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Comparison of non-invasive methods for quantifying population density of the fiddler crab *Uca tangeri*

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Excavation counts (absolute density), burrow counts and surface active individuals visual counts in a *Uca tangeri* population were compared. Population density estimated from burrow counts was not significantly different from population density estimated by excavation, but the population density estimated with visual counts was significantly lower than the absolute density. Ovigerous females should be the main cause of these differences because they frequently remain underground, but other groups of crabs are also contributing to this difference. Burrow counting is an accurate and quick non-invasive method for the census of *U. tangeri*. Other population measures (e.g. sex ratio) are not obtainable by burrow counting and only further understanding of the activity patterns of this species along the tide cycle, moon phase and reproductive season will allow visual counting of surface active individuals to be used as quick, efficient and non-invasive method for crab density and population structure estimation.

Excavation is regarded as the most accurate method to estimate the density of fiddler crab populations but it is intrusive and labour intensive, compromising the equilibrium of mudflat and salt marsh habitats. Therefore, alternative methods have been used, such as burrow counting (e.g. Ens et al., 1993; Mouton & Felder, 1996) and visual counts of surface-active individuals (e.g. Zucker, 1978; Wolfrath, 1993; Oliveira et al., 2000).

Uca tangeri is the only fiddler crab to occur in Europe and is found on almost all mudflats and salt marshes at Ria Formosa. Fieldwork took place from May to July 1999 in two periods between full moon and waning moon, at Cacela Velha and Fuzeta (Ria Formosa Natural Park, Algarve, Portugal). The air temperature ranged from 21.0°C to 33.5°C, higher than the minimum temperature needed for *U. tangeri* surface activity (18°C), (Wolfrath, 1993). Each day, five square plots of 1 m^2 were placed on the ground and the counting of surface-active individuals was conducted on the same day, after the set-up. The observer would remain motionless for 15 min at a distance of 3.5 m from the squares, back towards the sun, before starting observations. A total of six counts were done with a 5 min interval between them, always during ebb tide. At each count, the number of males and females per square were recorded, using binoculars. Individuals difficult to sex at that distance were counted in a third category. In subsequent analysis, the average of the six observations was used when referring to visual counts of surface-active individuals. After the end of the observations, the number of burrows in each square was counted, and their diameter was measured using Vernier callipers (±0.01 mm). This procedure was done for six days, completing a total of 30 observed squares. The burrows were then excavated individually throughout their depth until the occupant was found or the burrow was found to be empty. The rest of each plot was also excavated to a depth of approximately 50 cm and all the crabs found in the soil were collected and sexed. It was also noted if females were ovigerous or not.

Burrow density (6.33 ±4.43 burrows m^{-2}) was not significantly different from absolute crab density (6.23 ±3.53 individuals m^{-2}), (Wilcoxon matched pairs test: N=30, P=0.63). However, the density estimated with visual counts (2.44 ±1.25 individuals m^{-2}) was significantly lower than absolute density (Wilcoxon matched pairs tests: N=30, $P<0.001$), underestimating it in 60.8%. In other studies with *Uca annulipes*, burrow density was shown to overestimate crab density from 25% (Macia et al., 2001) to 46% (Skov et al., 2002) due to unoccupied burrows. Underestimation through direct visual counting, may be due to a significant part of the total individuals that remain underground, in particular ovigerous females and moulting individuals. Even breeding females and courting males do not surface every low tide (Fiona Burford and Joana Jordão, personal observations), remaining inside their burrows. Several authors had already shown that visual counts underestimated absolute abundance in *U. annulipes*, going up to 51% (Skov & Hartnoll, 2001). *Uca tangeri* individuals only moult once a year at the end of the breeding season (von Hagen, 1962), so this group should not contribute to the underestimation through visual counting in this study, which was carried out in the peak of the breeding season. Of the total number of females collected (149), 51.7% were ovigerous and this group would be expected to be the main cause of the differences observed between the absolute density and the density estimated with visual counts. However, the density of visual counts (2.44 ±1.25 individuals m^{-2}) is still significantly lower than the density of excavated crabs, even if we discard ovigerous females from the excavated numbers (3.67 ±2.12 individuals m^{-2}), (Wilcoxon matched pairs test: N=30, $P<0.001$), suggesting that other groups of crabs were not surfacing.

The numbers of surface active females and the absolute number of females (observed: 1.46 ±1.18 individuals m^{-2}; absolute: 4.97 ±3.32 individuals m^{-2}) were significantly different (Wilcoxon matched pairs test: N=30, $P<0.001$), and the same is true for the males (observed: 0.39 ±0.49 individuals m^{-2}; absolute: 1.27 ±1.28 individuals m^{-2}; Wilcoxon matched pairs test: N=30, $P<0.001$). This suggests that both males and females are equally underestimated by visual counts in about 70%, and therefore the absolute sex ratio (males: females=0.26) did not differ from the observed sex ratio of surfacing individuals (males: females=0.27). However, some individuals that could not be sexed at the distance were discarded from the surfacing individuals sex ratio. Discarded crabs were on average

0.59 ±0.54 individuals m^{-2}, which is about 25% of the total individuals observed by visual counts (2.44 ±1.25 individuals m^{-2}). There are studies in this population of *U. tangeri* where the observed sex ratio by visual counts is male biased (e.g. Wolfrath 1993: males: females=1.5; Oliveira et al., 2000: males: females=1.18). There is a general tendency for the sex ratio estimated from surface active individuals to be more male-biased that the real sex ratio. This would suggest that probably most of the individuals that could not be sexed through binoculars are males (which would result in surface active individuals sex ratio of 0.67 males: females). In fact, there are no differences between absolute males and visually counted males if the unsexed individuals by visual observation are added to the males' numbers (Wilcoxon matched pairs test: N=30, P=0.27).

In summary, burrow counting is a reasonably accurate and quick non-invasive method for the census of density of *U. tangeri*, in this population. Direct observation of surface-active crabs, as shown here, is not as accurate for estimating density. In our results, the sex ratio recorded for surfacing individuals did not differ from the actual sex ratio but there seems to be a general tendency for visual counts to overestimate males in relation to females, considering data from other studies using the same method and in the same study population. These differences could reflect an inaccuracy in the method (some individuals being difficult to sex at the distance) or, most likely, changes in the population activity patterns through time. Macia et al. (2001) found differences in the observed sex ratio during the reproductive season for *U. annulipes*, accordingly to moon phase; Skov et al. (2002) also report a vast variation in the accuracy of visual counts for the estimation of sex ratio of *U. annulipes*, depending on moon phase and population; and there are data regarding this population of *U. tangeri* where the sex ratio estimation of surface active individuals ranges for 0.43 to 5.00 (males : females), depending on time of day (before or after low tide) and day in lunar cycle (Denise Pope, unpublished data). Further contributions on the activity patterns of this species along the tide cycle, moon phase and reproductive season would be necessary in finding correction factors, allowing visual counting of surface active individuals to be used as a quick, efficient and non-invasive method for crab density and population structure estimation. The measurement of burrow entrance diameter can give additional information on the biomass values of the population, given the high correlation between burrow diameter and carapace size in this population (Lourenço et al., 2000), but it still lacks on the information of whether the occupant is a male or a female.

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