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Validation of Traditional Species Identification Techniques for Juvenile Mangrove Crabs (*Scylla* spp.)

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Species identification of mangrove crabs at the juvenile stages is crucial for maximizing production. Traditional knowledge in identifying juvenile mangrove crabs (*Scylla* spp.) is mainstream although the techniques vary with locations and are yet to be validated for their effectiveness. In this study, widespread traditional techniques for identifying juvenile *Scylla* were validated by looking for patterns through principal component analysis in dorsal carapace features – including bumps, black spots, coloration, and shape – as determined by CL:ICW. The less prominent bumps on the dorsal carapace and the lower CL:ICW of *S. olivacea* were seen to have the potential for nationwide adoption to distinguish this least preferred species. The dorsal carapace color varies across species but is inconsistent across regions. The presence of black and white spots, texture features such as depression on the center of the dorsal carapace, and growth rates of the samples were found to have no diagnostic applications for juvenile *Scylla.* This study confirmed the utility of some traditional techniques to identify juvenile *S. olivacea* without the aid of a device, but no consistent method to differentiate *S. serrata* from *S. tranquebarica* was found. Image analysis data on these validated techniques can be added to the machine learning algorithm of existing species identification tools to increase accuracy.

Keywords: aquaculture, fisheries, *Scylla*, taxonomy, traditional ecological knowledge

INTRODUCTION

Mangrove crabs (*Scylla* spp.), locally known as "alimango," are commodities of high value owing to their good quality meat. Three of the four *Scylla* have been recorded in wild populations in the Philippines. These are *Scylla serrata* (Forskål, 1775), *Scylla olivacea* (Herbst, 1796), and *Scylla tranquebarica* (Fabricius, 1798) (Keenan *et al.* 1998). Although the grow-out of mangrove crabs is a fast-growing industry, it is limited by the supply of crablets from hatcheries. The majority of mangrove crabs for grow-out are still sourced from wild populations in different geographic areas (VinceCruz-Abeledo *et al.* 2020).

Grow-out pond owners and traders have differing species preferences, depending on their goals. *Scylla olivacea* is preferred for soft-shell farming, as it is smaller in size. It is also preferred for fattening in some coastal areas in the country (Rodriguez *et al.* 2003). *Scylla serrata* is preferred for grow-out culture (juvenile to market size) and fattening due to its faster growth rate and tolerance for a wider range of conditions [Hastuti *et al.* (2019), as cited in VinceCruz-Abeledo *et al.* (2020)]. The wild population is a mix of the three species due to their overlapping ranges (Walton *et al.* 2006). Thus, farmers are not guaranteed to grow their preferred species. To maximize production gain, fishermen need to distinguish them before crab farming.

Farmers from different regions speak of a variety of traditional techniques they use to identify species of juvenile *Scylla*. This includes observation of the size, texture, and color of the claws; the color of the ventral and dorsal carapace; the presence of spots around the claws

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*Corresponding author: hilarie.orario@dlsu.edu.ph and on the dorsal carapace; the texture of the carapace; venation at the back and the legs of the crab; and even observation of the mouth or how the crabs walk (Orario *et al.* 2021). These techniques are hit-or-miss in terms of effectiveness and have yet to be validated.

In this study, select widespread traditional techniques for identifying juvenile *Scylla* were validated by looking for patterns – using principal component analysis (PCA) – of dorsal carapace features including bumps, black spots, coloration, and shape, as determined by the ratio of carapace length to internal carapace width (CL:ICW). Further analyses were done to determine the potential scale of the utility of these features. This study confirmed the utility of traditional techniques to identify juvenile *Scylla* without the aid of a device. Quantitative measurement of the features of validated techniques may also be done to establish patterns for developing a technology that relies on these traditional techniques as a basis for species identification.

MATERIALS AND METHODS

Collection of Samples

Ninety (90) mangrove crabs (*Scylla* spp.) in early juvenile stages were caught from the wild, either as early juveniles or earlier developmental stages from three locations in the Philippines – Cagayan, Camarines Norte, and Sorsogon, which are known to have high production rates of mangrove crabs in the country in the recent years. The crabs from Cagayan were sourced in March 2022, those from Camarines Norte in April 2022, and those from Sorsogon in June 2022. As the samples included in this study were caught from the wild, the number of samples for each species could not be guaranteed. Hatchery rearing in the country also focuses on *S. serrata*, as the farming of this species is more economically viable than the other two species (Quinitio *et al.* 2018). Juvenile mangrove crab availability in the Philippines is seasonal following patterns of spawning, recruitment season, rainfall, and salinity, but traders mentioned that the peak season for *S. serrata* and *S. tranquebarica* were January–March in Northern Samar and Camarines Norte and April–June in Cagayan, whereas the peak season for juvenile *S. olivacea* was from July–October in Camarines Norte (Gaillard 2010). The carapace width (CW) of the samples for this study was 42.4 ± 7.3 mm. Proper protocols for the care and use of animals were followed under the ethical clearance EXT.008.2018-2019.T2.COS of the Research Ethics Office of the De La Salle University. Crabs were transported in containers that had enough moisture and ventilation to keep them alive. Flash freezing at –20 °C was the method employed for sacrificing the samples before tissue extraction.

Morphological Analysis

Seven dorsal carapace features were analyzed considering the widespread traditional techniques used by fishermen in identifying juvenile *Scylla* (Orario *et al.* 2021). This includes color, bumps, depressions, white spots, black spots, shape based on CL:ICW, and growth rate based on the difference between CL post-acclimation and CL pre-acclimation divided by the difference between ICW post-acclimation and ICW pre-acclimation (Figure 1). Morphological analysis is done pre- and post-acclimation. Pictures of crab samples were taken in a photo box using a standardized setting in an iPhone 12 pro camera (*i.e.* 12-in distance of the sample from the camera, exposure of +2.0, ambient light). Dorsal carapace color was documented using both quantitative analysis of the pixels of the picture of each sample's dorsal carapace and qualitative scoring of the color of each sample. Texture features of the dorsal carapace – including the bumps, depressions, and spots – were

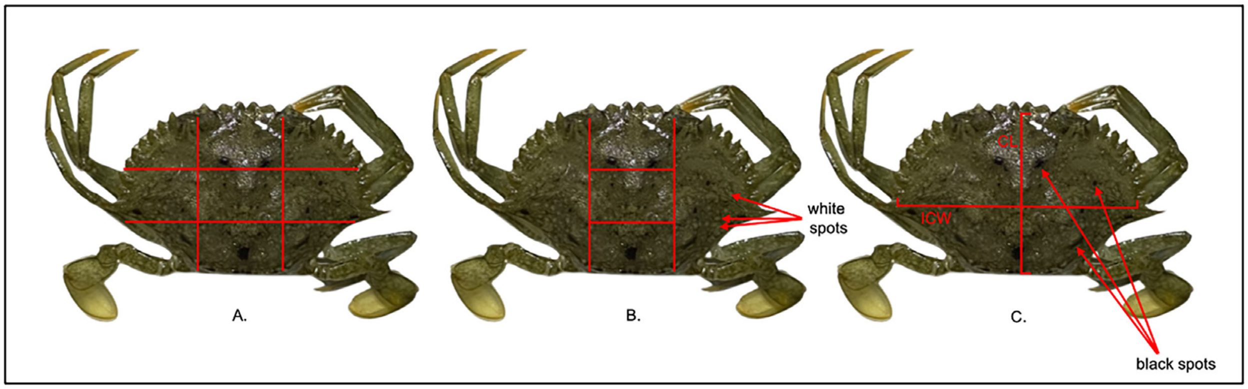

Figure 1. [A] Positions on the dorsal carapace where bumps can be found; [B] positions on the dorsal carapace where depressions can be found, and sample of white spots; [C] The carapace length (CL), internal carapace width (ICW), and sample of black spots.

qualitatively scored. Morphometric characters were measured from the image of each sample. Qualitative observations were converted into discrete values (*i.e.* the presence or absence of bumps on specific positions on the dorsal carapace were recorded as 1 or 0, respectively) and were standardized using a z-score alongside numerical measurements.

Acclimation of Crabs

Three rounds of a common garden experiment were set up. In each round, 30–50 samples from one site were reared for 30 d to remove the effect of the environment on the characteristics of the samples. Each sample was assigned a number in their respective groups per source and kept individually in 17.5 x 10 x 8 cm cages that were set in 60 x 40 x 20 cm tanks. Five cages are placed in each tank. The following environmental parameters recorded throughout the duration of acclimation were as follows: temperature of 27–30 °C, salinity of 10–25 ppt, and oxygen concentration of 3–5 ppm. The samples were fed with a mixture of trash fish and seafood entrails at 50–80% of total body weight per day [SEAFDEC-AQD (2016): Farming Mangrove Crab (Mud Crab)].

Confirmation of Species through Molecular Method

The species identity of each sample was confirmed using the 16S rRNA gene marker. DNA was extracted from the muscle tissue using the KAPA Express Extract kit, and the 16S rRNA sequence was amplified using the primers and protocols, following Imai *et al.* (2004). The presence of 16S rRNA genes in the amplified PCR products was checked on 1.0% agarose gel with a 1000-bp ladder. The PCR products were sent to a sequencing facility for DNA purification and sequencing. The resulting sequences were processed in such a way that the series of bases that mostly have less than 40 Q scores at the beginning and end of the sequence were deleted. The processed sequences were entered as a query sequence in NCBI BLAST (Basic Local Alignment Search Tool) for nucleotide sequences to find matching sequences from the database. Sequences were aligned using ClustalW and neighbor-joining analysis was used to generate a phylogenetic tree to verify species identification.

Statistical Analysis

The PCA was performed to check if the morphological variations of the traditional techniques would result in species clusters. Paired sample t-test was used to compare means before and after acclimation. One-way analysis of variance was done to compare the means of the three species for specific variables.

RESULTS

Species Identity of Samples Confirmed by Molecular Methods

A total of 85 samples were identified by molecular means. Twenty-five (25) out of the 30 samples from Cagayan were found to be *S. serrata*, whereas the remaining five were found to be *S. tranquebarica.* All 25 samples from Camarines Norte were found to be *S. olivacea*. The 30 samples from Sorsogon were all found to be *S. serrata*. Figure 2 shows the closer genetic relationship between *S. serrata* and *S. tranquebarica* compared with the genetic relationship of *S. olivacea* with either of the two.

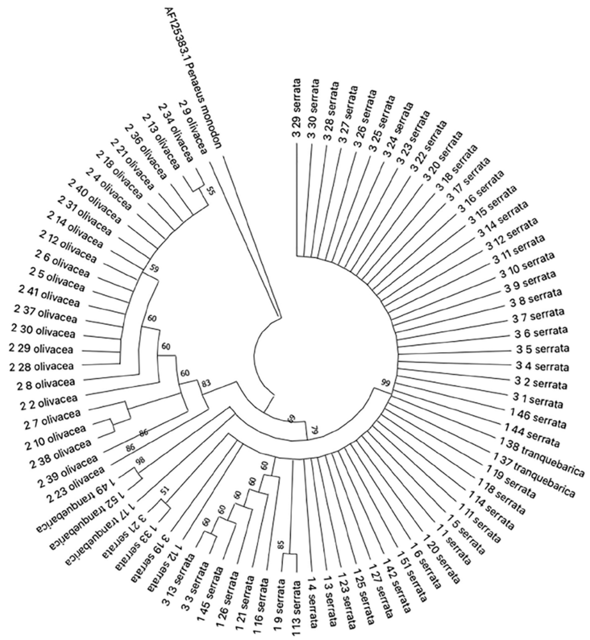

Figure 2. Neighbor-joining phylogenetic tree demonstrating the genetic relatedness of the three *Scylla* species found in wild populations in the Philippines. *Scylla serrata* and *S. tranquebarica* have a notably closer genetic relationship compared to *S. olivacea*, which can also be observed in their morphology.

Species Clusters Generated from Principal Component Analysis (PCA)

The PCA of dorsal carapace features including bumps, black spots, coloration, and shape as determined by CL:ICW only revealed two clusters with *S. olivacea* forming a separate group from *S. tranquebarica* and *S. serrata* (Figure 3). These features were observed consistently to separate the samples into two groups pre- and post-acclimation.

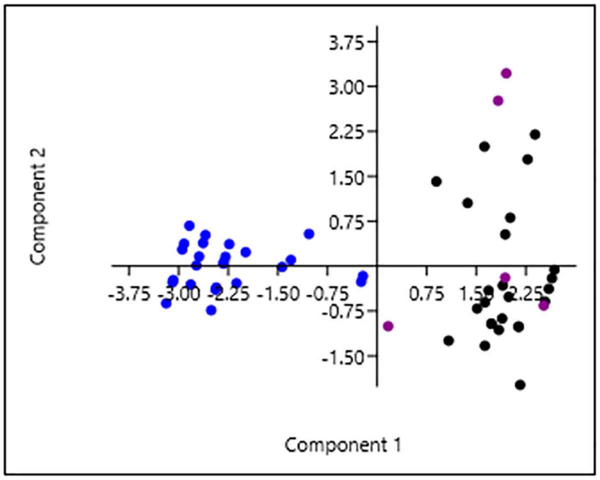

Figure 3. *S. olivacea* samples (blue dots) forming a separate cluster from *S. serrata* species (black dots) and *S. tranquebarica* samples (purple dots).

Traditional Techniques That May Be Adopted for Nationwide Use

Morphological variations that cluster species samples and which do not differ significantly pre- and post-acclimation are traditional practices that have the potential for nationwide adoption.

Bumps. *S. olivacea* samples exhibit less prominent or no bumps compared to *S. serrata* and *S. tranquebarica* (Figure 4) both pre- and post-acclimation. This supports the claim of fishers from Cagayan (VinceCruz-Abeledo *et al.* 2020) and Sorsogon that *S. olivacea* has a smoother dorsal carapace compared to the other two species.

Shape. Mean CL:ICW is smaller in *S. olivacea* compared to *S. serrata* and *S. tranquebarica* pre-acclimation (p = 0.0000000023) and post-acclimation (p = 0.00039). This observation is consistent with claims of fishers from Sorsogon that *S. olivacea* has a wider shell or lower CL:ICW ratio (Figure 5) and that *S. serrata* and *S. tranquebarica* have a more curved carapace compared to *S. olivacea*.

Debunked Traditional Techniques Due to Variations across Species and Environmental Conditions

Color. The PCA of the color intensities of the pixels of the image of each sample's dorsal carapace separated *S. olivacea* samples from *S. serrata* and *S. tranquebarica* samples pre- and post-experiment (Figure 6). These observations are supported by the results of the analysis of colors by human perception. The dorsal carapace color exhibited by most samples of the same species remained the same pre- and post-experiment, but variation in color is still seen for 36–40% of the samples. Interestingly, the majority of samples of the same species sourced from different locations exhibited different colors with 32% of *S. serrata* from Cagayan having an olive green dorsal carapace, whereas 57% of the same species sourced from Sorsogon were blackish.

These observations suggest that there is a specific coloration characteristic to the species but are influenced by the environment, as reports say [Gong *et al.* (2015); Fushimi and Watanabe (1999), as cited by VinceCruz-Abeledo *et al.* (2020); Parkes *et al.* (2011)]. Based on the results of this study, the dorsal carapace color varied across species but was inconsistent across regions.

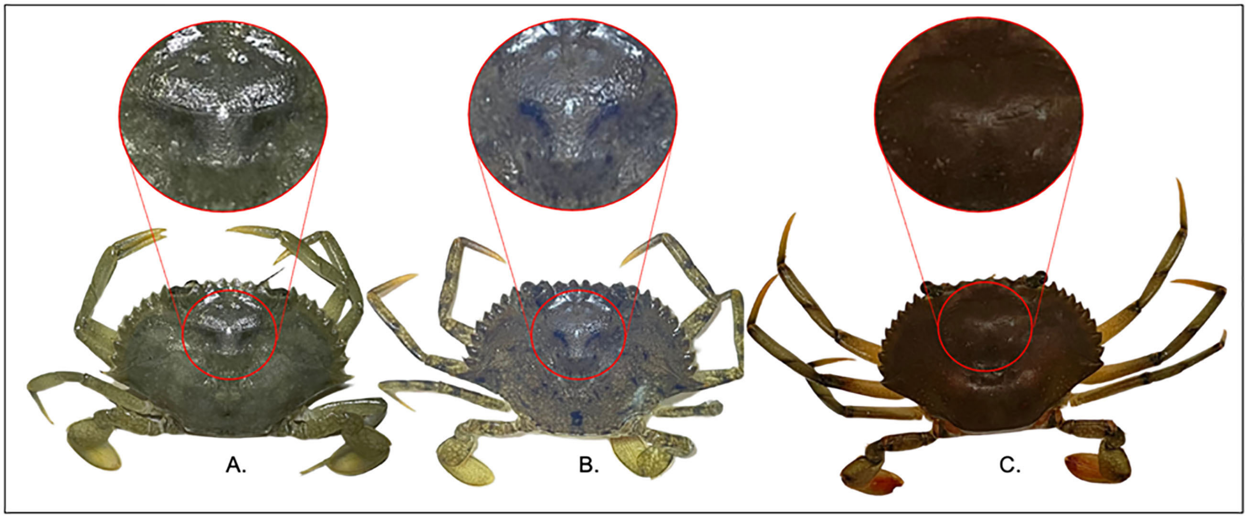

Figure 4. Dorsal surface of confirmed sample of [A] *S. serrata*, [B] *S. tranquebarica,* and [C] *S. olivacea*. The zoomed-in part of the dorsal carapace is where prominent bumps in *S. serrata* and *S. tranquebarica* are found, but which is less prominent or smooth in *S. olivacea.* Legs are incomplete as the chelipeds, which bear the claws, were removed for transport.

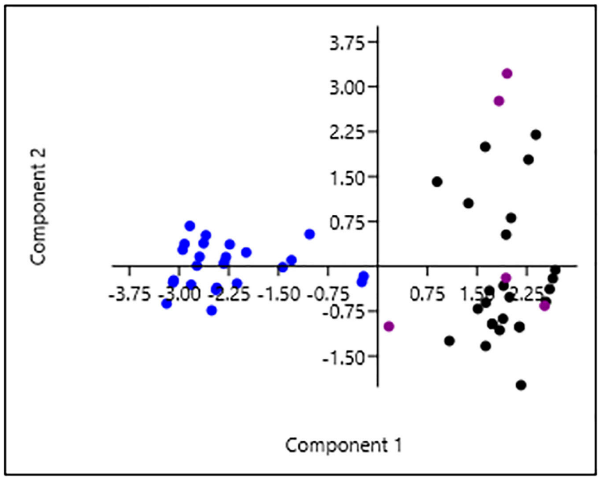

Figure 5. Mean CL:ICW ratios of species pre- and post-experiment with 95% confidence interval error bars. Mean CL:ICW is 0.729 and 0.712 for *S. serrata*, 0.692 and 0.697 for *S. olivacea*, and 0.759 and 0.732 for *S. tranquebarica* for pre- and post-experiment, respectively.

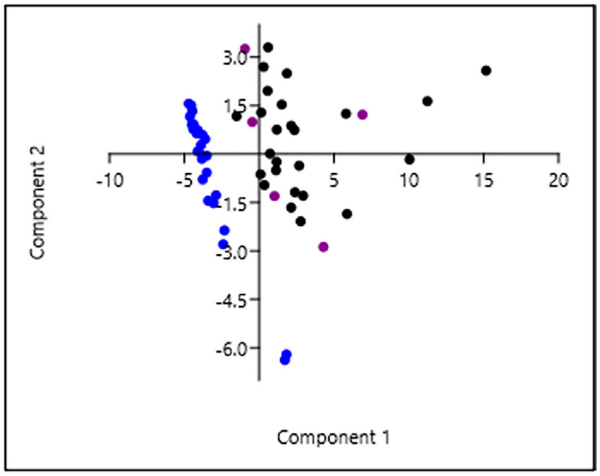

Figure 6. PCA of the color intensities of the dorsal carapace of the samples, with a total variation of 73.98%. *S. olivacea* samples (blue dots) form a cluster separate from *S. serrata* (black dots) and *S. tranquebarica* (purple dots) samples.

White spots. There was no difference in the patterns and frequency of white spots across species pre- and post-experiment, as well as in similar species sourced from different regions. Tiny white spots occurred in great amounts scattered all over the dorsal carapace, which served no distinguishing function.

Black spots. There was no observable pattern in terms of placement and frequency of black spots across species. Characteristic black spots may be a function of environmental adaptation, development, characteristic color, or other factors, although further studies should be conducted to confirm this.

Depressions. The presence of a depression at the middle part of the dorsal carapace served no distinguishing function, as it is a characteristic of all three species of mangrove crabs. The emergence of a depression at the top part of the dorsal carapace was also exhibited by several samples across all three species after the experiment.

Growth of the crablets. A comparison of the mean shell growth for the three species showed no significant difference ($p = 0.74$) (Figure 7).

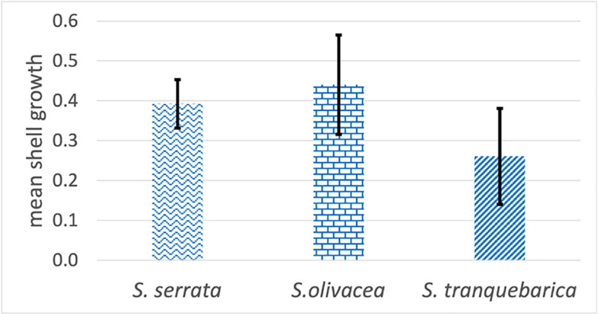

Figure 7. Mean shell growth rates of the species with standard error for error bars.

DISCUSSION

Difficulty in Differentiating *S. serrata* from *S. tranquebarica*

The PCA of the features was only able to separate *S. olivacea* from either *S. tranquebarica* and *S. serrata* but not the latter two species from each other. A similar result was observed in the study by VinceCruz-Abeledo *et al.* (2020), which found that the only significantly different morphometric ratio that can separate juveniles into species clusters was the frontal median spine height relative to frontal width (FMSH/FW) of *S. olivacea* with either *S. serrata* or *S. tranquebarica.* The close morphological similarity between *S. serrata* and *S. tranquebarica* could be traced to their close genetic relationship (Keenan *et al.* 1998). *S. serrata* has been mistakenly reported as *S. tranquebarica* in many Indian scientific literature works [Jithendran *et al.* (2010); Mohanty *et al.* (2006), as cited in Balasubramanian *et al.* (2014)] due to the overlapping morphometric ratios and continuity in the diagnostic characters of the two species (Jirapunpipat *et al.* 2008). This issue has already been resolved using molecular data (Keenan *et al.* 1998; VinceCruz-Abeledo and Lagman 2018), and a morphological marker that differentiates adult *S. serrata* from *S. tranquebarica* has been defined (VinceCruz-Abeledo and Lagman 2018), but no method with a glaring difference can distinguish the two species at earlier stages yet.

Implications on Practice of Limitations in Species Identification

Focused group discussion with locals revealed that catchers, growers, and traders do not bother sorting or differentiating *S. serrata* apart from *S. tranquebarica*, as long as there is none of the less preferred, potentially more destructive (Gaillard 2010) *S. olivacea* in their catch, seed, or produce. There is currently no report similar to this from other countries, but it is likely that the same compromise is being made by mangrove crab fishers due to the similarity between *S. serrata* and *S. tranquebarica*. This poses a problem for the fishers as *S. serrata* was reported to grow to a maximum weight of 2.86 kg at maturity, whereas *S. tranquebarica* was able to grow to only a maximum of 1.21 kg in the study by VinceCruz-Abeledo and Lagman (2018). This implies that production gain is not at maximum if a fisher continues to make the compromise of growing *S. serrata* and *S. tranquebarica* together (Table 1).

The Use of Being Able to Delineate *S. olivacea*

The use of the shape and the bumps on the dorsal carapace, which were found to have potential for nationwide adoption, provides mangrove crab stakeholders with a method to identify juvenile *Scylla olivacea* species that is obvious and not dependent on technology. This is useful for stakeholders who are not well acquainted with the morphology of mangrove crabs, for stakeholders who prefer the use of traditional techniques over automated ones (VinceCruz-Abeledo *et al.* 2020), and for stakeholders who do not have easy access to technology. The methods are also useful to prevent the smaller *S. olivacea* from being grown together with the bigger species *S. serrata* and *S. tranquebarica*. Separating *S. olivacea* from the bigger species increases its chances of survival so that it may be used for other purposes. *S. olivacea*'s smaller size makes it suitable for soft-shell farming. There is currently a high market demand for soft-shell crabs globally, and *S. olivacea* is one of the few species that are studied for the production of soft-shell crabs (Lahiri *et al.* 2021; Fujaya *et al.* 2020; Rahman *et al.* 2020).

Recommendations for Policy and Technology Improvements

Stakeholders, most especially catchers and growers, argue that it is better to trade crabs at the megalopa stage than trade juveniles. There is, however, Fisheries Administrative Order (FAO) No. 264, Series of 2020 in the country that prohibits the trade of mangrove crabs with carapace width smaller than 5 cm for grow-out purposes. Stakeholders mentioned that it is easier to transport megalopa for grow-out in other regions. Megalopae are transported in cool oxygenated seawater (24–25 °C) contained in two layers of plastic bags at a loading density of 50 individuals/L. Transporting juveniles, on the other hand, requires the extra step of having each of the juvenile crabs' pincers tied or trimmed to prevent cheliped-inflicted injuries or deaths during transport in containers with enough moisture and ventilation (Quinitio and Parado-Estepa 2003; Shelley and Lovatelli 2011). Stakeholders also claimed that they experienced minimal losses as megalopae will have a higher survival rate after transport compared to juveniles. This observation from mangrove crab stakeholders seems to differ from existing studies where juveniles, especially *S. serrata* were found to have wider salinity and temperature tolerance than megalopae (Baylon 2011; Alberts-Hubatsch *et al.* 2016). Since megalopae are delicate and not all crab growers know how to handle this delicate stage, traders or grow-out pond owners may as well use crab juveniles with about 2 cm CW from the nursery or hatchery since these can be transported even without much water, and there is no need to cut chelipeds. Additionally, according to FAO No. 264, the collection method for megalopae primarily uses a push net or scissor net, which inadvertently catches the juveniles of non-target species and contributes to environmental damage. Revisions to the FAO may prove to be helpful not only for mangrove crab catchers, growers,

Table 1. Total earnings when a fisher is not able to secure the preferred *S. serrata* and grows a mix of the two species together *vs.* total earnings when a fisher is able to accurately identify and secure *S. serrata* for grow-out.

	Total weight of produce in kilograms (kg) (no. of species * maximum weight of species at maturity)	**Total earnings in USD [weight in kg * USD 7.25 (price of mangrove crab per kg regardless of species)]**
Growing a mix of 25 pieces *S. serrata* and 25 pieces *S. tranquebarica*	25 * 2.86 kg = 71.5 kg *S. serrata*	71.5 kg * 7.25/kg = USD 518.38
	25 * 1.21 kg = 30.25 kg *S. tranquebarica*	30.25 kg * 7.25/kg = USD 219.32
		Total: USD 737.7
Securing and growing 50 pieces *S. serrata*	50 * 2.86 kg = 143 kg *S. serrata*	143 kg * 7.25/kg = USD 1,036.75
		Total: USD 1,036.75

and traders but also in conserving viable populations in the wild. Studies on the identification of species at the early juvenile stages and better optimization of nursery protocols would also be imperative if trading can start as early as the early juvenile stage (*i.e.* 2 cm CW).

CONCLUSION

This study validated the use of traditional techniques that look at the dorsal carapace shape and bumps for distinguishing juvenile *S. olivacea* from *S. serrata* and *S. tranquebarica*, thereby providing stakeholders with an obvious method to identify the species without the aid of technology. It will be useful not only in minimizing losses brought about by deaths of the smaller *S. olivacea* from predation but also in identifying it for use in the soft-shell industry. The use of dorsal carapace color, spots, depressions, and growth rate were found to have no distinguishing function due to variations across species and environmental conditions. There is still no obvious morphological feature found to delineate juvenile *S. serrata* from *S. tranquebarica*. Image analysis data on the two validated techniques can be added to the machine learning algorithm of existing species identification tools to increase accuracy. Stakeholders have also expressed their call to have FAO No. 264, S. 2020 revised to allow the trade of juveniles at earlier stages (*i.e.* 2 cm CW) for grow-out, which were seen to have potential advantages for both the stakeholders and the wild population of mangrove crabs.

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STATEMENT ON CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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