Int. J. Aquat. Biol. (2024) 12(5): 452-458
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

A comparative study of photography and mud excavation methods for estimating population density, sex ratio, body size, and claw size of the fiddler crab *Austruca perplexa* (Brachyura, Ocypodidae)

Kanitta Keeratipattarakarn[1], Fahmida Wazed Tina[*2,3]

[1]Data Analysis and Digital Technology Program, Faculty of Science and Technology, Nakhon Si Thammarat Rajabhat University, Nakhon Si Thammarat 80280, Thailand.
[2]Mathematics Program (Statistics Major), Faculty of Science and Technology, Nakhon Si Thammarat Rajabhat University, Nakhon Si Thammarat 80280, Thailand.
[3]Creative Innovation in Science and Technology Program, Faculty of Science and Technology, Nakhon Si Thammarat Rajabhat University, Nakhon Si Thammarat 80280, Thailand.

Abstract: Though fiddler crabs are considered ecosystem engineers in mangroves, accurately estimating their density, sex ratio, and body sizes is challenging. The conventional method of mud excavation is frequently employed to estimate these parameters, yet it proves destructive because it destroys crab burrows and stresses the crabs. In this study, we compared a non-destructive photography method with the mud excavation method in a fiddler crab, *Austruca perplexa*, population in southern Thailand. Ten 0.25 m^2 quadrats were fixed, and 4 photographs of the crabs inside each quadrat were taken. Subsequently, the mud within the quadrat was excavated to 30 cm, and all crabs were collected. Parameters such as crab numbers, sexes, body sizes, male handedness (right/left major claws), and male major claw sizes were estimated. Afterward, the photographs were processed in GIMP (GNU Image Manipulation Program) to estimate the same parameters. These parameters were then compared between the two methods. We observed that the photography method was as effective as the mud excavation method in measuring most parameters, except female density, which was higher in the mud excavation method. This study reveals that the photography method could be used effectively instead of the mud excavation method in estimating several population parameters in fiddler crabs while acknowledging its limitation in accurately estimating female density.

Article history:
Received 6 September 2024
Accepted 25 October 2024
Available online 25 October 2024

Keywords:
GNU Image Manipulation Program (GIMP)
Mud excavation
Photography

Introduction

Fiddler crabs inhabit a diverse range of ecosystems, including tropical and subtropical estuaries, mangrove swamps, salt marshes, mudflats, and sandy beaches, and they are the most conspicuous group of invertebrates (Hartnoll et al., 2002; Costa and Negreiros-Fransozo, 2003). Extensive research has been conducted on their distribution, population structure, and taxonomic status (Crane, 1975). Notably, female fiddler crabs possess two small feeding claws employed to scoop sediment from the substrate surface and put it into the mouth (Tina et al., 2016). In contrast, males exhibit one small feeding claw and another large (major) claw utilized to attract mates and engage in male-male combats (Tina et al., 2016, 2019). The orientation of the major claw is equally likely to be either on the left- or right-hand side of the body (i.e., handedness of males) (Crane, 1975).

Both male and female fiddler crabs engage in burrowing activities, which, coupled with their feeding activities, significantly influence the function and structure of their habitats. These activities contribute to improving sediment oxidation and drainage, enhancing the growth of microorganisms in the substrate, and increasing the decomposition rate of plant debris within sediments, earning fiddler crabs the designation of ecosystem engineers (Kristensen, 2008; Smith et al., 2009).

Since fiddler crab populations are beneficial for their habitats, it is necessary to estimate the key population parameters such as crab density, sex ratio,

[*]Correspondence: Fahmida Wazed Tina
E-mail: fahmida_tina@nstru.ac.th
DOI: https://doi.org/10.22034/ijab.v12i5.2354

body sizes of males and females, claw sizes of males, and male handedness within a given population. Despite the ecological significance of these estimations, field assessments of these parameters are challenging, particularly in mangrove sites characterized by high root densities. Traditionally, population density, sex ratio, and the body/claw sizes of crabs are assessed through widely employed methods such as mud/soil excavation (where mud/soil is excavated to a depth of 25-50 cm) and burrow excavation (focused solely on crab burrows) (Frith and Brunenmeister, 1980; Litulo, 2006; Silva and Calado, 2013). While the mud excavation method yields a precise estimation of the population density, sex ratio, and body/claw sizes of the crabs, it is destructive, resource-intensive, and time-consuming (Hubbard, 2008). In contrast, burrow excavation, although an easier and less destructive method since the whole sampling area is not excavated, still destroys crab burrows and interferes with their activities. Notably, this method may underestimate crab density when individuals remain concealed within closed burrows. Various non-destructive methods have been explored to mitigate the limitations of destructive methods, including crab burrow counts, visual counts, and photography. While the burrow count method aligns closely with the crab density estimated by the mud excavation method (Jordão and Oliveira, 2003), it cannot measure the sex ratio, body size, and claw sizes of the crabs. The visual count method, utilizing binoculars to count the surface-active crabs, is unable to count the underground crabs and results in an underestimation of crab density compared to the crab density obtained in the mud excavation method (Skov and Hartnoll, 2001; Jordão and Oliveira, 2003). Moreover, it lacks the capability to measure the body and claw sizes of the crabs.

The photography method presents a promising alternative, involving capturing images of surface-active crabs for subsequent estimation of population parameters (Keeratipattarakarn et al., 2021). A recent study (Keeratipattarakarn et al., 2021) compared the efficacy of photography with the burrow excavation method and found that photography is as effective as the burrow excavation method in estimating crab density, sex-ratio, and body/claw size distribution of fiddler crab populations. However, that study did not assess the effectiveness of the photography method with the mud excavation method, which gives a more accurate estimation of fiddler crabs' population parameters. Addressing this gap is crucial for establishing the broader applicability of the photography method in the comprehensive assessment of fiddler crab populations. Therefore, this study aims to assess the efficacy of the photography method in comparison to the conventional mud excavation method for estimating various crucial parameters within a fiddler crab, *Austruca perplexa*, population, namely male and female density, sex ratio, male and female body sizes, male major claw sizes, and male handedness (distinguishing between right- and left-clawed males). We predict that there will be no significant difference in estimating these parameters between photography and mud excavation methods.

Materials and Methods

Study site: This study was conducted in Ban Nai Thung village, Thasala district, Nakhon Si Thammarat province, southern Thailand (8°38'41.76''N, 99°57'04.54''E). The fiddler crab, *A. perplexa* population occupied approximately 300 m^2. The study was conducted in September 2023 from 9:30 a.m. to 3:30 p.m. and during low tides.

Data collection: The density, sex ratio, body size of males and females, major claw sizes of males, and male handedness of *A. perplexa* were estimated using ten 0.25 m^2 quadrats. The quadrats were set randomly inside the crab population. After fixing one quadrat, a small piece of paper with the quadrat's number and a 30 cm ruler were placed in the middle of the quadrat. The ruler was used as a scale for measurement (Keeratipattarakarn et al., 2021). Afterward, we waited for 15-20 minutes for the crabs to come out from their burrows and start surface activities. Then, 4 photos were taken using the Canon EOS M100 camera. Each photo was taken at 5-minute intervals from a distance of 1.5 m (Keeratipattarakarn et al.,

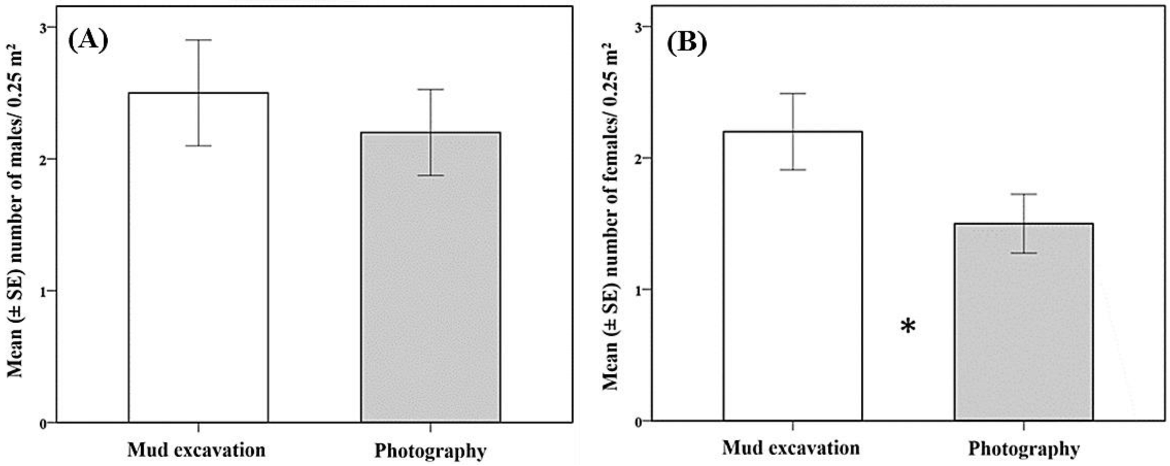

Figure 1. The density of *Austruca perplexa*, compared between 'mud excavation (white bars)' and 'photography' (grey bars) methods: (A) male density, and (B) female density. The asterisk indicates a statistically significant difference ($P<0.05$).

2021). While taking photos, the photographer did not change his/her position since a little movement forces the crabs to enter their burrows. After taking photos, all the crab burrows were excavated to catch the fiddler crabs from their burrows. Then, the mud inside the quadrat was excavated to a depth of 30 cm, and all underground crabs were caught. The number of males and females was counted, and the carapace width (mm) of males and females and the major claw length (mm) of males were measured using Vernier callipers. The handedness (right- or left-clawed) of males was also recorded.

In the lab, an open-source image analysis software GNU Image Manipulation Program (GIMP), version 2.10 (http://www.gimp.org/) was used to process the photographs (Dos Reis et al., 2016; Brown and Wells, 2020; Keeratipattarakarn et al., 2021). After opening the GIMP program on the computer, one photograph from one quadrat was selected and opened. The default unit for the ruler in GIMP canvas is pixels. That unit was changed from pixels to millimetres to measure the body and claw sizes of the crabs. First, the number of males and females were counted and recorded. Then, the carapace width of each crab and the major claw length of each male were measured using the scale (mm) of the ruler used in the photograph as a measurement tool. The handedness of each male was checked and recorded. If the carapace width or major claw length of any crab was not clear or not measurable, the other photographs were opened in the GIMP program and checked. However, all three remaining photographs were checked, and if any additional crab was observed, it was recorded, and its body size, claw size, and handedness were measured.

Statistical analysis: The normality of the data was assessed using the Skewness, Kurtosis, and Shapiro-Wilk tests before starting data analysis. The differences in mean crab (male and female) density (crab numbers/0.25 m^2) between the 'mud excavation' and 'photography' methods were tested using a paired-sample *t*-test. Fisher's exact probability tests were used to determine whether the sex ratio and the ratio of right- and left-clawed males differed between the two methods. Body size-frequency distributions (i.e., carapace width) of both sexes and claw size-frequency distributions (i.e., major claw length) of males were compared between two methods using Kolmogorov-Smirnov test. All data were reported as mean ± standard error (SE), and all tests were considered statistically significant at the 0.05 level.

Results

The density of *A. perplexa*: Male density was not different between the 'mud excavation' and 'photography' methods (t_9 = 1.964, P = 0.081), but female density was significantly higher in the 'mud excavation' method than in the 'photography' method (t_9 = 3.280, P = 0.010) (Fig. 1).

Sex-ratio of *A. perplexa*: The sex-ratio of males and females was not significantly different between the

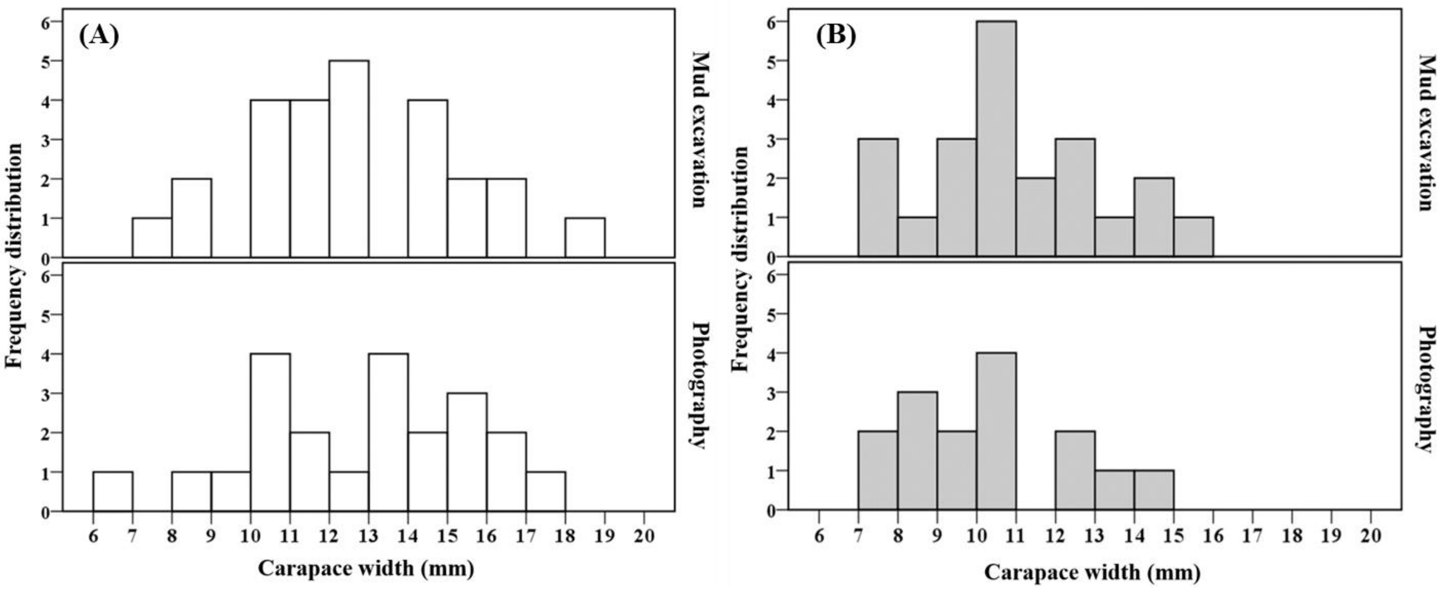

Figure 2. Size-frequency (carapace width) distributions of *Austruca perplexa* obtained in the 'mud excavation' and 'photography' methods: (A) males (white bars), and (B) females (grey bars).

'mud excavation' and 'photography' methods (mud excavation- male: 25, female: 22; photography-male: 22, female: 15; Fisher's exact test: $P = 0.660$).

Size-frequency distributions of *A. perplexa*: The body size-frequency (i.e., carapace width) distributions of males and females were not different between 'mud excavation' and 'photography' methods (males: Kolmogorov-Smirnov, $Z = 0.634$, $P = 0.816$ (Fig. 2A); females: Kolmogorov-Smirnov, $Z = 0.697$, $P = 0.716$ (Fig. 2B)). Moreover, major claw length distributions of males were also not different between two methods (Kolmogorov-Smirnov, $Z = 0.305$, $P = 1.000$ (Fig. 3)).

Handedness of *A. perplexa*: The ratio of right and left major claws did not show any difference between 'mud excavation' and 'photography' methods (handedness: mud excavation- right-clawed males: 15, left-clawed males: 10; photography- right-clawed males: 15, left-clawed males: 7; Fisher's exact test: $P = 0.762$).

Discussions

No significant difference was observed in male density between the mud excavation and photography methods, aligning with the findings of Keeratipattarakarn et al. (2021), which also reported no significant difference in male density when comparing the photography method with the burrow excavation method. These results suggest the efficacy of the photography method in accurately measuring male density within a fiddler crab population.

Conversely, the mud excavation method yielded a higher female density than the photography technique, indicating that the latter missed the females who were concealed inside closed burrows. *Austruca perplexa* is an underground mating species (i.e., resource-based mating system) where males construct breeding burrows and attract females towards those burrows by waving their major claws (Tina et al., 2018; Tina and Muramatsu, 2022). Females, in turn, search for males and visit multiple mating burrows before selecting a suitable one for breeding. Afterward, the pair mates underground, and the female stays with her partner for one to four days until she ovulates the eggs (Nakasone and Murai, 1998; Backwell et al., 2006; Murai and Backwell, 2006). After ovulation, the male leaves the burrow, and the female conceals the burrow entrance (Backwell et al., 2006). The female then uses that burrow for 14-15 days for egg incubation, and during egg incubation, she does not come to the surface for feeding or other activities (Nakasone and Murai, 1998). The reason for being underground during the egg incubation period is that the females of this species are broad-fronted (Henmi, 2003) and have

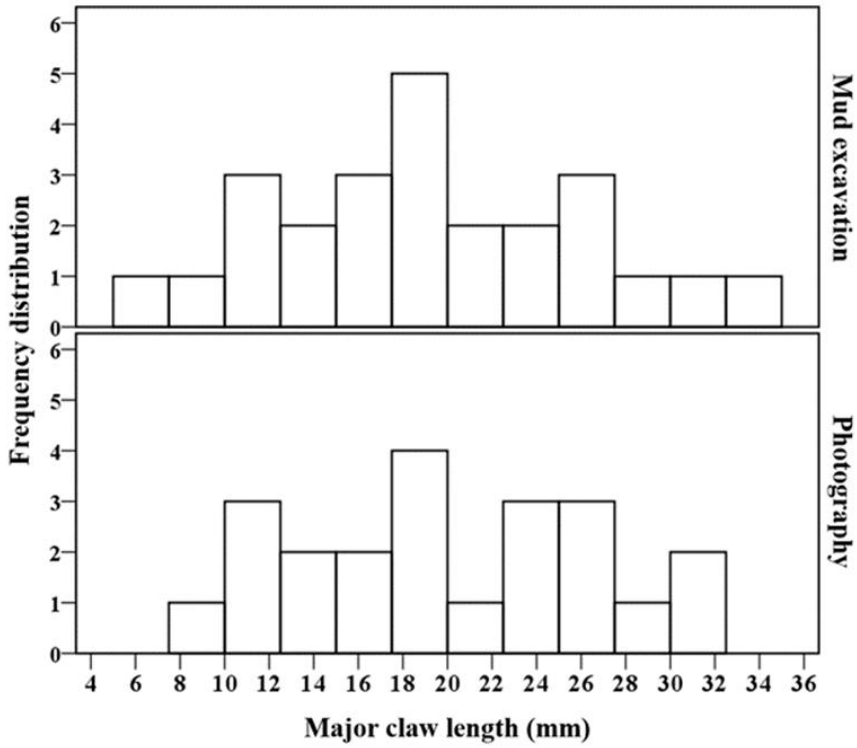

Figure 3. Size-frequency (major claw length) distributions of *Austruca perplexa* males obtained in the 'mud excavation' and 'photography' methods, as indicated on the figures.

large egg clutches that are not completely covered with the abdominal flaps, and thus, there is a chance of desiccation during feeding or other activities on the surface. Therefore, they stay underground during egg incubation and rarely or do not come to the surface until their eggs hatch (Christy and Salmon, 1991). This could be the reason why underground females were not captured in the photography method. However, the photography method might be useful to estimate female density in the fiddler crab species where females show surface mating (i.e., a resource-free mating system). In surface mating, females build breeding burrows, and they mate with the males at the surface next to their burrows. Afterward, females incubate eggs inside their breeding burrows (Christy and Salmon, 1984). Females of surface mating fiddler crab species are typically narrow-fronted and have small egg clutches that are covered and protected by the abdominal flaps therefore, they commonly feed and do other activities on the surface while their eggs develop (Christy and Salmon, 1991) such as *Tubuca dussumieri*, *Gelasimus vocans* and *T. rosea* (Murai et al., 1996; Henmi, 2003). However, further research is required to test whether the photography method could estimate female density accurately in surface mating and narrow-fronted fiddler crab species.

The photography method showed efficiency in determining the sexes and estimating the sex ratio of fiddler crabs. The clarity in the photographs allowed for reliable identification of the minor (feeding) and major claws of the crabs, facilitating the differentiation of sexes. This study and a previous study (Keeratipattarakarn et al., 2021) have found that the photography method is as effective as the mud excavation and burrow excavation methods during assessing the sex ratio of fiddler crabs. The photography method is also able to determine the males lacking a major claw and can differentiate the male from a female, which is difficult in the binocular observation method (Macia et al., 2001).

This study reveals that the photography method is similar to the mud excavation method while measuring the body size of males and females, the claw size of males, and male handedness. In contrast, a few alternative non-destructive methods, like visual count (i.e., binocular) and burrow count methods, are not capable of accurately measuring the body and claw sizes of the fiddler crabs. In the burrow count method, it might be possible to make a regression equation first between the burrow diameter and carapace width of resident fiddler crabs and later calculate the crab size of the population based on the burrow diameters (Skov and Hartnoll, 2001). However, in the burrow count method, it is impossible to determine the sex of the resident crabs based on the burrows.

Conclusion

This investigation demonstrates the comparable effectiveness of the photography method to the traditional destructive mud excavation method in estimating male density, sex ratio, body sizes of males and females, major claw sizes of males, and male handedness in *A. perplexa*. However, the photography method exhibited limitations when estimating female density due to the underground mating behaviour of *A. perplexa*. The effectiveness of the photography method may vary depending on the mating system and behaviours exhibited by the species under investigation. Since the photography method is capable of estimating most of the population parameters of fiddler crab species and is environmentally friendly, it could be used as an alternative to the mud excavation method, which is a destructive and time-consuming method. Further research is warranted to explore the broader applicability of the 'photography' method across diverse fiddler crab and other crab populations.

Animal ethics

Before commencing the research endeavours in Ban Nai Thung village, Thasala, permission was sought and obtained from the head of the village. Additionally, all procedures involving the handling of fiddler crabs throughout the course of this study strictly adhered to the ethical standards outlined in the 'Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes' by the National Research Council of Thailand (User Application ID U1-10997-2566). It is imperative to note that no crabs were harmed or sacrificed during the research process, and upon completion of data collection, they were promptly released back into their natural habitat. Through this commitment, we underscore our dedication to ethical and responsible conduct in scientific investigations.

Acknowledgements

The authors would like to thank the undergraduate students of the Mathematics and Statistics Program, and Computer Science Program under the Faculty of Science and Technology, Nakhon Si Thammarat Rajabhat University, who helped during data collection from the field.

References

Backwell P., Jennions M., Wada K., Murai M., Christy J. (2006). Synchronous waving in two species of fiddler crabs. Acta Ethologica, 9: 22-25.

Brown M.B., Wells E. (2020). Skeletal dysplasia-like syndromes in wild giraffe. BMC Research Notes, 13: 1-6.

Christy J.H., Salmon M. (1984). Ecology and evolution of mating systems of fiddler crabs (Genus *Uca*). Biological Reviews, 59(4): 483-509.

Christy J.H., Salmon M. (1991). Comparative studies of reproductive behavior in mantis shrimps and fiddler crabs. American Zoologist, 31(2): 329-337.

Costa T.M., Negreiros-Fransozo M.L. (2003). Population biology of *Uca thayeri* Rathbun, 1900 (Brachyura, Ocypodidae) in a subtropical South American mangrove area: results from transect and catch-per-unit-effort techniques. Crustaceana, 75(10): 1201-1218.

Crane J. (1975). Fiddler crabs of the world (Ocypodidae: genus *Uca*). Princeton University Press, Princeton.

Dos Reis F.J., De Barros E Silva V., De Lucena R.N., Mendes Cardoso B.A., Nogueira L.C. (2016). Measuring the pain area: an intra- and inter-rater reliability study using image analysis software. Pain Practice 16(1): 24-30.

Frith D.W., Brunenmeister S. (1980). Ecological and population studies of fiddler crabs (Ocypodidae: genus *Uca*) on a mangrove shore at Phuket Island, western Peninsular Thailand. Crustaceana, 39: 157-184.

Hartnoll R.G., Cannici S., Emmerson W.D., Fratini S., Macia A., Mgaya Y., Porri F., Ruwa R.K., Shunula J.P., Skov M.W., Vannini M. (2002). Geographic trends in mangrove crab abundance in east Africa. Wetlands Ecology and Management, 10: 203-213.

Henmi Y. (2003). Trade-off between brood size and brood interval and the evolution of underground incubation in three fiddler crabs (*Uca perplexa*, *U. vocans*, and *U. dussumieri*). Journal of Crustacean Biology, 23(1): 46-54.

Hubbard C.R. (2008). A comparison of invasive and non-invasive techniques for measuring fiddler crab density in a salt marsh. M.Sc. thesis, Georgia Southern University, Statesboro, USA.

Jordão J.M., Oliveira R.F. (2003). Comparison of non-invasive methods for quantifying population density of the fiddler crab *Uca tangeri*. Journal of the Marine Biological Association of the United Kingdom, 83(5): 981-982.

Keeratipattarakarn K., Tina F.W., Sangngam R., Thongsri K., Suphap A. (2021). 'Photography'as a useful method for estimating the density, sex-ratio and body size of the surface-active *Austruca perplexa* (H. Milne Edwards, 1852) (Brachyura, Ocypodidae). Crustaceana, 94: 1429-1440.

Kristensen E. (2008). Mangrove crabs as ecosystem engineers; with emphasis on sediment processes. Journal of sea Research, 59(1-2): 30-43.

Litulo C. (2006). Population and reproductive biology of the fiddler crab *Uca chlorophthalmus* (Brachyura: Ocypodidae) from Inhaca Island, southern Mozambique. Journal of the Marine Biological Association of the United Kingdom, 86(4): 737-742.

Macia A., Ivaldo Q., Paula J. (2001). A comparison of alternative methods for estimating population density of the fiddler crab *Uca annulipes* at Saco mangrove, Inhaca Island (Mozambique). Hydrobiologia, 449: 213-219.

Murai M., Backwell P.R. (2006). A conspicuous courtship signal in the fiddler crab *Uca perplexa*: female choice based on display structure. Behavioral Ecology and Sociobiology, 60: 736-741.

Murai M., Goshima S., Kawai K., Yong H.S. (1996). Pair formation in the burrows of the fiddler crab *Uca rosea* (Decapoda: Ocypodidae). Journal of Crustacean Biology, 16(3): 522-52.

Nakasone Y., Murai M. (1998). Mating behaviour of *Uca lactea perplexa* (Decapoda: Ocypodidae). Journal of Crustacean Biology, 18 (1): 70-77.

Silva W., Calado T. (2013). Number of ghost crab burrows does not correspond to population size. Open Life Sciences, 8(9): 843-847.

Skov M.W., Hartnoll R.G. (2001). Comparative suitability of binocular observation, burrow counting and excavation for the quantification of the mangrove fiddler crab *Uca annulipes* (H. Milne Edwards). Hydrobiologia, 449: 201-222.

Smith N.F., Wilcox C., Lessmann J.M. (2009). Fiddler crab burrowing affects growth and production of the white mangrove (*Laguncularia racemosa*) in a restored Florida coastal marsh. Marine Biology, 156: 2255-2266.

Tina F.W., Jaroensutasinee M., Jaroensutasinee K. (2016). A note on behavioural and morphological compensations of male *Uca vocans* (Linnaeus, 1758) relative to females, for the loss of one functional feeding claw. Crustaceana, 89(8): 975-981.

Tina F.W., Jaroensutasinee M., Jaroensutasinee K. (2018). Claw regeneration, waving display and burrow characteristics of *Austruca perplexa* (H. Milne Edwards, 1852) (Brachyura, Ocypodidae) from southern Thailand. Crustaceana, 91(10): 1247-1257.

Tina F.W., Keeratipattarakarn K., Jaroensutasinee M., Jaroensutasinee K. (2019). Time allocations for different activities in the fiddler crab *Tubuca rosea* (Tweedie, 1937) (Brachyura, Ocypodidae). Journal of Animal Behaviour and Biometeorology, 7: 60-65.

Tina F.W., Muramatsu D. (2022). Males signal their breeding burrow characteristics to females in the fiddler crab *Austruca perplexa*. Behaviour, 159(11): 1045-1062.