocator* (Herbst, 1804). Proportion of ovigerous females sampled by CPUE (catch-per-unit-effort – gray columns) and QT (quadrat technique – white columns) throughout the year, in the studied mangroves.
Figure 6. Mean density (standard deviation) of burrows (white columns) and *Minuca vocator* (Herbst, 1804) (gray columns), recorded with quadrat technique (QT) throughout the year, in the studied mangroves. *, statistical significance. ns, no significant (Student’s test).

**Discussion**

Our results suggest that sampling procedure can induce differences in estimates of population structure in fiddler crabs. Body size estimation and frequency distribution obtained by each sampling procedure did not coincide within populations. Using CPUE, adult crabs and the largest crabs were sampled more frequently, while using QT, a greater proportion of smaller crabs was found, showing that these population parameters were influenced by the sampling technique. Costa and Negreiros-Fransozo (2003), studying *L. thayeri*, also sampled larger fiddler crabs using the CPUE procedure. This suggests that a better estimate of crab sizes may be achieved by combining the data obtained using both sampling procedures.

Since in QT, the quadrat area was exhaustively scanned for *M. vocator* specimens, we assumed that the density estimates thereby obtained were accurate (Skov and Hartnoll, 2001). Although CPUE is often used as an abundance index (Richards and Schnute, 1986), we considered that it underestimated the size of *M. vocator* populations, despite the higher total number of fiddler crabs sampled with this procedure (Fig. 2). Richards and Schnute (1986) also showed that CPUE was a poor abundance estimator for marine species. Additionally, based on the spread of data recorded with each procedure, we could assume different spatial
distribution patterns for *M. vocator*. CPUE suggests a uniform or random distribution, while QT infers some degree of aggregation (Taylor, 1961; Kristensen et al., 2013). More specific studies are required to define the actual distribution pattern of *M. vocator*.

Settlement and subsequent recruitment to the adult population are processes that influence the population dynamics and densities of intertidal crabs (Flores et al., 2002; Émond et al., 2015). Precise information on juvenile density is therefore central to population ecology, but accurate estimations are difficult to obtain due to sampling constraints (Skov and Hartnoll, 2001; Almeida et al., 2008). Flores et al. (2002) used a core-type sampler (15 cm diameter and 10 cm deep), a sieve to sort sediment (upper 3-cm layer of a quadrat), and visual scanning of the surface for *Perisesarma guttatum* (A. Milne-Edwards, 1869) juveniles in a mangrove area. They found that the latter procedure provided the best results with less sampling effort. Almeida et al. (2008) assessed that the recruitment rates for *Carcinus maenas* (Linnaeus, 1758) estimated by pitfall traps were not related to natural densities of the species. In our study, both sampling procedures recorded the same continuous recruitment pattern for all three populations of *M. vocator*. However, the proportion of juveniles obtained with QT was higher than with CPUE, suggesting that recruitment rates estimated by QT were more suitable.

The presence of juveniles all year round indicates a continuous breeding season for *M. vocator*. In tropical and subtropical mangroves, fiddler crabs usually exhibit continuous reproduction, probably due to the constancy of environmental conditions and resource availability throughout the year (Colpo and Negreiros-Fransozo, 2004; Litulo, 2005; Benetti et al., 2007; Costa and Soares-Gomes, 2009; Martins and Masunari, 2013). The data on ovigerous frequency obtained by CPUE corroborated the continuous breeding hypothesis in Itapanhau and Itamambuca. However, the proportion of ovigerous females found by QT was very low and did not enable a suitable estimation of the reproductive period. As a broad-front species, *M. vocator* remains in the burrow while eggs are incubating (Crane, 1975; How et al., 2009; Ribeiro et al., 2010), which hinders ovigerous female sampling. CPUE appears to have been more efficient for providing ovigerous-dependent information than QT. At Indaia, the high recruitment rate contrasted with the low proportion of ovigerous females. This population may depend on larvae supply from a neighboring population (Bogazzi et al., 2001; Laurenzano et al., 2012).

In general, both sampling techniques showed predominance of males in all three *M. vocator* populations. The sex ratio biased in favor of males is a widely accepted trend for adult fiddler crabs, since male dominance has been recorded for many species collected by different sampling procedures (Johnson, 2003). The causes of this sex ratio pattern are historically related to differential growth rates, migration, spatial and temporal variation in the use of resources, and differential behavior patterns related to sex (Montague, 1980; Salmon, 1987; Caravello and Cameron, 1987; Spivak et al., 1991; Macia et al., 2001; Costa and Negreiros-Fransozo, 2003; Hayes et al., 2013). However, Johnson (2003) concluded that high female mortality rate, probably due their high energy investment in reproduction, is the principal cause of the notable sex ratio bias toward males in fiddler crabs populations.

Mangrove ocypodids start burrowing in the post-settlement stage (Macintosh, 1988; Kristensen, 2008). It would be possible to evaluate seasonal changes in size composition of the population through the density and size of burrows; however, estimates may be somewhat skewed. Skov and Hartnoll (2001) found a highly significant coefficient of determination ($r^2 = 0.98$) for carapace width and burrow diameter in *Uca annulipes* (H. Milne Edwards, 1837) [=*Austruca perplexa* (H. Milne Edwards, 1852) as mentioned by Shih et al., 2016]. However, Macia et al. (2001) observed that burrow counting, for this same fiddler crab species, overestimated the population by 25%. Recently, Schlacher et al. (2016) reviewed the accuracy of density estimation by means of burrow entrance counts. They found no relationship between number of burrows and population size. There is thus considerable uncertainty in predicting the density of benthic organisms according to the number of holes. In this study, the population density estimation based on burrows overestimated the natural density of *M. vocator* in all mangroves. The higher number of burrows than the existing number of fiddler crabs may be because they dig more than one burrow to escape from predators, to avoid conspecific aggression, or even that they abandoned or lost their burrows (Macintosh, 1988). Additionally, *M. vocator*
burrows have two entrances (personal observation), leading to overestimations of burrow density. Another point to consider is that other fiddler crab species were found coexisting with *M. vocator* in the sampling areas (*L. thayeri, L. uruguayensis, M. mordax, M. rapax, and M. burgersi*). Of the total fiddler crabs collected by QT, *M. vocator* represented 83% at Itapanhau, 73% at Indaia and 51% at Itamambuca. Evidently, some of the counted holes were not burrowed by *M. vocator*. The presence of sympatric burrowing species also discredits the use of indirect census techniques to measure population sizes.

To sum up, the accurate estimation of body size and size–frequency distribution requires the combination of data obtained by both CPUE and QT procedures. QT provided a reliable evaluation of population density and juvenile recruitment rates, whereas CPUE was more efficient for providing oviigerous-dependent information. However, the sex ratio in the study populations favored males, regardless of the sampling procedure. These results suggest that neither CPUE nor QT individually were accurate sampling techniques, but together provided reliable assessments of fiddler crab populations. The choice of an appropriate sampling procedure thus depends on the study goals, work scale, sampling time, existence of sympatric species and kind of environment.

**Acknowledgements**

This paper is dedicated to Professor Nilton J. Helbling who has inspired generations of Brazilian carcinologists. We thank CNPq for a fellowship to the first author, FAPESP (#98/03134-6) for financial support to the second author, SESC (Bertioga) for providing lodging, and NEBECC co-workers for their assistance with field work. All samplings were in accordance with Brazilian State and Federal laws concerning wild animals.

**References**


