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Cover Photo
Pothos insignis is one of the 55 known species of flowering plants in the genus *Pothos* under the family Araceae, (tribe Potheae). This tree-climbing species is only distributed in the islands of Borneo and Palawan. They are often found at the base of the mountains, especially near the rivers. The cream-white inflorescences are sequentially produced (Photo: Jonah van Beijnen). This is the first in-situ photo-documented of the Palawan population of this species.

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EDITORIAL

The Palawan Scientist, the official scientific journal of the Western Philippines University, started out by mainly publishing the research outputs of the university. It then progressed as a regional journal by publishing papers that are related to Palawan and its neighboring provinces. Now, even if it has still retained its name, the journal has a global reach. It currently publishes articles that dealt not only with research studies done in Palawan but also reviews and accepts papers from researchers all over the Philippines and overseas. This is clearly an indication that The Palawan Scientist is gaining momentum and establishing its reputation as a journal that publishes high quality research papers. As Palawan Scientist become more visible and widely accessed by majority of the population because of internet, scientific information covering wide range of topics practically reaches almost everyone in the scientific community.

Despite of the current pandemic, this volume contains eleven scientific articles composed of ten original research articles and a research note. These papers cover topics from fisheries and aquaculture, to environmental sciences and biodiversity to educational and social sciences. There are two papers that focused on aquaculture, seven papers on environmental science, conservation and biodiversity, and two papers on education and social sciences. Seven articles came from Filipino researchers, and four articles were outputs of collaborative studies between Filipino and foreign authors.

This year also marks the second year that The Palawan Scientist got included in the Clarivate Analytics database. This indexing body contains all journals under the Web of Science Core Collection. The journals that are included in this collection demonstrate high levels of editorial rigor and best practice. Currently, there are 24 Philippine-based journals in this database, and the country ranked fifth among the ASEAN countries, with Singapore as the highest with 128 journals. The Palawan Scientist is aiming to be at par with other ASEAN journals by increasing its scientific impact. Hence, the goal of the journal should now be geared towards increasing citations of its published papers and by encouraging submissions from authors based abroad.

We are encouraging authors to submit papers that are multidisciplinary in nature and papers that are outputs of collaborative research. Review articles that discuss timely and relevant topics and are

within the scope of the journal can be solicited from well-published authors. This strategy can contribute towards increasing the impact and citations of the journal as well as gaining a positive reputation among researchers.

I am looking forward to continue working with the Editorial Board and together with the author-contributors, we can make The Palawan Scientist become a reputable journal that publishes high quality and high-impact scientific articles.

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Botanical observations from a threatened riverine lowland forest in Aborlan, Palawan, Philippines

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ABSTRACT

This research provided a general overview of the vegetation structure of the Talakaigan watershed, Aborlan, Palawan, Philippines, with highlights on some ecological aspects of selected flora and intent of providing urgently needed data supporting existing conservation efforts in the area. Observations were carried during regular trekking activities in the watershed and surrounding areas from 2009 to 2016. Photographs were taken to facilitate species identification. Several noteworthy observations are presented, including a new locality for *Begonia palawanensis*, a short description of several new species of *Begonia* and notes on a large population of the Critically Endangered *Orania paraguanensis,* including details on the early life history of these palm. A large number of anthropogenic disturbances were observed in the area, including well-intended forestation and development efforts by the local and provincial government. Since the watershed does not hold any formal protective status, these disturbances pose a serious threat to the future existence of this unique watershed and some of the endemic species it contains. Our findings support the call to declare the entire watershed as an official protected area.

Keywords: floral inventory, lowland rainforest, conservation, Southeast Asia, *Begonia, Orania*.

INTRODUCTION

Palawan is a large island province in the southwest of the Philippines, northeast of Borneo. Because of the island's low population density and the fact that its forests contain relatively few valuable hardwood species, it has been spared from massive deforestation that has plagued the rest of the Philippines (Vitug 1993). Subsequently, the 450 km long island still has a forest cover of approximately 50% (PCSDS 2015). In Southeast Asia forests have been decimated and continue to disappear at a rapid and unprecedented pace (Hughes 2018). The high forest cover in Palawan is thus exceptional, but unconfirmed reports suggest that the island is quickly being deforested and heading down the same road.

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Previous local bureaucratic barriers made biological research difficult to conduct in the province and as a result, Palawan's biodiversity and the status of its forests are still poorly mapped and understood. However, renewed interest in research and conservation from both the local and provincial government has recently resulted in a number of new research initiatives to map the island's biodiversity. A collaborative framework of local stakeholders have engaged in a research and conservation initiative that focuses on the Cleopatra's Needle Mountain Range in northern Palawan (van Beijnen and Hoevenaars 2015; Vermeer et al. 2016). This initiative opened the gateway for researchers to record and publish several new island records including a very large freshwater fish (van Beijnen and Jose 2016), rediscovered two lost amphibian species (Jose and van Beijnen 2017), and described a number of ants and damselflies that are new to science (General and Buenavente 2015; Villanueva et al. 2018). Moreover, recent botanical expeditions in central and northern Palawan, resulted in the description of several new *Begonia* species for the island (Hughes et al. 2010, 2011, 2018).

Other parts of Palawan, especially south of the island's capital city of Puerto Princesa, have been much less explored for biological research. Updated records on the distribution, natural history and behavior of species from these areas, particularly locally endemic ones, will be of great use to both conservationists and policy makers. Especially given the paucity of information on species in this region, baseline data is lacking and essential for further studies and conservation, thereby empowering conservation efforts.

One of the least explored areas in Palawan is the municipality of Aborlan, located 69 km south of the island's capital city. This municipality covers 807.33 km^2 and contains one small town, with most of the surrounding countryside being covered by primary forest and secondary vegetation (PCSDS 2015). This forest is part of the Victoria-Anipahan Mountain Range that stretches from the municipality of Narra in the south up to Puerto Princesa. In Aborlan, three rivers spring from this mountain range, of which the Talakaigan River and its accompanying watershed contain some of the least disturbed lowland riverine forest remaining on the island. The forested area in this municipality is entirely listed as a Key Biodiversity Area in the Philippines (Ambal et al. 2012) and it is part of the ancestral lands of the Tagbanua tribe, who still depend on these forests for a large portion of their livelihood (Sopsop and Buot Jr. 2011). However, these forests, including the Talakaigan River watershed, largely remain unprotected and are in peril due to the influx of outsiders that bring in unsustainable harvest practices of a variety of forest products (Sopsop and Buot Jr. 2011; Bernardo 2016), and the provincial government push for so-called economic development, including a proposed hydropower (Tabuada 2015).

This research provided a general overview of the vegetation structure of the Talakaigan watershed and to highlight some ecological aspects of selected flora with the intent of providing urgently needed scientific data to support existing conservation efforts in the area.

METHODS

As part of their weekly exercise routine, two biologists that are based in Palawan made bimonthly treks through the Talakaigan watershed and surrounding areas (Figures 1 and 2) between 2009 and 2016. The watershed was crossed mostly at random, following the river upstream and several of its side creeks uphill. All interesting encounters with different plant species were photographed using a Canon 6D Camera with high-resolution capabilities and GPS coordinates were recorded automatically. All photographs were taken by the first author. Species identifications were verified through consultation with taxonomic experts for each species group. The Co's Digital Flora of the Philippines online platform was extensively used for this purpose. Ecological observations, including records on vegetation structure, were noted down after each trip and stored for future reference. Based on the observations of selected threatened and undescribed species, a family by family, genus by genus approach were carried out to describe the sub-canopy and understory of the study site and highlight the importance of protecting its biodiversity. This sometimes required the team, during for example the germination of palm seeds, to make follow up visits to specific areas in the watershed within a short timeframe.

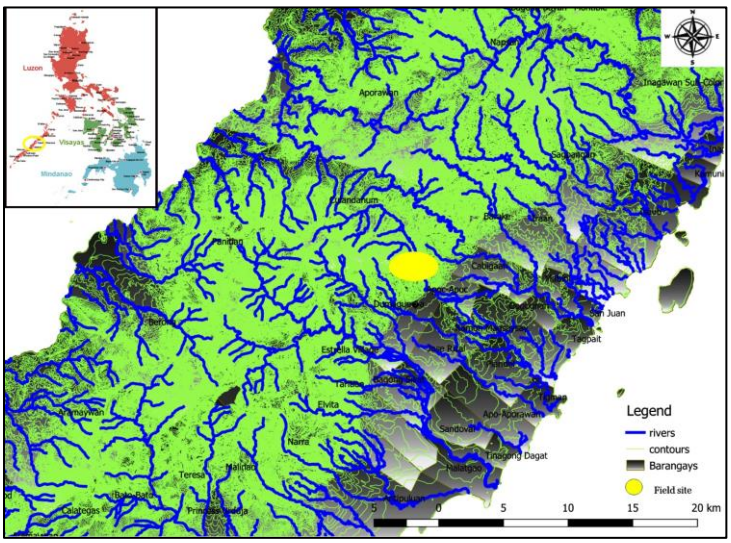

Figure 1. Map of the Philippines (inset), Aborlan and the location of the Talakaigan watershed (yellow circle).

Figure 2. The upper reaches of Talakaigan River characterized by rocky substrate, and shallow clear waters with lush vegetation along the banks.

As this research had been based on opportunistic non-professional observations only, no specimens were hurt, mishandled, or collected for the production of this research and besides photographs with GPS coordinates, no actual measurements were taken.

RESULTS

The vegetation structure of the Talakaigan watershed was a characteristic for riverine lowland forest in central and southern Palawan (Figure 3); with the canopy along the river dominated by massive *Koompassia excelsia* (Becc.), *Dipterocarpus,* and *Ficus* species. Some large *Agathis philippinensis* (Warb.) are still present in the canopy layer, especially on hill tops. The under canopy was characterized by a high density of *Orania paraguanensis* (Becc.) and *Barringtonia acutangula* (L.), as well as some *Artocarpus* species. The understory was dominated by *Myrmeconauclea strigosa* (Korth.) at the riverbank, mixture of *Etlingera* spp., *Pinanga* spp., *Caryota* spp., *Alocasia* spp., *Areca* spp., *Begonia* spp., and a variety of other species.

Figure 3. Views of the steep riverbank (a) with assorted palm trees (b) that characterize a large portion of the watershed of the Talakaigan River.

Arecaceae: *Orania paraguanensis*

A notably large population of *O. paraguanensis* (Becc.), consisting of at least several hundred individuals, was encountered at the watershed. This species seems to prefer the riverbank as they mainly occurred in close vicinity of the river, where they grow predominantly on very sandy loam or sand, sometimes mixed with limestone. Palm trees were observed flowering in June and July, during the peak of the rainy season, with fruits maturing around November (Figure 4). Trees carry several inflorescences, totaling most likely over 100 fruits. The seeds were perfectly round, very hard, and approximately 5 cm in diameter (Figure 4c). When seeds mature, they fall off and, in most cases, they ended up in the river because of the steeply inclining riverbank. This method is presumably their main mode of dispersal, and this would explain why the presence of these palm trees is concentrated around a small number of larger rivers, underlining the importance of protecting upstream populations.

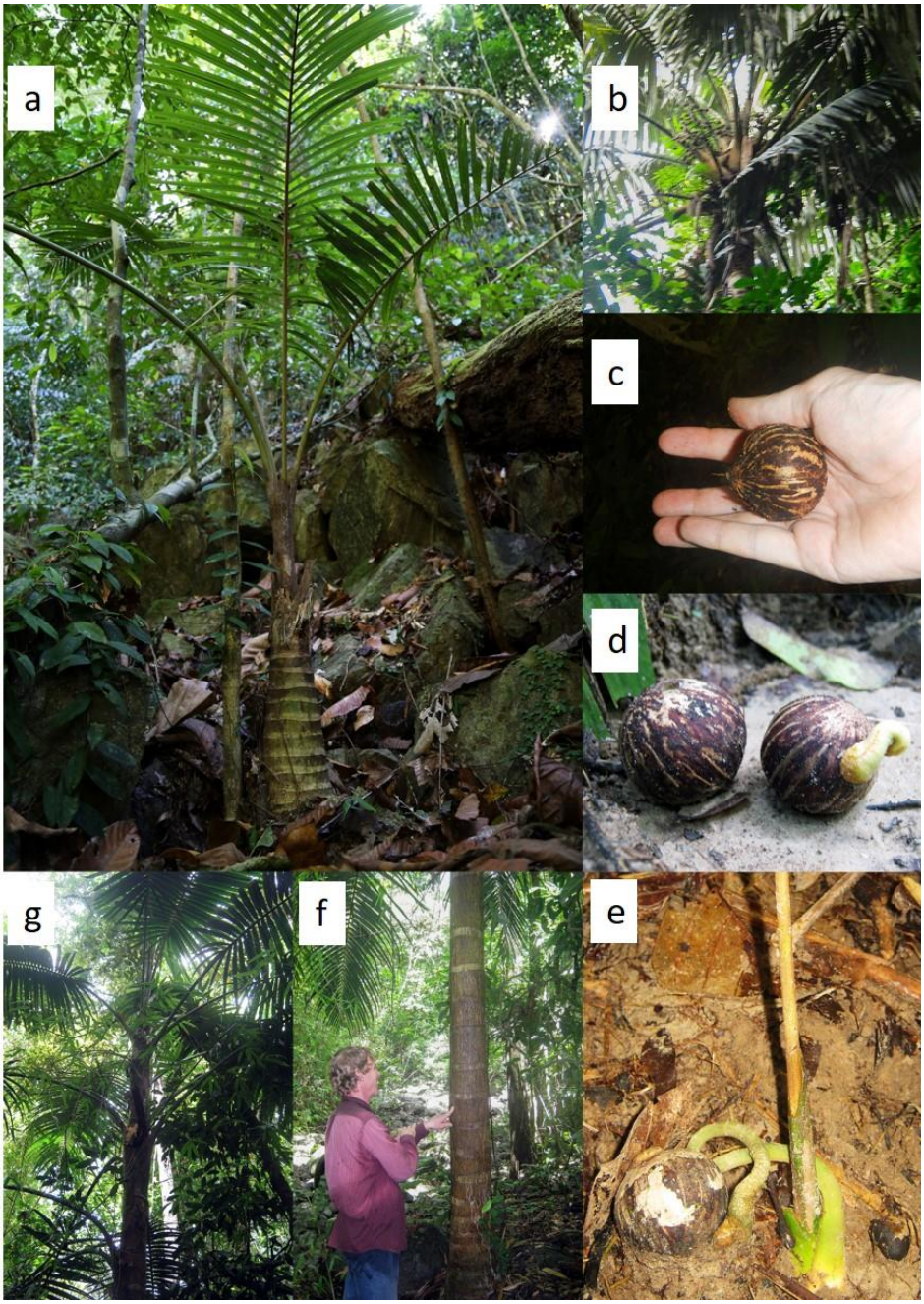

Figure 4. Different developmental stages of *Orania paraguanensis*: Sub-adult tree (a), fruiting tree (b), freshly fallen seed (c), germinating seeds (d), germinated and settled seedling (e), close-up of the trunk of a mature tree (f) and close-up of the canopy of a mature tree (g).

Begoniaceae: *Begonia palawanensis, Begonia mindorensis* and several new *Begonia* species

On 20 March 2015, about 5-km hike upstream along the watershed a very small number of plants were found. Three non-flowering individuals of *Begonia palawanensis* were observed. Plants measured about 10 to 15 cm tall and this small size in combination with the absence of flowers likely indicates that the plants were still immature. All observed plants were growing in loamy clay, mixed with loose limestone. The plants were overhanging a steep bank of a side-creek of the Talakaigan River (Figure 5), approximately 2 m from the creek bed and roughly 150 m above sea level.

Figure 5. Immature *Begonia palawanensis* growing in-situ along a small creek in Aborlan, Palawan. This is the first published in-situ photograph of the species.

Additionally, further upstream, one epiphytic individual that somewhat seems to resemble *B. mindorensis* has been observed (Figure 6). The plant is characterized by having long petioles (about 10-20 cm), surrounded with reddish brown hairs. Leaves are medium-size, ovate-lanceolate shape. We found the plant inhabiting a tree trunk shared with dense fern-moss species. However, *B. mindorensis* is said to be restricted in the forest floor. Apparently, our observation of the species could be the first record of its epiphytic habit.

Figure 6. An undescribed epiphytic *Begonia* found in the area.

Besides *B. mindorensis* (Merr.), three other *Begonia* phenotypes had been observed in this particular watershed and surrounding areas. The leaves of this deciduous *Begonia* measure only 1-2 cm in diameter (Figure 7). During the dry season leaves were absent and plants survived in tuberous form.

Figure 7. An undescribed miniature deciduous *Begonia* found in the area (a), and a different flowering individual from the same area (b).

Zingiberaceae: *Wurfbainia palawanensis*

Another noteworthy finding was the endemic *Wurfbainia palawanensis* (previously *Amomum palawanense,* Elmer) on 13 July 2015. Several hundred individuals were found growing in a relatively open secondary forest area on sandy clay. Some of these plants were flowering (Figure 8). This rare, large-size ginger plants bear flowers sprouting from its rhizomes. Our record is the first photograph and known in-situ season of its inflorescence.

Figure 8. Inflorescence of *Wurfbainia palawanensis*.

Araceae tribe Potheae: *Pothos insignis*

Several fruiting individuals of the liana *Pothos insignis* (Becc.) were recorded on 12 November 2013. These fruiting individuals (Figure 9) were part of a large group of plants that were found growing in the trunk of a large unidentified tree just along the river. This climbing aroid was commonly growing and climbing on large trees along the forest of the Talakaigan watershed.

Figure 9. Fruits (a) and habit (b) of *Pothos insignis*.

Araceae: *Homalomena palawanensis*

On 12 November 2013, *Homalomena palawanensis* (Merr.) was also recorded in the watershed (Figure 10). A small group of these semiaquatic clump-forming perennials was found growing along the side of the river where the water reached a depth of 15–20 cm. Aborlan, and most likely this particular watershed, is the type locality of this species.

Miscellaneous recordings

Other plants that have been recorded in the watershed by this study and that have been identified by consulted experts include *Pothos dolichophyllus* (Merr.), *Globba aurea* (Elm.), *Geocharis fusiformis* (Ridl.), *Citrus macroptera* (Mon.), *Boesenbergia* spp., *Amischotolype marginata* (Hassk.), *Leea* spp., *Psychotria* spp., *Alocasia culionensis* (Engl.), *Pinanga curranii* (Becc.), *Caryota* spp., *Hoya* spp., and *Piper* spp.

Figure 10. Semi-aquatic *Homalomena palawanensis* growing in shallow water along the river.

DISCUSSION

Orania paraguanensis is endemic to Sabah and Palawan (Keim and Dransfield 2012). It is classified as Critically Endangered by DENR Administrative Order 2017-11 but has not been yet assessed for IUCN (2018). Mature individuals can reach an average height of 9 to 12 m, with literature references of up to 18 m and a trunk diameter of 20–30 cm (Keim and Dransfield 2012). Almost all populations of *O. paraguanensis* in Palawan are located on the east coast of the island, where higher rainfall occurs, with the species dominating only a few kilometers from the seashore, where seeds can be carried inland during high tide and strong winds. However, a small number of individuals are found pretty far from the riverbanks and it could be that the wild boar, large flying foxes, rodents (squirrels) and birds (hornbills), of which Palawan harbors several species, disperse some of the seeds as well. Dispersal by flying foxes has been recorded for *Orania sylvicola*, a species that occurs throughout most of Southeast Asia and which has a very similar seed size (Zona 2006). Seeds were observed to germinate rapidly after landing on the forest floor, often within 1 or 2 weeks. The seeds germinate remotely and the first structure to emerge from the seed is the cotyledonary petiole (Meerow and Broschat 2017). The petiole will securely anchor the seed at a depth of 20–30 cm after which it swells and develops the first root, and eventually a single shoot. From this shoot, the first leaf will emerge and

seedlings add 2 or 3 more leaves in the two following months, before the start of the dry season in January. It should be noted that the species has been officially recorded for Palawan and the province of Sabah in Malaysia. However, the presence of this species in Malaysia is based on a single observation from 1961 (Keim and Dransfield 2012), with no other records known. As such, it is safe to assume that the center of distribution for this species is located in Palawan.

Since the original discovery of the species in 1910, there have been only three recorded observations of *B. palawanensis* in Palawan; first in 1991 in Brooke's Point (Hughes et al. 2010), approximately 100 km southeast from the original type locality. Additionally in 2011 and 2014 two other observations have been made in the vicinity of the Salakot waterfalls in Puerto Princesa (Hughes et al. 2018). Besides *B. mindorensis* (Merr.) which is restricted to the forest floor, three other *Begonia* phenotypes have been observed in this particular watershed and surrounding areas. This includes a still to be described minute deciduous *Begonia* (Hughes pers. comm.) that limits its occurrence to some rocky outcrops along the river. A colleague, Esquerion Prieto found a very similar *Begonia* (Figure 11) in the Inagawan watershed, just 20 km north of the Talakaigan watershed. Actual specimens and further research are needed to determine if this *Begonia* is just an anomaly or if it represents a new species. More research is needed to determine if they belong to the same species or if they show enough variation to be classified as different species.

Figure 11. An undescribed *Begonia* from Inagawan watershed (a), and a different flowering individual from the same area (b).

Wurfbainia palawanensis is somewhat uncommon Zingiberaceae, and has only been recorded a few times (Nickrent et al. 2018), not because of its rarity but more likely because it rarely flowers and is consequently often overlooked in botanical surveys.

Pothos insignis is an uncommon aroid known only from Borneo and Palawan (Boyce and Hay 2001) and this is the first publication of in-situ photographs and inflorescence season of the species (Boyce pers. comm.). A literature review confirmed only one observation of the species since its original description: an observation that was made by Leonard Co in the same area in 2011 (Nickrent et al. 2018).

The above observations considerably increase our insights in the general vegetative structure of the watershed and the ecology and distribution of several endemic and threatened species. To further update the botanical record for this watershed, Leonard Co identified a number of other species and genera in the watershed in 2011 that have not been published, and which were not observed by this team. These species include: *Aerva lanata* (Schult.), *Pomatocalpa bicolor* (J.J.Sm.), *Ptyssiglottis* spp., *Arenga brevipes* (Becc.), *Habenaria muricata* (Barb. Rodr.), *Rinorea* spp., *Jatropha gossypifolia* (L.), *Mycetia javanica* (Hook.), *Trigonostemon villosus* (Hook.) and *Selaginella* spp. Photographs of these species are available from Nickrent et al. (2018). Altogether, these recorded observations demonstrate that the study area has interesting diversity, and includes a number of noteworthy species of conservation importance. More studies are needed to compare the results of this study with other sites to demonstrate how remarkable the diversity and conditions of this primary forest are, but based on their years of experience the authors believe this particular watershed clearly stands out.

Besides the noted species records and vegetation analysis, the team observed a number of anthropogenic disturbances in the watershed and surrounding areas and over the years, the severity of these disturbances has increased. Most notably these include the well-intended forestation and development efforts (Figures 12 and 13) by the local and provincial government, land grabbing and consequent clearings for land speculation and illegal quarrying activities, again highlighting the urgency for further study and formal protection of the area. The clearing of primary vegetation to make place for non-native trees with a higher economical value, like mahogany and rubber, is a common development approach used by government agencies across Palawan (CALG 2015), even in protected areas like national parks. Although this "greening" policy stems from poorly guided but well-intended efforts to sustain and improve the livelihoods of local residents, the impact of this practice on the native biodiversity can be substantial. Most endemic species, like the discussed *O. paraguanensis* and *Begonia* spp., have very specific habitat preference and small tolerance range in terms of temperature, humidity, and light influx (Işik 2011). When clearing the understory, the

increased influx of light, increases the temperature while decreasing the humidity, and endemic species with their narrow niche are the first to disappear (Işik 2011). With the extremely limited distribution range of many *Begonia*s in Palawan, this practice clearly has the potential to push these species into extinction.

Figure 12. Well-intended clearing of the understory inside the Talakaigan watershed to make place for non-native trees.

More importantly, the Talakaigan watershed and surrounding forests are also vital to the survival of indigenous people, like the Tagbanua tribe that are mainly dependent on the forests for their livelihood (Sopsop and Buot Jr. 2011). These indigenous people primarily use native plant species to sustain their diet and livelihood and the practice of slowly replacing the original forest composition with non-native species will certainly affect their livelihood negatively. As such, it is highly recommended to the relevant government agencies to reevaluate this counterproductive "greening" and "livelihood" policy and modify it.

The livelihoods of indigenous people are more likely positively impacted by officially protecting these last remaining lowland forest habitats and by limiting the influx of informal settlers and other outsiders that often employ less sustainable methods of extracting forest resources. Additionally, when planting seedlings of tree species, it is highly recommended to solely use

native species that have germinated from seeds that have been sourced in the same area. Species like Almaciga (*A. philippinensis*), assorted dipterocarps and *Ficus* spp. are recommended as they play an important role in the livelihood of indigenous people while also performing a key function in these threatened forest ecosystems.

Figure 13. A public irrigation project in vicinity of the Aborlan River watershed.

Our observations also give us the opportunity to make some recommendations on the existing or proposed conservation status of the discussed species. *Orania paraguanensis* seems to be much more common than previously reported, which can probably be attributed to the limited amount of fieldwork in Palawan by previous authors. Over the years, the team has recorded the species in many coastal riverine areas along the east coast of the island. At least three of these rivers are located in the newly established protected area of the Cleopatra's Needle Critical Habitat (Dasgupta 2017) in central Palawan. However, the distribution of the species remains limited to riverbanks of larger rivers in lowland areas on the east coast of the island, and this specific habitat type is increasingly under pressure by anthropogenic land conversions. Considering that the species occurs in an area over 5,000 km^2, but of less than 20,000 km^2, and that the adult population most likely holds over 2,500 individuals, based on IUCN criteria it is recommended to update the status of this species to Vulnerable.

Begonia palawanensis has no longer been observed at its type locality in Napsan, Puerto Princesa, and the continued occurrence of the population from which the specimen that was collected in 1991 originated in Brooke's Point, could not be verified despite visiting the area. This makes the watershed of the Aborlan River and the forest around the Salakot waterfalls

in western Puerto Princesa the only two verified locations where the species currently occurs. The lack of any formal protective status of both of these sites, together with the large number of disturbances in the watershed, endanger the future existence of this species. Additionally, the area surrounding the Salakot waterfalls seems to have been opened up for development by the local government and the recent influx of numerous settlers have resulted in massive deforestation. With this extremely limited distribution range, the small number of mature plants that have been observed and the large number of threats to the species, the authors believe it is warranted to classify *B. palawanensis* as Endangered, supporting the earlier proposal by Hughes et al. (2010).

Despite the numerous treks made by the team around the entire municipality, the miniature deciduous *Begonia* and the epiphytic *Begonia* have both been recorded from a single location only. While the larger *Begonia* with an acuminate apex has been observed in two small localities, all localities spanning less than 1 km^2 per site, and with only a handful of individual plants observed. Considering this very limited distribution and adding the clearing of the understory in the watershed, there is an urgent need of taxonomic and ecological studies for the species. Results from these studies would be the basis of recommendations for IUCN status. With our current knowledge of these species, we argue recommending a data deficient status for each species based on IUCN criteria. *Wurfbainia palawanensis* is currently listed as Vulnerable and despite the fact that the species is uncommon throughout its range, it has a wide distribution and few threats, thus its conservation status is likely warranted. Lastly, the single observation of *P. insignis* and *H. palawanensis* confirmed that both species are still present in the study site.

The lowland forest of Aborlan still holds several endemic and threatened species and, as recorded by other studies, these forests continue to play a vital role in the survival of the island's indigenous people (Sopsop and Buot Jr. 2011). With the many threats present, immediate action to protect these forests is paramount or they, and the local indigenous people who depend on them for their survival, will be lost forever. There seems to be hope as the local government of Aborlan is planning to prioritize conservation and tourism in their future development plan (WWF 2016). Furthermore, with considerable support of the local government, a local conservation NGO is in the process of developing a plan to protect 164.789 hectares of old growth forest in the area (Magdayao 2018). With forests throughout Southeast Asia disappearing at an alarming rate (Hughes 2018), support and funding for these type of conservation projects and for more comprehensive, targeted surveys to fully document community composition and prevalence of rare and threatened species should be prioritized, as to hopefully safeguard Palawan's amazing forests for future generations.

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Developing STEAM educators' proficiency scoring framework

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ABSTRACT

The study aimed to develop the scoring framework for the self-rating proficiency indicators for Philippine STEAM educators. The study further sought to design mathematical framework and program for scoring STEAM educators' proficiency and validate the designed scoring program. About 1507 responses from the self-rating STEAM proficiency tool were used to undergo the three tier quantitative and qualitative validation. Mathematical equations were derived to direct the development of the scoring programs using Microsoft Excel and Fortran. Results show an agreement between the proficiency profiles generated from the Microsoft Excel and Fortran program. Using the online survey and the classroom observation rating, proficiencies were compared as determined through the Fortran program of the pre-determined career stage (distinguished, highly proficient, proficient, beginner). Lastly, qualitative validation was performed by comparing the generated codes in the interview transcripts and observation notes and the attributes in the PPST domains and TPACK dimensions. Qualitative validation indicates that the occurrences of the indicators in the interview and classroom observation matched with the expected attributes per career stage as per the PPST. This indicates that the validation of the scoring system developed for the online survey generate the STEAM educator proficiency. Further, the scope of the scoring framework developed is universal and may be adapted to suit any local setting. However, increasing the number of interviews and classroom observations to 10% of the sample population of teachers will produce a robust scoring program.

Keywords: STEAM education, TPACK dimension, PPST domain, STEAM educators' proficiency, STEAM proficiency scoring program

INTRODUCTION

Education seems to be the basis of every country's progress and groundwork for its future. This tagline exists for many generations taking a new form from one era after another. The entire timeline notes three major

education themes that directly influence the society: Education 1.0 underscored agriculture education for agriculture industry, Education 2.0 responded to industrial society, and Education 3.0 addressed the technology society (Diwan 2017). Currently, the quest for industrial revolution 4.0 (IR 4.0), dominated by global connectivity, smart machines and new media, dictates the new contour of education to foresee training and re- and upskilling of the future workforce of this era (Haron 2018). Education in this digital era known as Education 4.0 (E 4.0) and labeled as the "Dawn of Digital Monarchy" envisions to facilitate educating the Generation Z (13-19 years old) learners to imbibe life skills and skills of creating innovation to develop the future workforce 4.0 who exhibits the 4Is: intelligent, interconnected, instrumented, and innovative (Goldsberry 2018) with a "learning is a way of life" mindset (Shook and Knickrehm 2017). This education framework emphasizes a new vision for learning where content and subject matter are secondary to the knowledge of why you need something, and where to find it (Fisk 2017). It features learning together and peer learning where teachers primarily act as facilitators of the learning mechanism dictated by the learners, and machines aid the facilitators in tracking the learners' performance through data-based customization. This schema especially applies to STEM education, which later was known as STEAM (Ghanbari 2015) or Science, Technology, Engineering, Agri/Fisheries and Mathematics education in an effort to integrate design and creativity, - one of the most sought-after main pillars of knowledge-based society and economy (Ministry of Finance of the Slovak Republic 2018). While E 4.0 frames the new learning paradigm for workforce 4.0, the new education framework calls for quality, re and upskilled teachers to catalyze learning in IR 4.0.

Quality and re and upskilled teachers direct STEM/STEAM education to quality, which can prepare the future workforce with skills that match the new skill demands of IR 4.0 (Deloitte 2015). Furthermore, they need to facilitate STEAM learning for the Generation Z learners to acquire a strong background on the meta-discipline (Morrison 2006; Tsupros et al. 2009; Ejiwale 2013), and to obtain specific and highly intricate skills such as design thinking, time management and programming skills (Montero and Evans 2011; Mars et al. 2016) aside from life skills and the 4Is. STEAM educators perpetuate learning as a way of life for the Generation Z learners to survive and be successful in their future work environment (Renjen 2018).

The demand for quality STEAM education to be in the loop of Education 4.0 framework forges the need for re and upskilled competencies of STEAM teacher quality. As Wilson (2016) claimed, teaching in this era should go beyond the teaching of disciplinary subject matter, instead, teachers should focus on the integrative presentation of lesson to learners, e.g. authentic/problem-based learning and design thinking, emphasizing interdisciplinary approach using STEAM field to teach a STEAM course.

Feedback system and assessment should emphasize formative development of students' skills to demonstrate their learning and its relevance to society (Miller 2017). Finally, quality STEAM teachers should showcase dynamic professional development as pathways to success (e.g. collaborative/partnerships, community service for service learning, and professional learning). These traits feature the new skills of quality STEAM teachers who are driven by what they know and what they can do with what they know (Obama 2016) to train our Generation Z learners to learn the trick of how to solve real problems and be contributory to the society.

There are already numerous efforts to provide quality education by improving the quality of teachers. Training and upskilling of teachers in other countries commenced to attain the aforementioned traits and new skill set required to be quality STEAM teachers of the Generation Z learners which calls for monitoring and assessment tools. In fact, the National Research Council proposed two indicators for STEM teacher quality: teachers' science and content knowledge for teaching and teachers' participation in STEM-specific professional development. Similar efforts surfaced in the STEAM world (Kim and Kim 2016) extending the attempt from identifying and polishing the new teacher quality competencies to developing STEAM quality teaching indicators and rating tools.

In the Philippines, standards for professional teachers Philippine Professional Standards for Teachers (PPST) set the tone of research activities related to teacher quality (Department of Education-Teacher Education Council 2017). Specifically, PPST outlines the desired competencies and skills of quality teachers to enable them to handle and manage emerging global frameworks. However, PPST targets the primary, junior, and senior education level with no existing elaborations on subject matter or content, and teaching and learning of complex skills in the tertiary level, thus, enjoining the group of Morales et al. (2019) to design a self-rating proficiency indicator for tertiary STEAM (with "A" for Agri/Fisheries) teachers framed from the paradigms of PPST; Policies, Standards and Guidelines of the Philippine Commission on Higher Education (PCHED); and the theoretical underpinnings of Technological Pedagogical Content Knowledge (TPACK) (Mishra and Koehler 2006). This self-rating tool boosts the disciplinal mapping and matching of the seven domains of PPST and the seven TPACK dimensions to capture the entire spectrum of competencies expected of a Philippine tertiary STEAM quality teacher. Apparently, Kim and Kim (2016) tracked similar path specifying constructs and domains to which teacher quality may be gauged through a self-rating tool (Kim and Kim 2016). However, minority among the identified studies in an exhaustive literature search present the assessment framework of the developed self-rating tool, thus the current investigation focuses on developing the corresponding assessment framework for the self-rating proficiency indicators for Philippine STEAM educators that highlights

the scoring system for the self-rating tool. Specifically, the study sought answers to the following objectives: 1. Design the mathematical framework of scoring STEAM educators' proficiency; 2. Design the program for scoring STEAM educators' proficiency; 3. Validate the designed assessment/scoring program; and 4. Try-out and pilot test STEAM educator's proficiency scoring framework.

METHODS

The proficiency scoring framework crafted and elucidated in the following is a product in response to a fundamental question that we think the formalism should be able to answer: How do we extract and therefore determine ones proficiency from the self-rating survey data alone without any external assumption and thereby self-contained? The answer to this is exemplified in the next paragraphs, including an illustration determining the proficiency of a higher education STEAM teacher. A three tier validation, involving both quantitative and qualitative validations, were performed to the scoring framework and program. The results of pilot testing can be seen in the next section.

We wish to know the national STEAM educators' proficiency profile in a) the seven domains of the PPST, and b) the seven TPACK dimensions, from data gathered in the online administration of the developed self-rating survey (Morales et al. 2019) to n higher education STEAM teachers nationwide. The PPST's seven domains are in 1) content knowledge and pedagogy, 2) learning environment, 3) diversity of learners, 4) curriculum and planning, 5) assessment and reporting, 6) community linkages and professional engagement, and 7) personal growth and professional development. The seven TPACK dimensions are 1) pedagogical content knowledge (PCK), 2) technological pedagogical knowledge (TPK), 3) technological pedagogical content knowledge (TPCK), 4) technological content knowledge (TCK), 5) technological knowledge (TK), 6) pedagogical knowledge (PK), and 7) content knowledge (CK). We express the STEAM educators' proficiency as a) beginner, b) proficient, c) highly proficient, and d) distinguished in each of the seven domains and seven dimensions. We include as well the proficiency profile and proficiency in the overall domain.

National Proficiency Profile

Let R_{ijk}, the STEAM educator's proficiency profile of the j^{th} higher education STEAM teacher-respondent in the k^{th} PPST domain, be defined as

$$R_{ijk} = \frac{\text{total } i^{th} \text{ choice } j^{th} \text{ respondent chose in items in the } k^{th} \text{ domain}}{\text{total items in the } k^{th} \text{ domain}}, \quad (1)$$

where $i = 0, 1, 2, 3, 4$, with $i = 0$ corresponding to choice "Not Applicable" (NA), $i = 1$ corresponding to choice "Rarely true to myself" (Rarely-ttm), $i = 2$ corresponding to choice "Occasionally true to myself" (Occasionally-ttm), $i = 3$ corresponding to "Often true to myself" (Often-ttm), and $i = 4$ corresponding to "Always true to myself" (Always-ttm), as choices on all items of the self-rating survey; $j = 1, 2, \ldots, n$; and $k = 1, 2, \ldots, 7$. Similarly, we define the STEAM educator's proficiency profile of the j^{th} higher education STEAM teacher-respondent in the k^{th} TPACK dimension, T_{ijk} as

$$T_{ijk} = \frac{\text{total } i^{th} \text{ choice } j^{th} \text{ respondent chose in items in the } k^{th} \text{ dimension}}{\text{total items in the } k^{th} \text{ dimension}}. \quad (2)$$

R_{ijk} and T_{ijk} obey the normalization property

$$\sum_{i=0}^{4} R_{ijk} = \sum_{i=0}^{4} T_{ijk} = I_{jk} = 1, \quad (3)$$

because each teacher-respondent is required to respond on every item by choosing one and only one choice.

The national STEAM proficiency profile of the sample population corresponding to the i^{th} choice in the k^{th} domain and k^{th} dimension are measured with the following means given by

$$\overline{R}_{ik} = \frac{1}{n}\sum_{j=1}^{n} R_{ijk}, \qquad \overline{T}_{ik} = \frac{1}{n}\sum_{j=1}^{n} T_{ijk}. \quad (4)$$

Each domain contributes equally to the overall proficiency profile of the sample population. The domain-based overall proficiency profile then is

$$\overline{G}_i = \frac{1}{7}\sum_{k=1}^{7} \overline{R}_{ik}. \quad (5)$$

The national STEAM educators' proficiency profiles in eq. (4) and eq. (5) satisfy the normalization conditions

$$\sum_{i=0}^{4} \overline{R}_{ik} = \sum_{i=0}^{4} \overline{T}_{ik} = I_k = 1, \text{ and } \sum_{i=0}^{4} \overline{G}_i = 1, \tag{6}$$

which follows directly from eq. (3).

Knowing Ones Proficiency Profile and Proficiency

Any STEAM teacher wishing to check his/her level of proficiency may do so by taking the 60 item self-rating survey. Once completed, the teacher's proficiency profiles are calculated using

$$R_{ik} \equiv R_{i1k} = \sum_{j=1}^{n} \delta_{1j} R_{ijk}, \qquad T_{ik} \equiv T_{i1k} = \sum_{j=1}^{n} \delta_{1j} T_{ijk}. \tag{7}$$

where δ_{lj} is the Kronecker delta having the property $\delta_{lj} = \begin{cases} 0 & l \neq j \\ 1 & l = j \end{cases}$. A STEAM teacher's proficiency profile also satisfy the normalization conditions

$$\sum_{i=0}^{4} R_{ik} = \sum_{i=0}^{4} T_{ik} = I_k = 1. \tag{8}$$

A STEAM teacher's proficiency profile is then compared to the national STEAM educators' proficiency profile, calculated using eq. (4), by taking their difference like so $\Delta R_{ik} = R_{ik} - \overline{R}_{ik}$ and $\Delta T_{ik} = T_{ik} - \overline{T}_{ik}$. The proficiency profiles' normalization properties in eq. (3), (6), and (8) will ensure that, for any PPST domain or TPACK dimension, any one and only one of the five differences $\Delta R_{4k}, \Delta R_{3k}, \Delta R_{2k}, \Delta R_{1k}$, and ΔR_{0k} ($\Delta T_{4k}, \Delta T_{3k}, \Delta T_{2k}, \Delta T_{1k}$, and ΔT_{0k}) will obtain the greatest positive difference, thereby determining uniquely the teacher's proficiency using the difference-proficiency association in Table 1. Print out or email generated by the program spells out the general attributes and the per domain attributes of the teacher's proficiency level.

Table 1. Translating difference in proficiency profile to teacher's proficiency.

Greatest Positive Difference	STEAM Teacher's Proficiency
$\Delta R_{4k}(\Delta T_{4k})$	Distinguished (i = 4) in domain (dimension) *k*
$\Delta R_{3k}(\Delta T_{3k})$	Highly Proficient (i = 3) in domain (dimension) k
$\Delta R_{2k}(\Delta T_{2k})$	Proficient (i = 2) in domain (dimension) k
$\Delta R_{1k}(\Delta T_{1k})$	Beginner (i = 1) in domain (dimension) k
$\Delta R_{0k}(\Delta T_{0k})$	Not Observed (i = 0) in domain (dimension) k

In the following illustration, the scoring framework is applied to $n = 1507$ higher education STEAM educators who had participated in the online self-rating survey at the time this investigation started which is roughly 78% of the total sample population of the study provided by 123 randomly selected universities and colleges from a total of 2,299 Philippine higher education institutions.

For instance, the self-rating survey data says that the national STEAM educators' proficiency profile of higher education institutions corresponding to PPST's domain on content knowledge and pedagogy, calculated using eq. (4) with $k = 1, n = 1507$, are as follows explicitly: Always-ttm ($i = 4$), $\overline{R}_{41} = 0.37$; Often-ttm ($i = 3$), $\overline{R}_{31} = 0.44$; Occasionally-ttm ($i = 2$), $\overline{R}_{21} = 0.14$, Rarely-ttm ($i = 1$), $\overline{R}_{11} = 0.03$, NA ($i = 0$), $\overline{R}_{01} = 0.02$. Suppose now, a higher education STEAM teacher who has taken the 60-item self-rating survey have a proficiency profile in the PPST's content knowledge and pedagogy domain given explicitly by: Always-ttm ($i = 4$), $R_{41} = 4/19 \cong 0.21$, Often-ttm ($i = 3$), $R_{31} = 6/19 \cong 0.32$, Occasionally-ttm ($i = 2$), $R_{21} = 5/19 \cong 0.26$, Rarely-ttm ($i = 1$), $R_{11} = 3/19 \cong 0.16$, NA ($i = 0$), $R_{01} = 1/19 \cong 0.05$. Using Table 1, we say that the teacher is a beginner STEAM teacher in the PPST's content knowledge and pedagogy domain. The illustration just shown is extended to the other PPST domains, TPACK dimensions and overall PPST domain to complete the level of proficiency unique to the teacher.

Scoring Program and Validation

The derived mathematical eq. (4) and eq. (5) directed the development of the scoring programs using Microsoft excel and Fortran. Three tier validation (quantitative and qualitative) through participant responses determined the robustness and soundness of the scoring program. For the quantitative validation, the sampling ensured nationwide coverage. Respondents received the survey and replied to them online using google form. Once all prospective replies in google form are in, these are converted into Excel file for the convenient and automatic calculation of the a) ratios in eq. (1) and (2); and b) means in eq. (4) and (5). Likewise, the proficiency profiles in eq. (4) and (5) are calculated independently using Fortran. The Fortran codes are produced and saved as f95 file with respondents' replies converted into input txt file. These files are compiled to produce the proficiency profiles. Comparison and equivalence of the proficiency profiles established through the scoring programs using Microsoft Excel and Fortran determined the first-tier quantitative validation of the programs.

The second tier validation involves the calculation and comparison of the proficiencies of STEAM teachers, who 1) have taken the online, self-rating survey, and 2) were observed in classrooms. The proficiencies were determined through the Fortran implementation of the scoring framework

using data from a) the self-rating survey, and b) the classroom observation ratings of observers. Validity is established once the program results show the presence of agreement on the proficiencies of teachers obtained a) according to oneself, and b) from an observer in the classroom.

The third tier emphasized a qualitative validation by comparing generated codes in the interview transcript and observation notes of the participant in each career stage emerging as incurring the same measure in the online survey and in the classroom rating scale (2nd tier) and the significant attributes underscored in all PPST domains and strands in each domain and TPACK dimensions. The proponents noted and assessed the equivalence of the indicators in the online survey as clustered in each domain for equivalence and presence in the interview transcript and observation notes to establish the validity.

RESULTS

The implementation of the analytic expressions of the proficiency profiles, including the determination of proficiencies of teachers, specified in the methods section are herein explained in detail. The results of validations and pilot tests are presented here as well.

Figure 1 shows the result (the value appearing in element T597) of the Excel implementation of eq. (1) corresponding to a teacher-respondent's ($j = 597 - 1$) "always true to myself" choice ($i = 4$) on the 19 items of Domain 1 ($k = 1$). The ratios appearing in columns U, V, W, and X, were for "often true to myself" ($i = 3$), "occasionally true to myself" ($i = 2$), "rarely true to myself" ($i = 1$), and "not applicable" ($i = 0$) choices, respectively. In each and every row, the elements in columns T, U, V, W, and X, sums up to unity, consistent with the normalization property, eq. (3), of the proficiency profile. The same Excel implementation of eq. (1) was done to the other 6 domains. The same Excel implementation was employed to the 7 dimensions using eq. (2).

Figure 2 shows the results of the Excel implementation of eq. (5). Elements BI1533, BJ1533, BK1533, BL1533, and BM1533, corresponding to $\overline{G}_4, \overline{G}_3, \overline{G}_2, \overline{G}_1$, and $\overline{G}_0$ in eq. (5), respectively, are collectively called the overall national STEAM proficiency profile in the PPST domain. Element BN1533 confirms the normalization property of the $\overline{G}_i$'s in eq. (6). The numbers appearing in elements BI1512, BJ1512, BK1512, BL1512, BM1512 are results of Excel implementation of $\overline{R}_{41}$, $\overline{R}_{31}$, $\overline{R}_{21}$, $\overline{R}_{11}$, and $\overline{R}_{01}$ in eq. (4), respectively. These elements collectively correspond to the national STEAM proficiency profile in the PPST's content knowledge and pedagogy domain. Elements BN1512, BN1515, BN1518, BN1521, BN1524, BN1527, and BN1530 verify that the $\overline{R}_{ik}$'s satisfy the normalization property eq. (6).

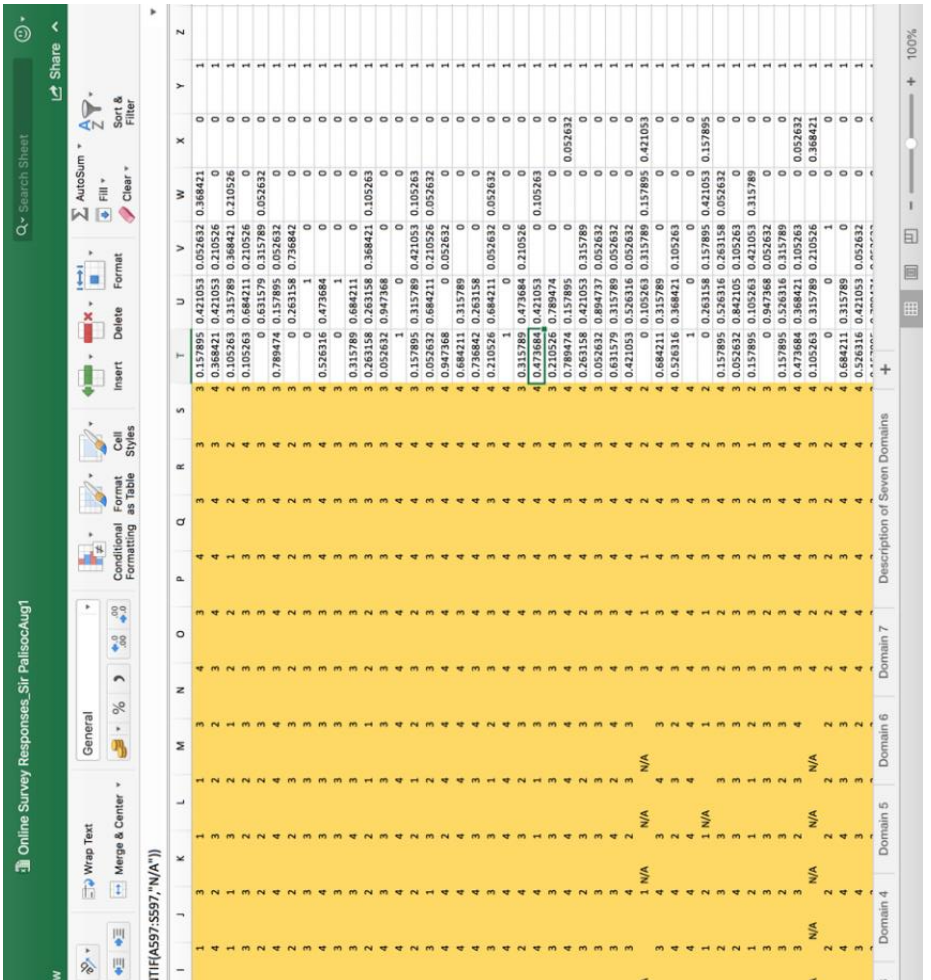

Figure 1. Excel implementation of equation 1.

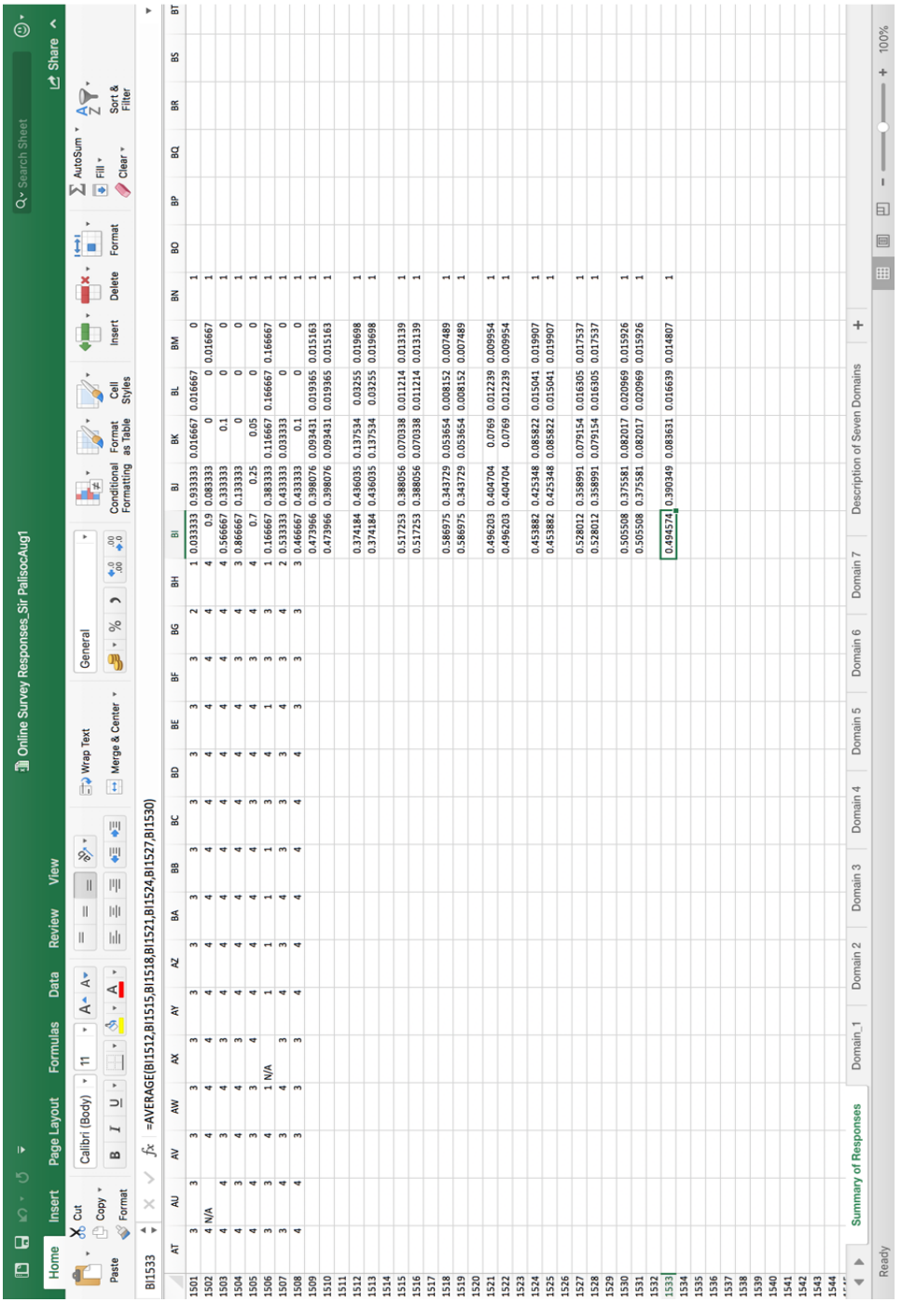

Figure 2. Excel implementation of equation 5.

Figure 3 shows the initial part of the Fortran program code named proficiency that implemented eq. (1), eq. (4) and eq. (5). It starts by uploading (using the Fortran command open(10,file='Domain1.txt')) the responses of the 1,507 teacher-respondents to the 19 items covering domain one stored in a data file named Domain1.tex. Henceforth, until after the 4th "end do" Fortran statement, eq. (1) is implemented, for each response of every teacher-respondent in domain 1, and stored in codes R4(j), R3(j), R2(j), R1(j), and R0(j).

```
program proficiency
integer, dimension(2000,20)  :: f,ff
real, dimension(10,13) :: NAF1
real, dimension(2000) :: R4
real, dimension(2000) :: R3
real, dimension(2000) :: R2
real, dimension(2000) :: R1
real, dimension(2000) :: R0
integer               :: i
integer               :: j
real :: answer,x,y,x1,y1,z1
real :: DIF10,HDF10,PRF10,BIF10,NAF10
real :: DIF11,HDF11,PRF11,BIF11,NAF11
real :: NORMAL,NORMAL1,NORMAL2
real :: RR4,AVR4,AVR41,AVR42
real :: RR3,AVR3,AVR31,AVR32
real :: RR2,AVR2,AVR21,AVR22
real :: RR1,AVR1,AVR11,AVR12
real :: RR0,AVR0,AVR01,AVR02
real :: NORMAL3,NORMAL4,NORMAL5
real :: AVR43,AVR44,AVR45
real :: AVR33,AVR34,AVR35
real :: AVR23,AVR24,AVR25
real :: AVR13,AVR14,AVR15
real :: AVR03,AVR04,AVR05
real :: NORMAL6,NORMAL7
real :: AVR46,AVR47
real :: AVR36,AVR37
real :: AVR26,AVR27
real :: AVR16,AVR17
real :: AVR06,AVR07
open(10,file='Domain1.txt')
do j=1,1507
do i=1,1
read(10,*) f(j,i),f(j,i+1),f(j,i+2),f(j,i+3),f(j,i+4),f(j,i+5),f(j,i+6),f(j,i+7),f(j,i+8),f(j,i+9),f(j,i+10),f(j,i+11) &
  ,f(j,i+12),f(j,i+13),f(j,i+14),f(j,i+15),f(j,i+16),f(j,i+17),f(j,i+18)
end do
end do
do j=1,1507
DIF11=0
HPF11=0
PRF11=0
BIF11=0
NAF11=0
DIF10=0
HPF10=0
PRF10=0
BIF10=0
NAF10=0
do i=1,19
        if (f(j,i)==4) then
        DIF11=DIF10+1.0
        else if (f(j,i)==3) then
        HPF11=HPF10+1.0
        else if (f(j,i)==2) then
        PRF11=PRF10+1.0
        else if (f(j,i)==1) then
        BIF11=BIF10+1.0
        else if (f(j,i)==5) then
        NAF11=NAF10+1.0
        else
        print *, f(j,i)
OverallDomain.f95
```

Figure 3. Fortran implementation of equation 1.

Then, from the last "do – end do" loop statement until the start of the implementation of domain 2, eq. (4) is implemented for domain 1, stored in codes AVR41, AVR31, AVR21, AVR11, AVR01, and printed, as shown in Figure 4.

```
      HPF10=0
      PRF10=0
      BIF10=0
      NAF10=0
      do i=1,19
            if (f(j,i)==4) then
            DIF11=DIF10+1.0
            else if (f(j,i)==3) then
            HPF11=HPF10+1.0
            else if (f(j,i)==2) then
            PRF11=PRF10+1.0
            else if (f(j,i)==1) then
            BIF11=BIF10+1.0
            else if (f(j,i)==5) then
            NAF11=NAF10+1.0
            else
            print *, f(j,i)
            end if
            DIF10=DIF11
            HPF10=HPF11
            PRF10=PRF11
            BIF10=BIF11
            NAF10=NAF11
      R4(j)=DIF11/i
      R3(j)=HPF11/i
      R2(j)=PRF11/i
      R1(j)=BIF11/i
      R0(j)=NAF11/i
      NORMAL=R4(j)+R3(j)+R2(j)+R1(j)+R0(j)
      end do
      end do
      RR4=0
      RR3=0
      RR2=0
      RR1=0
      RR0=0
      do j=1,1507
         RR4=RR4+R4(j)
         RR3=RR3+R3(j)
         RR2=RR2+R2(j)
         RR1=RR1+R1(j)
         RR0=RR0+R0(j)
      end do
      AVR4=RR4/(j-1)
      AVR3=RR3/(j-1)
      AVR2=RR2/(j-1)
      AVR1=RR1/(j-1)
      AVR0=RR0/(j-1)
      NORMAL=AVR4+AVR3+AVR2+AVR1+AVR0
      AVR41=AVR4
      AVR31=AVR3
      AVR21=AVR2
      AVR11=AVR1
      AVR01=AVR0
      NORMAL1=NORMAL
      print *, AVR41, j-1
      print *, AVR31, j-1
      print *, AVR21, j-1
      print *, AVR11, j-1
      print *, AVR01, j-1
      print *, NORMAL1
      !Start of Domain 2
:
```

Figure 4. Continuation of Figure 3 including the Fortran implementation of eq. (4)

The procedure just described is repeated for the remaining 6 domains in preparation for the implementation of eq. (5) shown in Figure 5. The Fortran code program proficiency is then compiled to run the program and extract the results into a default file a.out, the result of which is shown in Figure 6 and the basis of Table 2.

The first tier validation says that the national STEAM proficiency profile for both PPST domains and TPACK dimensions determined through Microsoft excel agree with Fortran results with 1,507 as the total number of sample respondents.

```
              DIF10=DIF11
              HPF10=HPF11
              PRF10=PRF11
              BIF10=BIF11
              NAF10=NAF11
        R4(j)=DIF11/i
        R3(j)=HPF11/i
        R2(j)=PRF11/i
        R1(j)=BIF11/i
        R0(j)=NAF11/i
        NORMAL=R4(j)+R3(j)+R2(j)+R1(j)+R0(j)
        end do
        end do
        RR4=0
        RR3=0
        RR2=0
        RR1=0
        RR0=0
        do j=1,1507
           RR4=RR4+R4(j)
           RR3=RR3+R3(j)
           RR2=RR2+R2(j)
           RR1=RR1+R1(j)
           RR0=RR0+R0(j)
        end do
        AVR4=RR4/(j-1)
        AVR3=RR3/(j-1)
        AVR2=RR2/(j-1)
        AVR1=RR1/(j-1)
        AVR0=RR0/(j-1)
        NORMAL=AVR4+AVR3+AVR2+AVR1+AVR0
        AVR47=AVR4
        AVR37=AVR3
        AVR27=AVR2
        AVR17=AVR1
        AVR07=AVR0
        NORMAL7=NORMAL
        print *, AVR47, j-1
        print *, AVR37, j-1
        print *, AVR27, j-1
        print *, AVR17, j-1
        print *, AVR07, j-1
        print *, NORMAL7
        AVR4=0
        AVR3=0
        AVR2=0
        AVR1=0
        AVR0=0
        NORMAL=0
        AVR4=(AVR41+AVR42+AVR43+AVR44+AVR45+AVR46+AVR47)/7
        AVR3=(AVR31+AVR32+AVR33+AVR34+AVR35+AVR36+AVR37)/7
        AVR2=(AVR21+AVR22+AVR23+AVR24+AVR25+AVR26+AVR27)/7
        AVR1=(AVR11+AVR12+AVR13+AVR14+AVR15+AVR16+AVR17)/7
        AVR0=(AVR01+AVR02+AVR03+AVR04+AVR05+AVR06+AVR07)/7
        NORMAL=AVR4+AVR3+AVR2+AVR1
        print *, AVR4
        print *, AVR3
        print *, AVR2
        print *, AVR1
        print *, AVR0
        print *, NORMAL
        end  program proficiency
(END)
```

Figure 5. Fortran implementation of equation 5.

```
~/Proficiency — -bash                                   ~/Proficiency — -bash
Last login: Fri Dec  7 08:32:08 on ttys001
TPACK-STEAMs-MacBook-Pro-3:~ tpack-steam$ cd Proficiency/
TPACK-STEAMs-MacBook-Pro-3:Proficiency tpack-steam$ gfortran OverallDomain.f95
TPACK-STEAMs-MacBook-Pro-3:Proficiency tpack-steam$ ./a.out
  0.374184340           1507
  0.436035812           1507
  0.137534022           1507
   3.25497948E-02       1507
   1.96975358E-02       1507
   1.00000143
  0.517253101           1507
  0.388055980           1507
   7.03382790E-02       1507
   1.12143420E-02       1507
   1.31386947E-02       1507
   1.00000036
  0.586974680           1507
  0.343728870           1507
   5.36544099E-02       1507
   8.15243460E-03       1507
   7.48886215E-03       1507
  0.999999225
  0.496282856           1507
  0.484704273           1507
   7.69005343E-02       1507
   1.22391675E-02       1507
   9.95354448E-03       1507
   1.00000036
  0.453882277           1507
  0.425348103           1507
   8.58217999E-02       1507
   1.58409220E-02       1507
   1.99071132E-02       1507
   1.00000024
  0.528012897           1507
  0.358990401           1507
   7.91545659E-02       1507
   1.63048655E-02       1507
   1.75372064E-02       1507
  0.999999846
  0.505507231           1507
  0.375581056           1507
   8.20171237E-02       1507
   2.09688265E-02       1507
   1.59256775E-02       1507
  0.999999940
  0.494573802
  0.390349209
   8.36315304E-02
   1.66386198E-02
   1.48069477E-02
   1.00000012
TPACK-STEAMs-MacBook-Pro-3:Proficiency tpack-steam$
```

Figure 6. Compilation of Fortran code with gfortran and viewing of results with ./.

Table 2 shows the national STEAM proficiency profile based on the PPST domains. On the average, it appears that, overall nationally, for a "rarely true to oneself" choice chosen on an item, five other items received "occasionally true to oneself" response, while "often true to oneself" choice were chosen in 24 other items, and 30 other items got "always true to oneself" choice.

Table 2. National STEAM educator's proficiency profile in the Philippine Professional Standards for Teachers (PPST) domains. ** true to myself are herein implied for simplicity from here onwards.

PPST Domain	Always**	Often**	Occasionally**	Rarely**	NA
Content Knowledge and Pedagogy	0.37	0.44	0.14	0.03	0.02
Learning Environment	0.52	0.39	0.07	0.01	0.01
Diversity of Learners	0.59	0.34	0.05	0.01	0.01
Curriculum and Planning	0.50	0.40	0.08	0.01	0.01
Assessment and Reporting	0.45	0.42	0.09	0.02	0.02
Community Linkages and Professional Engagement	0.53	0.36	0.08	0.02	0.01
Personal Growth and Professional Development	0.50	0.38	0.08	0.02	0.02
Overall	0.49	0.39	0.08	0.02	0.02

Table 3 shows the national STEAM educator's proficiency profile based on the 7 TPACK dimensions. On the average, out of the 13 items of the self-rating survey dealing directly on the pedagogical knowledge dimension, around seven items received "always true to myself" response, roughly five other items got "often true to myself" answer, and close to an item obtain an "occasionally true to myself" reply. Choices "rarely true to myself" and "not applicable" were hardly chosen in this dimension.

For the second-tier validation, the online, self-rating survey's sample population used was 1,455 teacher-respondents, the proficiency profiles of which (see Table 4) in domains 1) content knowledge and pedagogy, 2) learning environment, and 3) diversity of learners, formed the basis for determining the proficiency level of 52 other teachers who took the online survey and were observed in classroom as well.

Table 3. National STEAM educator's proficiency profile in the Technological-Pedagogical-Content-Knowledge (TPACK) dimensions.

TPACK Dimension	Always	Often	Occasionally	Rarely	NA
Pedagogical Content Knowledge	0.54	0.36	0.07	0.01	0.02
Technological Pedagogical Knowledge	0.50	0.41	0.07	0.01	0.01
Technological Pedagogical Content Knowledge	0.30	0.44	0.18	0.05	0.03
Technological Content Knowledge	0.43	0.43	0.10	0.02	0.02
Technological Knowledge	0.48	0.38	0.09	0.02	0.03
Pedagogical Knowledge	0.56	0.37	0.06	0.01	0.00
Content Knowledge	0.45	0.44	0.09	0.01	0.01

Table 4. STEAM proficiency profile of 1455 online survey teacher-respondents.

Domain	Always	Often	Occasionally	Rarely	NA
Content Knowledge and Pedagogy	0.38	0.43	0.14	0.03	0.02
Learning Environment	0.52	0.39	0.07	0.01	0.01
Diversity of Learners	0.59	0.34	0.05	0.01	0.01

Finally, Table 5 presents the third tier of validation focused on identifying presence or occurrence of indicators in the interview transcripts and classroom observation notes matched with the expected attribute per career stage as per PPST.

Table 6 shows the STEAM proficiency profiles of a teacher classified as distinguished overall (Teacher A), including in all domains except in Domain 5, where the teacher appears to be highly proficient only; a teacher envisaged as highly proficient overall, including in all domains except in Domain 7, where the teacher appears to be distinguished (Teacher B); and a teacher proficient in Domain 3 and beginner in Domain 1 (Teacher C).

Table 5. Qualitative validation of the scoring program. The shaded portion indicate non-match of interview transcript or classroom observation notes with the expected attribute per career stage as per Philippine Professional Standards for Teachers (PPST). Data element having two values mean data comes from two respondents.

Domain	Distinguished		Highly Proficient		Proficient		Beginner	
	I	CON	I	CON	I	CON	I	CON
Content Knowledge and Pedagogy • composed of 7 strands • with 19 items (Online Survey)	18	3		14 6	11	10	10	10
Learning Environment • composed of 6 strands • with 10 items (Online Survey)	3		6 4	3 2	5	4	6	6
Diversity of Learners • composed of 5 strands • with 7 items (Online Survey)	3	1	5 2	2 1	1	5	6	3
Curriculum and Planning • composed of 5 strands • with 9 items (Online Survey)	3	3	3 4	4 3	5 4	5 5	0	4
Assessment and Reporting • composed of 5 strands • with 3 items (Online Survey)	1	1	3 3	0 1	3 1	1 1	1	1
Community Linkages and Professional Engagement • composed of 4 strands • with 7 items (Online Survey)	5	1	5	0	3	0	4	2
Professional Growth and Professional Development • composed of 5 strands • with 5 items (Online Survey)	3		2	1	1	1	3	1

I-Interview, CON – classroom observation notes

Table 6. STEAM proficiency profiles of higher education teachers A (TA), B (TB), and C (TC), determined, respectively, as distinguished overall, highly proficient overall, and proficient in domain 3 and beginner in domain 1 in the PPST domain.

Domain	Always			Often			Occasionally			Rarely			NA		
	TA	TB	TC	TA	TB	TC	TA	TB	TC	TA	TB	TC	TA	TB	TC
Content Knowledge and Pedagogy	0.47	0.31	0.21	0.42	0.53	0.42	0.00	0.16	0.10	0.11	0.00	0.16	0.00	0.00	0.11
Learning Environment	1.00	0.00	0.50	0.0	0.90	0.50	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Diversity of Learners	0.86	0.43	0.57	0.14	0.57	0.29	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00
Curriculum and Planning	0.56	0.44	0.56	0.33	0.56	0.11	0.11	0.00	0.22	0.00	0.00	0.11	0.00	0.00	0.00
Assessment and Reporting	0.00	0.00	0.67	0.67	1.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00
Community Linkages and Professional Engagement	0.86	0.43	0.86	0.14	0.43	0.00	0.00	0.14	0.00	0.00	0.00	0.14	0.00	0.00	0.00
Personal Growth and Professional Development	0.80	0.80	0.80	0.20	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00
Overall	0.65	0.34	0.59	0.27	0.60	0.24	0.02	0.06	0.07	0.01	0.00	0.09	0.05	0.00	0.01

DISCUSSION

A self-contained mathematical framework, free from external assumptions, able to unambiguously determine a higher education STEAM teacher's proficiency is formulated in this study. On the basis of this framework, a program is designed and subjected to quantitative and qualitative validation showing meaningful and consistent prediction of STEAM teachers' proficiency. A pilot test of this program to three STEAM teachers of varying levels of proficiency indeed shows its capacity to identify uniquely a STEAM teacher's proficiency. These results are necessary to make sense of the developed self-rating tool in Morales et al. (2019) in providing STEAM teachers with their equivalent rating in terms of STEAM Education proficiency. Consequently, the STEAM educator's proficiency scoring framework ably determines the proficiency profile of a target population and predict unambiguously individual teacher-respondent's proficiency level. The outputs of the previous work and the current study form part of a bigger project on developing the Philippine STEAM Education Model for higher and advanced learning. The novelty of this study (a) is that it provided the self-rating tool in Morales et al. (2019) a programmed framework of scoring which other and most developed instruments lack, and (b) lies in the quantitative and qualitative combination of approaches to validation of the STEAM educators' scoring framework, which, to the best of our knowledge, is unique to this study.

The developed framework, including the program it took form, in determining a Philippine higher education STEAM teacher's proficiency is self-contained, universal, albeit shaped by local peculiarities, transparent, and technology-enhanced (Steel 2015). The determination of ones' proficiency depends entirely and sufficiently on the population of the self-rating survey through the national STEAM educators' proficiency profiles as shown in Tables 2 and 3. External assumption is not necessary. The scoring framework's adaptability is universal provided the survey used require one and only one response on every item, which may be adapted to suit any local setting—a trait matching the characteristics of the seven principles of universal design (Center for Excellence in Universal Design 2019). The formulated framework and program give results that any interested investigator may verify independently given the same set of data.

The framework's and program's unique determination of ones' proficiency have been quantitatively and qualitatively validated with varying presence of agreement. The presence of agreement in the proficiency level of teachers according to oneself and from an observer in the classroom is shown in Table 7. Out of the 52 teachers who took the online survey and were likewise observed in class, three teachers were determined as proficient in domain on diversity of learners in both online survey and classroom observation, which

translates to 50% agreement, considering there were six teachers predicted as proficient in the domain on diversity of learners through the online survey. Except in the domains on learning environment and on diversity of learners, both of which practically have no beginners, distinguished teachers in the domain on learning environment exhibits the least amount of agreement with 7%.

Table 7. Extent of agreement on proficiency level of teachers according to oneself and from an observer in the classroom.

Domain	Distinguished	Highly Proficient	Proficient	Beginner
Content Knowledge and Pedagogy	1 [11] 9%	3 [20] 15%	5 [15] 33%	2 [6] 33%
Learning Environment	1 [14] 7%	8 [32] 25%	1 [5] 20%	0 [1] 0%
Diversity of Learners	6 [19] 32%	7 [26] 27%	3 [6] 50%	0 [1] 0%

The occurrence of indicators may mean that outputs generated by the scoring program match the indicators as perceived by classroom observers and interviewers of the participants identified for validation test (Table 5). The decreasing trend may imply that in most of the domains, 4 of 7, proficiency in STEAM teaching may be dictated by the number of indicators exhibited by the STEAM educator(s) in the following domains: domain 1-content knowledge and pedagogy, domain 5-assessment and reporting, domain 6-community linkages and professional engagement, and domain 7-professional growth and development, with the greatest number of combination for the distinguished career stage and the least for the beginner stage. For example, distinguished teacher in domain 1 as rated by the scoring program based on the online self-rating survey exhibited 18 out of 19 indicators as per interview transcripts and 3 out of 19 as per classroom observation notes with a decreasing combination until the beginner stage. However, three of the seven domains did not exhibit the same trend, noting greater number of indicators exhibited by other career stages compared to the distinguished career stage. Analysis of the sample interview transcripts and classroom observation notes in Table 8 shows that although the three domains (Learning Environment, Diversity of Learners, and Curriculum and Planning) manifested a different trend in terms of number of exhibited indicators, the minimally exhibited indicator encompass large number of indicators with complex attribute.

Table 8. Sample interview transcripts and classroom observation notes per career stage.

Domain	Indicator	Distinguished	Highly Proficient	Proficient	Beginner
2: Learning Environment	13. Models various scientific attitude and STEAM professional traits	Researches for problem solving Conducts research to help industry in the province and to ease environmental difficulty	Read books; critical thinking; preparedness	Dedication to work	facilitated students' smooth/emotional disposition during the presentation upgrade self regularly
4: Curriculum and Planning	43. Designs, communicates, and implements STEAM-related activities in partnership with the community/industry.	Produces products like lutana tea, aspherkol and other innovative products	Ask students to develop a project to identify a burglar from not a burglar using machine vision (using GPS)	Actively participates in professional activities	Conduct capstone project and look for possible problems in the industry

The distinguished teacher's conduct of STEAM research and utilization of such research in STEAM teaching traverse wide array of domains that includes all the three aforementioned domains (Hazelkorn et al. 2015). Comparing this exhibited attribute, teachers in other career stages specified minute attributes compared to those exhibited by the distinguished teacher, indicative of validation of the scoring system developed for the online survey to generate the STEAM educator proficiency.

The scope of the scoring framework developed is universal and may be adapted to suit any local setting. Although all necessary aspects of validation were done and exhibited favorable results, increasing the number of interviews and classroom observations to 10% of the sample population of teachers will produce a robust scoring program.

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Antimicrobial property of the epidermal mucus of Tilapia *Oreochromis* spp.

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ABSTRACT

This study was conducted to determine and compare the antimicrobial property of the epidermal mucus of Tilapia (*Oreochromis* spp.) from two environmental conditions, the fish tank and fishpond. The antimicrobial property was determined using Filter Paper Disc Diffusion Method with Amoxicillin and Nystatin as the positive controls and distilled water as the negative control. Results showed significant differences in the effects of the treatments when tested against *Escherichia coli, Staphylococcus aureus*, *Bacillus cereus, Bacillus subtilis, Bacillus megaterium, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger* and *Aspergillus flavus* ($p<0.05$). The Duncan's Multiple Range Test further proved that the mucus of Tilapia from fishpond was significantly higher in terms of antibacterial property compared to mucus of Tilapia from fish tank. On the other hand, both of the epidermal mucus of Tilapia from fishpond and fish tank did not show any inhibitory effect against *P. aeruginosa* and *A. niger*. The epidermal mucus of Tilapia showed bacteriostatic, fungistatic and bactericidal effects against test microorganisms. Based on the results, the mucus of *Oreochromis* spp. from fishpond and fish tank are potential sources of antimicrobial compounds.

Keywords: Tilapia, *Oreochromis* spp., antimicrobial property, epidermal mucus, fish tank, fishpond

INTRODUCTION

Fish farming is the growing of fish in a controlled environment (concrete or earthen ponds), vats (wooden or fiber glass) and plastics (Nwokoye et al. 2007; Osawe 2007). Fish tanks allow the culturist to manage environmental parameters such as water temperature, dissolved oxygen concentration, pH and waste that can be adjusted to promote maximum production (DeLong et al. 2009). Since fish have limited access to natural foods in tanks, they must be fed a complete diet containing vitamins and minerals (Rakocy 2005). On the other hand, fish on ponds ranges from extensive systems using only organic or inorganic fertilizers, to intensive systems using high-protein feed, aeration (Rakocy and McGinty 1989) and water exchange rich in oxygen and some nutrients (Nandlal and Pickering

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2004). Studies show that a special set of water chemistry requirements is essential to a healthy, balanced, and functioning aquaculture system (DeLong et al. 2009). The growth and health of different fish species are also influenced by a different range of factors, among them water quality parameters (Makori et al. 2017). In the Philippines, Tilapia is the second most farmed fish species next to milkfish (Guerrero III 2019).

Tilapia *(Oreochromis* spp.) is a freshwater fish belonging to the Family Cichlidae. The group consists of three important genera, *Oreochromis, Sarotherodon* and *Tilapia* (Tower 2005). They are native to Africa, but were introduced into many tropical, subtropical and temperate regions of the world, including the Philippines, during the second half of the 20th century (Pillay 1990). Tilapia has a number of characteristics that make them attractive for tank. They prefer water temperatures between 29°C and 31°C and tolerate wide range of salinity. They can tolerate the crowding and handling that is required in a tank-based facility (DeLong et al. 2009) and also low dissolved oxygen and high ammonia concentrations better than most aquaculture species (Boyd 1990). They are more resistant to viral, bacterial and fungal diseases than other aquaculture species (World Seafood Market 2005) and can inhibit the spread of Vibrio and other pathogenic bacteria through the secretion of its mucus (Caipang et al. 2011; Wibowo et al. 2015).

The fish skin mucus is the slimy and slippery layer covering the epithelial surfaces which provides a stable physical or chemical barrier against the invading pathogens (Dash et al. 2018). Mucus is the material that makes fish slippery to touch. Its slipperiness is the result of its high water content and the presence of high-molecular-weight, gel-forming macromolecules (Shephard 1994). Several roles for this sticky layer have been suggested. This layer acts as a lubricant (Rosen and Cornford 1971) and has mechanical protective function (Cameron and Endean 1973) involved in osmoregulation and locomotion playing a possible immunological role (Fletcher 1978) and controls the intra-specific chemical communication (Saglio and Blance 1989). There is also a growing evidence that lectins from the skin mucus of fish have the ability to agglutinate, opsonize and/or suppress microbial growth (Suzuki et al. 2003; Dutta et al. 2005; Tsutsui et al. 2006, 2007; Argayosa et al. 2011). The antimicrobial activity of epidermal mucus extracts against a broad range of microbial pathogens was observed by Hellio et al. (2002). Many researchers have proven that the mucus substances are good defense which can inhibit the spread of *Vibrio* and other pathogenic bacteria (Caipang et al. 2011; Wibowo et al. 2015) but not in the difference on the effects of mucus from Tilapia grown in the fish tank and in the fishpond.

Thus, this present study aimed to evaluate the antimicrobial property of the epidermal mucus of Tilapia. Specifically, this study aimed (1) to determine the antimicrobial property of the epidermal mucus of Tilapia from

two environmental conditions namely concrete tanks and fishpond against *Escherichia coli* (Escherich, 1885), *Staphyloccocus aureus* (Ogston, 1880), *Bacillus subtilis* (Ehrenberg, 1835), *Bacillus cereus* (Ehrenberg, 1835), *Pseudomonas aeruginosa* (Schroeter, 1872), *Bacillus megaterium* (Ehrenberg, 1835), *Aspergillus flavus* (Link, 1809), *Aspergillus niger* (Tiegh, 1867), and *Candida albicans* (Berkhout, 1923); and (2) if antimicrobial property is present, determine which of the epidermal mucus from two environmental conditions would show the higher antimicrobial property.

METHODS

Sample Collection and Locale of the Study

This study was conducted in November 2016 at the Microbiology Laboratory of the Western Philippines University-Puerto Princesa Campus (WPU-PPC), Puerto Princesa City, Palawan, Philippines. The epidermal mucus was obtained from Tilapia grown in fish tanks of Aquatic Sciences Laboratory, WPU-PPC and in fish pond of Iwahig Penal Farm which are both locally located in Puerto Princesa City, Palawan. The mucus samples were freshly collected by scrapping at the dorsal part of the fish using a sterile spatula. For each treatment, four fish samples were collected with common sizes ranging from 15 to 20 cm in total length. Mucus from the four samples for each treatment was mixed together to make one replicate and were stored in a sterilized vial at 4°C. It was then placed in a cooler and immediately transported to the laboratory for analyses. Four biological replicates of epidermal mucus were prepared for each environmental condition. The freshly collected mucus were assayed directly for antimicrobial property.

Culture Media Preparation

Culture media (Nutrient agar, Nutrient broth and Potato Dextrose agar) preparation was done following the protocol provided by the manufacturer (HIMEDIA).

Preparation of Microorganisms

The test microbes were the opportunistic *E. coli, B. cereus, P. aeruginosa, A. flavus* and *C. albicans*; disease causing bacteria *S. aureus* and *A. niger;* and non-disease causing bacteria *B. subtilis* and *B. megaterium*. The test microorganisms were purchased from stock cultures of the Mindanao State University, Marawi City. These cultures were used as representatives for the four groups of microorganisms such as Gram-negative bacteria, Gram-positive bacteria, molds and yeast. A 10 ml of the previously prepared nutrient broth was poured into test tubes with cotton plugs for sterilization at 121°C,

15 psi for 15 minutes. After sterilization, the culture medium was allowed to cool down. A loopful (0.01 ml) of each test bacterium from the selected microbes was then inoculated aseptically on the pre-labeled broth medium and covered with cotton plugs. The microbial subcultures were placed in the incubator for 24 hours at 37°C.

Antimicrobial Assay

The filter paper disc diffusion method was used to determine the antimicrobial property of the epidermal mucus of Tilapia. For the positive controls, a 500 mg Amoxicillin, which was dissolved in 10 ml sterile distilled water for the bacteria, and Nystatin (100, 000 units/ml) for fungi were used. On the other hand, 10 ml sterile distilled water was used as the negative control. The treatment designations were the following: T1 (positive control), T2 (negative control), T3 (Tilapia mucus from fish tank) and T4 (Tilapia mucus from fishpond). A loopful (0.01 ml) of test organisms, *E. coli, S. aureus*, *B. cereus, B. subtilis, B. megaterium, P. aeruginosa, A. niger, A. flavus* and *C. albicans* from the subcultures were inoculated into the sterile nutrient agar (20 ml) by direct seeding before pouring it into Petri dishes and allowed to solidify. The previously sterilized filter paper discs (cut by paper puncher to 6 mm diameter) were soaked to the freshly collected mucus samples (from fishpond and fish tank) and in the positive and negative control using sterile forceps. These impregnated discs were placed on the designated areas (4 discs on 1 plate) (Figure 1). Four replicates for each treatment were prepared. The Petri plates were incubated for 24 hours to allow microbial growth. After 24 hours, the plates were examined and zones of inhibition were measured using standardized transparent ruler (in mm).

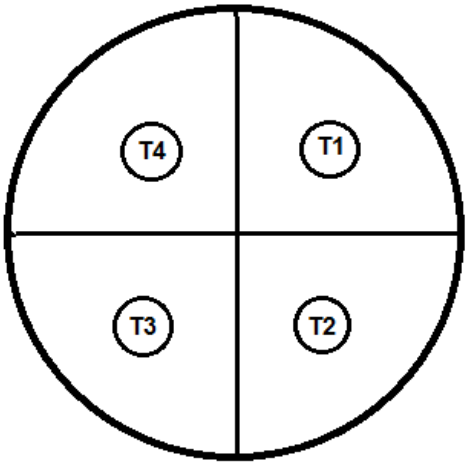

Figure 1. Filter Paper Disc Diffusion Test

Statistical Analyses

The data on the zones of inhibition of the treatments against *E. coli, S. aureus*, *B. cereus, B. subtilis, B. megaterium, P. aeruginosa, A. niger, A. flavus* and *C. albicans* were analyzed using one-way analysis of variance (ANOVA) to test the significant differences. The data were subjected to Duncan's Multiple Range Test (DMRT) to compare the means using IBM SPSS Statistics 20 software.

Diameter of Zones of Inhibition Interpretative Standard for Test Microorganisms

The zones of inhibition were interpreted using the Laboratory Manual of Standardized Methods for Antimicrobial Sensitivity Tests (Tendencia 2004; Table 1).

Table 1. Zones of Inhibition Interpretative Standard for Test Microorganisms.

Test Microorganisms	Interpretative Criteria		
	Sensitive	Intermediate	Resistant
Escherichia coli	≥18	14-17	≤13
Staphyloccocus aureus	20	-	19
Bacillus subtilis	≥18	14-17	≤13
Bacillus cereus	≥18	14-17	≤13
Bacillus megaterium	≥18	14-17	≤13
Pseudomonas aeruginosa	≥18	14-17	≤13
Candida albicans	≥18	14-17	≤13
Aspergillus niger	≥15	10-14	≤10
Aspergillus flavus	≥15	10-14	≤10

RESULTS

The epidermal mucus of Tilapia from fishpond showed higher inhibitory effect against *S. aureus*, *B. subtilis, B. cereus* and *C. albicans* compared to the effect of epidermal mucus of Tilapia from fish tank. It also showed inhibitory effect against *E. coli, B. megaterium* and *A. flavus* while the epidermal mucus of Tilapia from fish tank did not. Both of the epidermal mucus didn't show any zone of inhibition when tested against *P. aeruginosa* and *A. niger*. Table 2 shows the mean zones of inhibition of the treatments against the nine test microorganisms which were subjected to ANOVA and Post Hoc test (Duncan's test).

Table 2. Inhibitory effects of the treatments against Test Microorganisms. ** - highly significant at α= 0.01; * - significant at α= 0.05; Different letters signify significant difference at α=0.05.

Test Microorganisms/Treatment	Mean ± sd (mm) zones of inhibition	DMRT*	F-value	P-value
Escherichia coli				
Positive control	37.5±2.68	A	33.96**	0.000
Negative control	0	C		
Mucus of Tilapia from fish tank	0	C		
Mucus of Tilapia from fishpond	11.5±2.68	B		
Staphyloccocus aureus				
Positive control	15±1.19	A	52.82**	0.000
Negative control	0	C		
Mucus of Tilapia from fish tank	8.375±1.19	B		
Mucus of Tilapia from fishpond	8.75±1.19	B		
Bacillus subtilis				
Positive control	40±1.60	A	153.54**	0.000
Negative control	0	D		
Mucus of Tilapia from fish tank	9.25±1.60	C		
Mucus of Tilapia from fishpond	15.25±1.60	B		
Bacillus cereus				
Positive control	41.25±2.68	A	57.87**	0.000
Negative control	0	D		
Mucus of Tilapia from fish tank	12.75±2.68	C		
Mucus of Tilapia from fishpond	20±2.68	B		
Bacillus megaterium				
Positive control	20±0.88	A	226.68**	0.000
Negative control	0	C		
Mucus of Tilapia from fish tank	0	C		
Mucus of Tilapia from fishpond	8.5±0.88	B		
Pseudomonas aeruginosa				
Positive control	8.5±0.35	A	289.00**	0.000
Negative control	0	B		
Mucus of Tilapia from fish tank	0	B		
Mucus of Tilapia from fishpond	0	B		
Candida albicans				
Positive control	19.5±1.70	A	15.02**	0.000
Negative control	0	C		
Mucus of Tilapia from fish tank	13.6±1.70	B		
Mucus of Tilapia from fishpond	13.75±1.70	B		
Aspergillus niger				
Positive control	14.5±0.35	A	841.00**	0.000

Test Microorganisms/Treatment	Mean ± sd (mm) zones of inhibition	DMRT*	F-value	P-value
Negative control	0	B		
Mucus of Tilapia from fish tank	0	B		
Mucus of Tilapia from fishpond	0	B		
Aspergillus flavus				
Positive control	20±0.72	A	335.74**	0.000
Negative control	0	C		
Mucus of Tilapia from fish tank	0	C		
Mucus of Tilapia from fishpond	7.75±0.72	B		

Effects of Treatments against *E. coli*

In this study, epidermal mucus of Tilapia from fishpond showed zones of inhibition when tested against *E. coli* while the epidermal mucus from fish tank did not (Figure 2; ECR4). ANOVA proved that there were significant differences in the effects of the treatments when tested against *E. coli* while DMRT showed that the antimicrobial property of epidermal mucus from fishpond differ significantly compared to other treatments although not comparable to positive control when tested against *E. coli*.

Effects of Treatments against *S. aureus*

The epidermal mucus of Tilapia from fish tank and fishpond showed zones of inhibition when tested against *S. aureus* (Figure 2; SAR1). The ANOVA showed that there were significant differences in the effect of the treatments when tested against *S. aureus*. DMRT proved that the epidermal mucus from fishpond and fish tank are both comparable to each other but not to positive control when tested against *S. aureus* (Table 2).

Effects of Treatments against *B. subtilis*

The epidermal mucus of Tilapia from fishpond and fish tank both showed zones of inhibition against *B. subtilis* (Figure 2; BSR2). Between the two treatments that showed inhibitory effects, the epidermal mucus of Tilapia from fishpond showed the higher zones of inhibition. The ANOVA showed that there were significant differences in the effects of the treatments when tested against *B. subtilis*. DMRT further proved that epidermal mucus form fishpond was significantly higher in terms of antibacterial property but not comparable to positive control (Table 2).

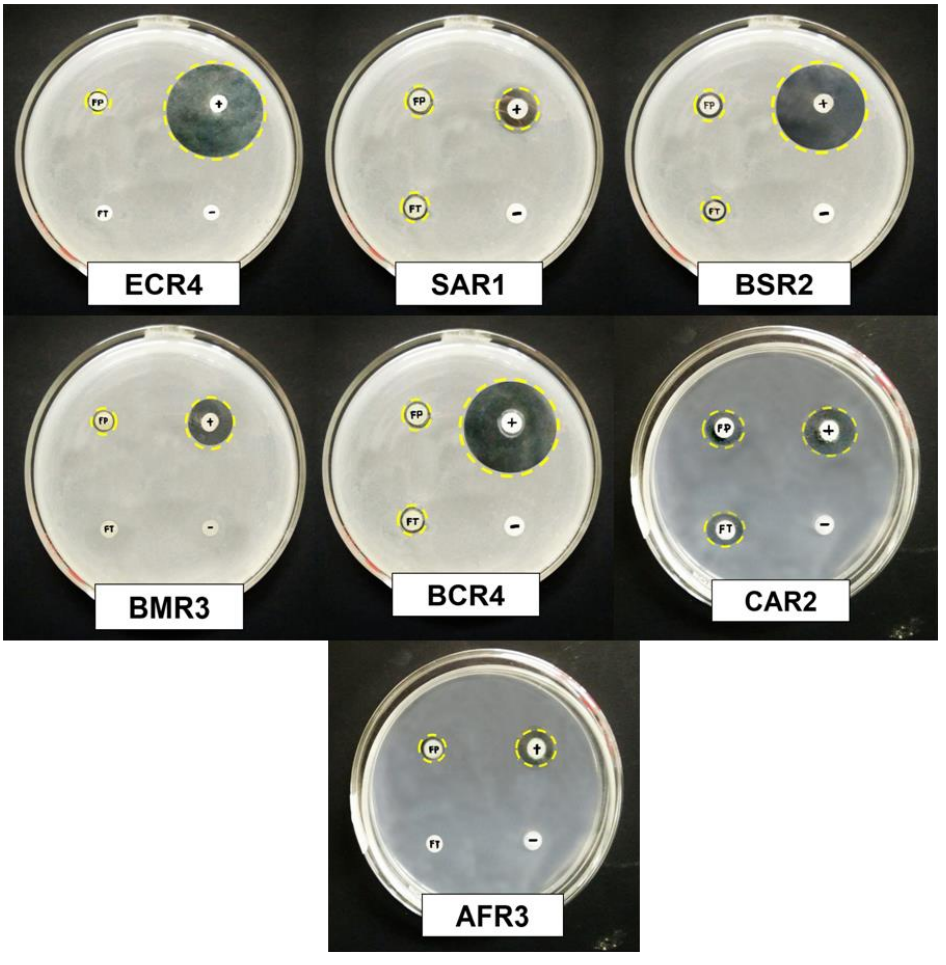

Figure 2. A representative replicate of epidermal mucus showing zones of inhibition against test microorganisms: *Escherichia coli* (ECR4), *Staphyloccocus aureus* (SAR1), *Bacillus subtilis* (BSR2), *Bacillus megaterium* (BMR3), *Bacillus cereus* (BCR4), *Candida albicans* (CAR2) and *Aspergillus flavus* (AFR3). FP stands for mucus of tilapia from fishpond and FT stands for mucus of tilapia from fish tank. Circles highlight the zones of inhibition.

Effects of Treatments against *B. cereus*

Both of the epidermal mucus from fish tank and fishpond showed zones of inhibition when tested against *B. cereus* (Figure 2; BCR4). The ANOVA showed that there were significant differences in the effects of the treatments against *B. cereus*. Duncan's test proved that the epidermal mucus of Tilapia from fishpond was significantly higher compared to epidermal mucus of Tilapia from fish tank but not comparable to positive control when tested against *B. cereus* (Table 2).

Effects of Treatments against *B. megaterium*

In this study, the epidermal mucus of Tilapia from fishpond showed zones of inhibition when tested against *B. megaterium* while the epidermal mucus of Tilapia from fish tank did not (Figure 2; BMR3). The ANOVA showed that there were significant differences in the treatments against *B. cereus*. DMRT proved that the epidermal mucus of Tilapia from fishpond is significantly higher from epidermal mucus from fish tank but not comparable to positive control when tested against *B. megaterium* (Table 2).

Effects of Treatments against *P. aeruginosa*

The epidermal mucus of Tilapia from fishpond and fish tank did not show any zone of inhibition against *P. aeruginosa* (Table 2).

Effects of Treatments against *C. albicans*

In this study, both of the epidermal mucus of Tilapia from fishpond and fish tank showed zones of inhibition against *C. albicans* (Figure 2; CAR2). The ANOVA showed that there were significant differences in the treatments against *C. albicans*. DMRT proved that the epidermal mucus of Tilapia from fishpond and the epidermal mucus of Tilapia from fish tank were not significantly different from each other and not comparable to positive control when tested against *C. albicans* (Table 2).

Effects of Treatments against *A. niger*

Both the epidermal mucus of Tilapia from fishpond and fish tank did not show any inhibitory effect towards *A. niger* (Table 2).

Effects of Treatments against *A. flavus*

The epidermal mucus of Tilapia from fishpond showed zones of inhibition when tested against *A. flavus* while epidermal mucus of Tilapia from fish tank did not (Figure 2; AFR3). ANOVA proved that there were significant differences in the effects of the treatments when tested against *A. flavus*. DMRT proved that the antifungal property of epidermal mucus from fishpond is not comparable to positive control when tested against *A. flavus* (Table 2).

Table 3 shows the average zones of inhibitions by the treatments where it shows that *P. aeruginosa* and *A. niger* were the most resistant test microorganisms while *B. subtilis* and *B. cereus* are the most susceptible.

Table 3. Average zones of inhibitions by the treatments towards the test microorganisms and their interpretations.

Test Microorganisms	Environmental Conditions	Mean ±sd (mm) of Zone of Inhibitions	Interpretation
Escherichia coli	Fishpond	11.5±2.68	Resistant
	Fish tank	0	Resistant
Staphyloccocus aureus	Fishpond	8.75±1.19	Resistant
	Fish tank	8.375±1.19	Resistant
Bacillus subtilis	Fishpond	15.25±1.60	Intermediate
	Fish tank	9.25±1.60	Resistant
Bacillus cereus	Fishpond	20±2.68	Sensitive
	Fish tank	12.75±2.68	Resistant
Bacillus megaterium	Fishpond	8.5±0.88	Resistant
	Fish tank	0	Resistant
Pseudomonas aeruginosa	Fishpond	0	Resistant
	Fish tank	0	Resistant
Candida albicans	Fishpond	13.75±1.70	Intermediate
	Fish tank	13.6±1.70	Intermediate
Aspergillus niger	Fishpond	0	Resistant
	Fish tank	0	Resistant
Aspergillus flavus	Fishpond	7.75±0.72	Resistant
	Fish tank	0	Resistant

DISCUSSION

The epidermal mucus of Tilapia collected from two environmental conditions, the fishpond and fish tank showed antimicrobial property. Fishes, like Tilapia, excrete mucus on their epidermal and epithelial cells (Pickering 1974; Ellis 1999) that acts as a lubricant (Rosen and Cornford 1971) and has its mechanical protective function (Cameron and Endean 1973) which involved in osmoregulation and locomotion, playing a possible immunological role (Fletcher 1978) that controls their intra-specific chemical communication (Saglio and Blance 1989). Mucus layer is a biological interface between fish and their aqueous environment that consists of biochemical diverse secretions. Over the past years, it has also been shown that mucus plays a pivotal role in the prevention of colonization by parasites, bacteria and fungi (Bragadeeswaran 2011) which this study has also proven.

Rao et al. (2015) found that Tilapia mucus has high protein content and acidic extract compared to other freshwater fish species. The acidic mucus extracts of Tilapia also showed potent bactericidal activity against a wide range of fish and human pathogens (Subramanian et al. 2008). One inherent property of tilapia such as the antimicrobial components in the acidic mucus

extracts of tilapia mucus are believed to have a key role in host defense against pathogenic infection (Rao et al. 2015) such as *Vibrio harveyii* (Forlenza et al. 2008) in the aqueous environment. Aside from the mucus, the skin also expresses genes that may enhance immune system including antimicrobial peptides, cytokines, complements, major histocompatibility complex (MHC) and immunoglobulins. These genes that are located in the skin produce substances which are then released to the surface and integrate with the mucus, thereby enhancing the first line of defense in fish against pathogens (Gonzalez et al. 2007).

The functional property of the mucus depends on its capacity to form gel on the epithelial surface (Bragadeeswaran 2011). This mucus is secreted by the epidermal goblet cells, composed mainly of water and gel forming macromolecules such as mucins and other glycoproteins (Martinez et al. 2006). In addition, fish mucus also contains a variety of biologically active substances such as lysozyme, lectins, flavoenzymes and immunoglobulin. It was reported that the epithelial tissues produce antimicrobial molecules which serve as the first line of a host's defense against microbial invasion in a variety of vertebrates (Villarroel et al. 2007).

In this study, the epidermal mucus of Tilapia obtained from fishpond has higher antimicrobial effect compared to epidermal mucus of Tilapia from fish tank. The two environmental conditions have differences in their environmental parameters (e.g., water temperature, dissolved oxygen concentration, pH) suggesting that there is a variation in type and level of innate immune factors in mucus between species inhabiting different ecological niches (Jensen 2015). Ponds have a wide variety of microbial life. Nutrients are brought to the pond by streams that feed into, run off during rain, or by the human anthropogenic activities (Ehiagbonare and Ogunrinde 2010). The water in soil, animal waste and decaying plant matter in the pond are broken down and used to fuel the pond ecosystem. Many animals that live in the surrounding area, migrating birds and nearby plants depends on these ponds for a rich source of nutrient and water (Ehiagbonare and Ogunrinde 2010). The presence of the following bacterial genera *Aeromonas, Klebsiella, Micrococcus, Alcaligenes, Vibrio, Flavobacterium*, *Bacillus, Pseudomonas* and *Coryneforms* and fungal species *Mucor, Aspergillus, Microsporum, Trichophyton* and *Chrysosporum* were more prevalent in pond water (Okaeme and Olufemi 1997). Some of these microbes have been implicated as the major causative organisms of known diseases of fish (Nahiduzzaman et al. 2000; Sarkar and Rashid 2012) and could also trigger the fish pond fishes to elicit protective mechanisms such as antimicrobial compounds in mucus which is more potent. On the other hand, using fish tanks allows the fish culturist to manage stocks and have a good deal of control over environmental parameters that can be adjusted to promote maximum production (DeLong et al. 2009). Since Tilapia has limited access to natural foods in tanks, they must

be fed a complete diet containing vitamins and minerals (Rakocy and McGinty 1989), though in this study, tilapia were only fed with floating pellets. In small tanks, it is practical and economical to treat diseases with therapeutants applied to the culture water (DeLong et al. 2009) suggesting that Tilapia may have depended their defense mechanism on this. Unlike on fishpond, fish are fed with rice bran, flour, peanut cake or pellet (Sarker et al. 2011).

Diets with functional ingredients are becoming a part of the preventive health strategy in fish farms (Bricknell and Dalmo 2005; Covello 2012). The strengthening of the skin and mucus layer through dietary modulation could play a role in preventing damage, parasite attachment, promote faster recovery of damaged skin (Jensen 2015) and could be source of nutrients and substrate for growth by certain bacteria (Shoemaker and LaFrentz 2015). In addition, the composition and rate of mucus secretion has been observed to change in response to microbial exposure or to environmental perturbation such as hyperosmolarity and acidity (Ellis 1999). The structure of fish skin is highly adapted to the aqueous environment (Jensen 2015). There is also evidence that the mucus composition varies with season, smoltification, salinity, stress, disease, parasite attack (Schrock et al. 2001; Mustafa et al. 2005; Roberts and Powell 2005; Easy and Ross 2009, 2010; Lü et al. 2012; Guardiola et al. 2014) and environmental conditions (Blackstock and Pickering 1982). Living in a pathogen-rich environment makes fish vulnerable to infections, and therefore reliant on a potent first defense line (Jensen 2015).

Differences in activities of antimicrobial enzymes, such as lysozyme and proteases, and how they relate to the structure and composition of mucus and epidermal layers, may also relate to the differences observed in disease resistance (Mozumder 2005) which may explain the observation why the epidermal mucus of Tilapia from fishpond smelled more fishy compared to the epidermal mucus of Tilapia from fish tank. The mucus holds some proteases (serine protease, cysteineprotease, metalloprotease and trypsin like protease) having strong antibacterial activity (Fast et al. 2002) such as in Tilapia (Sriket 2014).

Rao et. al (2015) compared the bactericidal activity of Tilapia to other fishes and it was found out that Tilapia and bagrid catfish have showed a broad spectrum of bactericidal activity against *E. coli*. The acidic mucus extract of tilapia and bagrid catfish were found to inhibit most of the human pathogens such as *E. coli*, *Streptococcus entericaserovar typhimirium*, *S. entericaserovar enteritidis*, *Klebsiella pneumoniae, P. aeruginosa, Methicillin-resistant S. aureus* (MRSA), *Micrococcus luteus, B. subtilis* and *Aeromonas hydrophila*. Similarly, the acidic mucus extracts of brook trout, haddock and hagfish showed bactericidal activity against a wide range of fish and human pathogens (Subramanian et al. 2008). This suggests that antimicrobial components in the acidic mucus extracts may have a key role in

host defense against pathogenic infection in the aqueous environment. Previous studies have shown a variety of antimicrobial proteins such as (paradaxinand pleurocidin) from fish mucus that is potentially involved in the protective function against invading pathogens (Cole et al. 1997).

On the other hand, this study found that the epidermal mucus of Tilapia from fishpond showed antimicrobial effects against most of test microorganisms except when tested against *P. aeruginosa* and *A. niger* for both exhibit cytotoxicity (Rivera et al. 2014) while epidermal mucus of Tilapia from fish tank showed only antimicrobial property when tested against *S. aureus*, *B. cereus, B. subtilis,* and *C. albicans.* The epidermal mucus of Tilapia from fishpond showed the higher antimicrobial property against *S. aureus*, *B. cereus, B. subtilis*, *C. albicans, E. coli, B. megaterium* and *A. flavus.* Gram-negative bacteria have an effective permeability barrier, comprised of a thin lipopolysaccharide exterior membrane, which could restrict the penetration of the epidermal mucus while Gram positive bacteria have a mesh-like peptidoglycan layer which is more accessible to permeation of epidermal mucus (Zgurska et al. 2015).

The present work supports the view that fish mucus could be a source of antimicrobial agent for animal pathogens. Further purification of the bioactive compounds is necessary in order to identify their chemical nature and to evaluate their potential as novel drug. Similar study can be done using the same or different species including other environmental conditions such as natural lakes.

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First record of *Thalassina kelanang* (Crustacea: Decapoda: Thalassinidae) from the Philippines

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ABSTRACT

The first record of mud lobster *Thalassina kelanang* (Moh and Chong, 2009) from the Philippines was documented in the mangrove forest of Suyac Island, Sagay City, Province of Negros Occidental. The male specimen with a total length of 16.5 cm and 4.5 cm carapace width was found surfacing the mangrove substrate in January 2019. Further survey yielded two more specimens. The specimens had an obtuse rostrum, and a deep median sulcus, which extends posteriorly, nearly the same length but behind the adrostral carinae. The slenderly elongate petasma bears conspicuous spines in the proximal lateral margin. The mounds of *T. kelanang* with heights ranging from 5 to 15 cm were smaller compared to those of *Thalassina anomala*. This is the fourth *Thalassina* species reported from the Philippines.

Keywords: mangrove forest, mud lobster, mounds, *Thalassina kelanang*, Suyac Island, Sagay City, Negros Occidental

INTRODUCTION

There is limited information on the diversity of mud lobsters *Thalassina* (Latreille, 1806) in the Philippines. At present, only three species were reported: these are *Thalassina anomala* (Herbst, 1804), *Thalassina squamifera* (De Man, 1915) (Ngoc-Ho and de Saint Laurent 2009), and the recently discovered *Thalassina spinosa* (Ngoc-Ho and de Saint Laurent, 2009) in Panay Island (Bedi and Primavera 2018). These three are among the 10 extant species under the genus *Thalassina*: *T. anomala*; *T. gracilis* (Dana, 1852); *T. squamifera*; *T. spinose*; *T. spinirostris* and *T. krempfi* (Ngoc-Ho and de Saint Laurent, 2009); *T. kelanang* (Moh and Chong, 2009); *T. australiensis* and *T. saetichelis* (Sakai and Türkay, 2012); and *T. pratas* (Lin et al. 2016) (Sakai and Türkay 2012; Lin et al. 2016).

Mud lobsters known in Western Visayas as “uson” (Hiligaynon) are well adapted for burrowing in muddy and sandy habitats and can live in

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anaerobic environments. They, therefore play a critical role in nutrient cycling (Malley 1977; Ng and Kang 1988). They usually inhabit mangrove forests and are known for their mound-building characteristics. However, recent discovery of the 10th species, *T. pratas* found in seagrass beds made an unusual pattern (Lin et al. 2016) from *Thalassina*'s previously known mangrove habitat. Nevertheless, deep burrows and cryptic behaviors of mud lobsters may have resulted for the species to be least studied compared to other crustaceans (Moh et al. 2015). A specimen of *T. kelanang* was found in Suyac Island, Sagay City, Negros Occidental. This is the fourth *Thalassina* species inhabiting the Philippine Islands.

METHODS

Mud lobsters examined in this study were collected from Suyac Island, Barangay Taba-ao, Sagay City, Province of Negros Occidental in January 2019. Suyac Island is almost 3 km from Old Sagay Port; accessible by a 15-minute boat ride. The roughly 2 ha island is surrounded by about 15 ha mangrove forest. The mangrove forest is composed mostly of matured (possibly century old) assemblages of *Sonneratia alba, Avicennia marina*, and *Rhizophora* spp. The island has four mangrove zones: *S. alba*, *Rhizophora* spp., *Aegiceras floridum*, and a mixed *S. alba–A. marina–Rhizophora* spp. zone. The island is sandy nearshore, and sandy-loam inward the mangrove forest.

One male *Thalassina* was found surfacing on the mangrove substrate during the mangrove rehabilitation and conservation planning with the community. The species is hardly known in the area, thus, the specimen was caught and brought to the Northern Negros State College of Science and Technology, Fisheries Research Center for identification. The organism appeared to have recently molted because of its slightly soft exoskeleton. The specimen was preserved in 10% buffered formalin and labeled as *Thalassina* sp., Suyac Island, 25 January 2019.

The species was morphologically identified as *T. kelanang*, a new record from the Philippines, therefore, another sampling was undertaken. The second sampling was done only for one day through hand picking during the low tide. *Thalassina* was observed to surface before the water fully subsided, thus, was easily captured. A 100-meter transect line was laid seaward, perpendicular to the Island; start of transect 10°95'03.91"N, 123°45'48.02"E, end of transect 10°95'04.51"N, 123°45'39.70"E. Five meters on both sides of the transect line was then scanned for surfacing mud lobsters. Two additional male specimens were caught during the sampling. One mound was excavated to acquire an additional specimen but the hole was vertically directed downwards, reaching more than a meter. Since the area is densely covered with century old mangroves, the root system has made the digging

challenging. The excavation was stopped at 1 m deep so as not to cause further disturbance on the mangrove trees. It would be convenient to catch the species with the use bamboo traps (Bedi and Primavera 2018), however, since the species is not yet exploited in the Island, we defer the use of the device to avoid potential overharvesting. Each specimen was measured for total length (TL) and carapace width (CW), preserved for further examination and kept at the Northern Negros State College of Science and Technology, Sagay City, Philippines.

RESULTS

Measurements

The three male mud lobsters measured: 16.5 cm TL, 4.5 cm CW; 17.5 cm TL, 4.5 cm CW; 11.0 cm TL, 2.4 cm CW.

Habitat

The mounds ranging from 5-15 cm in heights scattered all over the sandy mangrove forest of Suyac Island. These mounds were more than a meter deep but the exact depth was not determined to avoid disturbance on mangrove roots.

Species Description

Carapace oval-shaped, bearing a visible and deep gastric region; connected to the linea thalassinica (Figure 1A). Linea thalassinica smooth, without spines on the dorsal aspect. Branchiostegal spines short, sharp, rigid; pointing anteriorly. Dorsal median margin of the carapace protrudes like a spine but does not reach the first abdominal tergite. Left cheliped larger than the right in all male specimens. Both dactyls double the length of the fixed finger, lined with small blunt spines. Adrostral carinae more than half the length of gastro-orbital carinae. Median sulcus deep, runs through the rostrum, and extends posteriorly, nearly the same length but behind the adrostral carinae (Figure 1B). First abdominal somite narrow, small, and with distinct inverted Y-shaped groove on the dorsal tergite (Figure 1C). The base of the second abdominal somite with visible sternal ridge having 3-6 teeth (Figure 1D). Petasma, the male copulatory organ is slender with pointed tip, half of the distomesial lobe bears hooklets in the proximal area and distally triangular. The proximal lateral margin of the petasma bears three conspicuous spines (Figure 1E). These morphological characteristics are consistent with the descriptions of *T. kelanang* (see Moh and Chong 2009; Sakai and Türkay 2012; Lin et al. 2016).

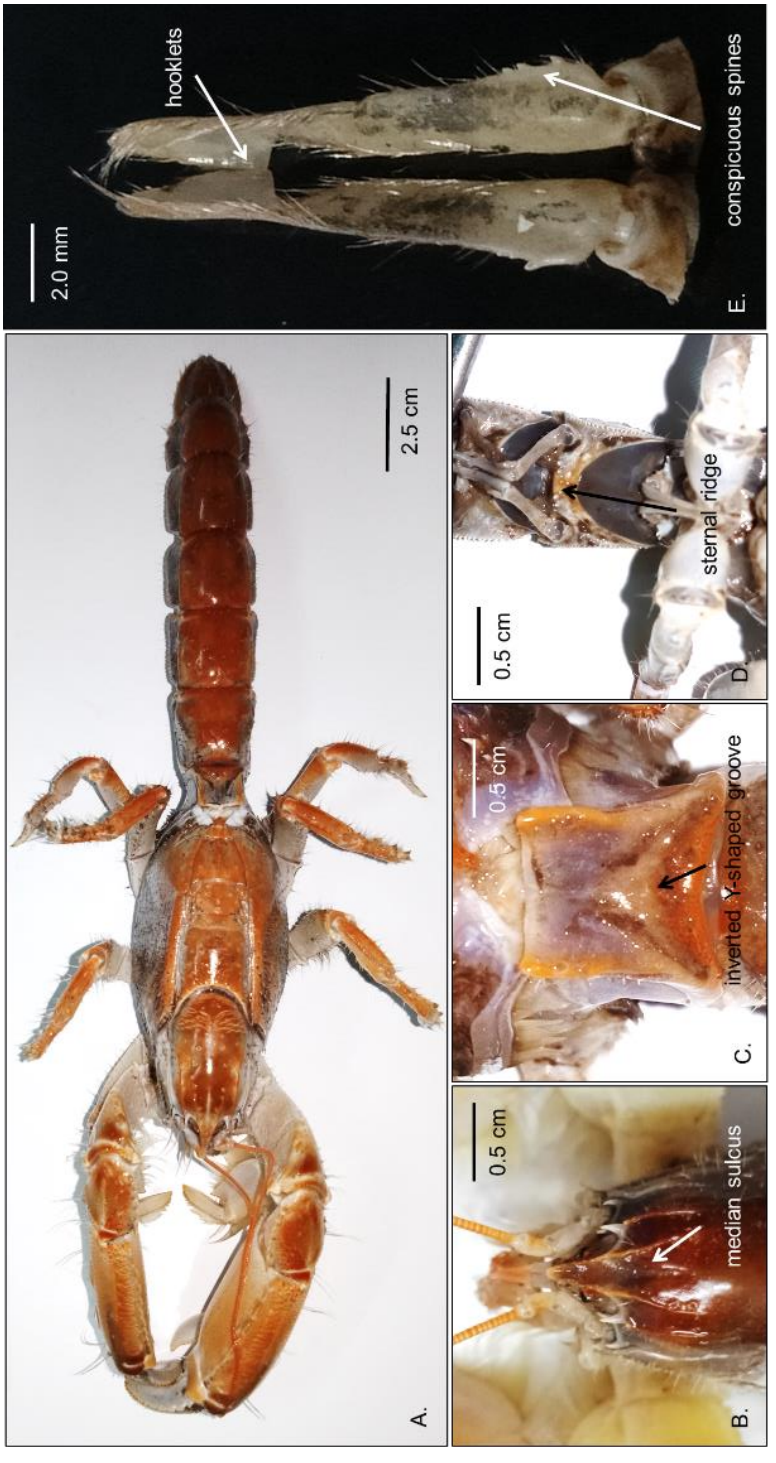

Figure 1. Dorsal view of male *Thalassina kelanang* collected from Suyac Island, Sagay City (A), anterior region of cephalothorax showing the position of rostral carinae (B), dorsal view of the first abdominal somite (C), ventral view of the second abdominal somite showing sternal ridge having 3-6 teeth (D), ventral view of the petasma (E).

DISCUSSION

The mud lobsters from Suyac Island were identified as *T. kelanang* as they fit the descriptions of Moh and Chong (2009), Sakai and Türkay (2012) and Lin et al. (2016). The species differ from *T. anomala*, a common species in the Philippines and mainland Negros Occidental, in many distinctive features such as rostrum, chela, carapace and somites. The rostrum of *T. anomala* is pointed; both chelae are smaller but with similar sizes; and its carapace has a dorsal median process which protrudes like a spine, reaching the first abdominal tergite. In *T. kelanang*, the rostrum is obtuse; chela is larger on one side; and the dorsal median process does not reach the first abdominal tergite. The first abdominal tergite of *T. kelanang* has inverted Y-shape groove rather than V-shape in *T. anomala* (Moh and Chong 2009). Although *T. kelanang* appears to be more similar to *T. squamifera* (Moh and Chong 2009), there are two major features that differentiate the species. These features are the rostrum and the petasma (in males) which are both agreed by Moh and Chong (2009) and Lin et al. (2016). On the contrary, Sakai and Türkay (2012) noted that the key difference can only be observed in the petasma. The latter have shown in their illustrations that median sulcus of *T. squamifera* extends behind adrostral carinae, similar to *T. kelanang*; which is contrary to that described by the two above-stated authors that median sulcus of *T. squamifera* does not extend behind adrostral carinae. Therefore, it is important to give more attention on the petasma when examining between these two species. The petasma of *T. kelanang* is slender while it is broad in *T. squamifera*; both species have hooklets in the proximal half of the distomesial lobe, but the lobes are distally triangular and distally rounded, respectively (Moh and Chong 2009; Sakai and Türkay 2012; Lin et al. 2016). Moh and Chong (2009) and Lin et al. (2016) also agreed that *T. kelanang* has 3-4 conspicuous spines at the proximal lateral margin of the petasma, but none in *T. squamifera*. While this characteristic was not mentioned by Sakai and Türkay (2012), their illustration of petasma (Figure 11 on Sakai and Türkay 2012) of *T. squamifera* does not exhibit any conspicuous spines proximally. This paper however considered all of those features that differentiated *T. kelanang* from *T. anomala,* a widely distributed species in the Philippines, and from *T. squamifera*, a closely similar species. The three specimens of *T. kelanang* was identified based on their deep median sulcus that extend behind the adrostral carinae, and slender petasma. The distomesial lobe of the petasma has hooklets in the proximal area and triangularly-shaped distally. Three conspicuous spines are also present at the proximal lateral margin making it easily distinguishable from *T. squamifera*.

The mounds of *T. kelanang* in Suyac Island were smaller compared to those of *T. anomala* that can reach more than a meter in height (Malley 1977). The mounds were rather similar to those of *T. kelanang* from Malaysia (Moh and Chong 2009; Moh et al. 2015) for having heights ranging between 5 and

15 cm, and basal diameter of about 21-38 cm. *Thalassina* spp. are nocturnal diggers or become active at twilight, with most of their mounds closed during the day (Dubey et al. 2012; Nur-Nadiah et al. 2019). However, *T. kelanang* collected from Suyac Island was found actively burrowing its mound around 14:00 in the afternoon. This may suggest a different burrowing behavior among Thalassinidae. *Thalassina* also rebuild their disturbed mounds but particular time is not confirmed (Hossain et al. 2019).

There are possible factors which affect the common distribution of *T. kelanang* in sandy and *T. anomala* in muddy substrates which may provide evidence of their adaptive mechanisms in these environments. Nevertheless, the discovery of this species in the Philippines corroborates the statement of Moh et al. (2015) suggesting that the species is widely distributed in the Southeast Asian Region. Although *T. kelanang* was never observed in the mainland of Negros Occidental, it was previously reported to cohabit with *T. anomala* (Moh and Chong 2009) in Malaysia which may suggest the same in the Philippines. Observations may be conducted on the occurrence of *T. kelanang* in Negros Island to determine its ecosystem connectivity (e.g. egg dispersal among group of islands). These observations may provide insights on the mangrove ecosystem connectivity between the islands since mud lobsters are often found below ground. A thorough assessment might be done to unveil the rich diversity of *Thalassina* in the Philippines.

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Note on the availability of Philippine Forest Turtle *Siebenrockiella leytensis* in online reptile markets

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ABSTRACT

The Philippine Forest Turtle *Siebenrockiella leytensis* is a critically endangered species endemic to the Palawan group of islands, where it is better known under the English name of Palawan Forest Turtle. No permits have been issued by the Philippine authorities to collect and/or trade in native live wild-caught reptiles since 2001, yet, the trade of captive-bred individuals is allowed. Regardless, evidence of this species breeding in captivity is very scarce and limited to two documented events. Although strictly protected, illegal trade seems to continue with sightings of this species in China, USA, and Europe. Here we report the results of a six-month survey in nine countries/regions carried out to estimate the availability of this species in the online pet trade. We monitored over 60 social media platforms (e.g. Facebook groups, MeWe) and reptile classified websites in Europe, United States of America, Malaysia, Japan, China, Taiwan, South Korea, Hong Kong SAR and the Philippines between 1 February 2019 and 31 July 2019. Only one advertisement offering two Philippine Forest Turtles was observed during the study period. On 30 June 2019, two adult Philippine Forest Turtles were offered for sale in a Japanese pet store. In addition, an advertisement was found outside the study period in Hong Kong, suggesting that there is still international demand for this critically endangered species.

Keywords: Philippine Forest Turtle, Palawan Forest Turtle, *Siebenrockiella leytensis,* Illegal Wildlife Trade, Social Media

INTRODUCTION

The Philippine Forest Turtle *Siebenrockiella leytensis*, endemic to the Palawan group of islands, is among the rarest of the world's chelonians. It is currently classified as Critically Endangered on the IUCN Red List of Threatened Species (Asian Turtle Trade Working Group 2000) and is listed as one of the world's 25 most endangered turtle species (Stanford 2018). With only four recorded specimens between 1921 and 1988 (both in the wild and in trade), very little was known about the Philippine Forest Turtle until the species' rediscovery in 2001 (Diesmos et al. 2004). The species is also listed as

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"critically endangered" under the Philippine national law (Republic Act No. 9147), making possession and trade of this species illegal and punishable by a maximum imprisonment of four years and/or a fine of PHP 300,000 (USD 5,760). The Philippine Forest Turtle is currently listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which means an export permit is required if one is to conduct any international trade in the species. No permits have been issued by Philippine authorities for native wild-caught reptiles, since 2001. However, illegal trade seems to continue with sightings of this species in China, USA, and Europe (Sy 2018; Sy et al. in prep.). Here we report the results of a six-month survey in nine countries/regions carried out to estimate the availability of this species in the online pet trade.

METHODS

We monitored social media platforms (e.g. Facebook, MeWe) and reptile classified websites in Europe, United States of America, Malaysia, Japan, China, Taiwan, South Korea, Hong Kong SAR, and the Philippines between 1 February 2019 and 31 July 2019. Exact sale platforms and keywords used are kept confidentially to prevent displacing trade to other potentially unknown platforms. The total number of platforms monitored encompassed over 60 known reptile classified platforms. For each advertisement, we collected 1) country where offered, 2) online platform, 3) price of animal (converted to USD), 4) origin, and 5) size of the animal. We monitored each platform twice a week and used keyword searches to look for our target species such as known code words or trade names, scientific names, and the name of this species in Mandarin and Japanese. We used the exchange rate of USD 1 = PHP 52.086 and USD 1 = JPY 107.74 (http://www.oanda.com as of 12 September 2019).

RESULTS

Only one advertisement, offering two Philippine Forest Turtles was observed during the study period. On 30 June 2019, two adult Philippine Forest Turtles were offered for sale in a Japanese pet store specializing in reptiles at JPY 400,000 (approximately USD 3,713), with the male having slight injuries. The same pair was offered for sale on a different website for JPY 500,000 (USD 4,641). No information was provided on the origin of these turtles.

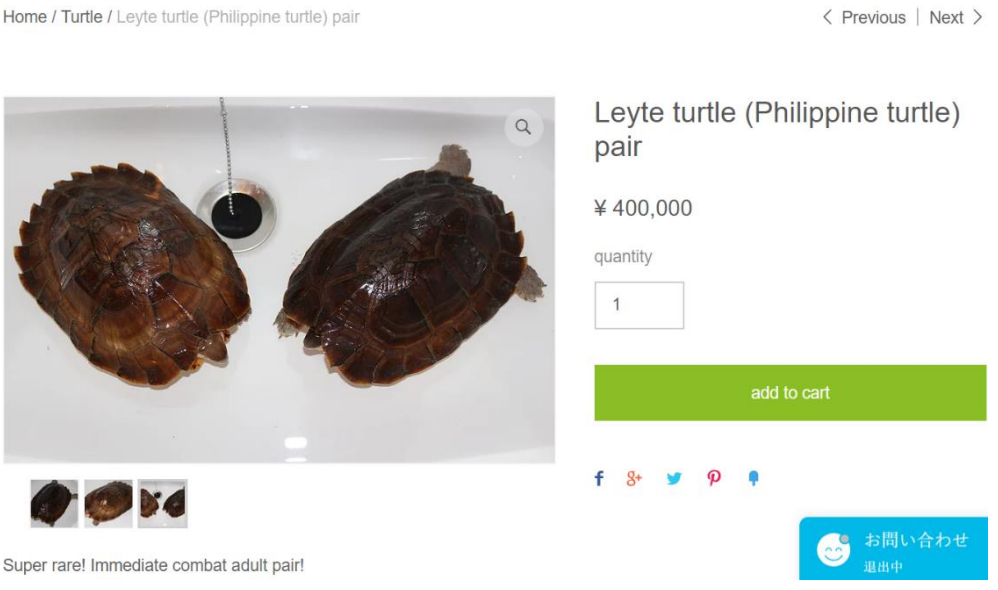

Figure 1. Advertisement of a pair of Philippine Forest Turtles observed for sale on 30 July 2019 in Japan. Advertisement translated using Google Translate.

DISCUSSION

Only two Philippine Forest Turtles were observed for sale over a six-month period, during a survey covering the most important reptile markets globally. Considering their rarity, this is not a surprise. The fact that both animals were adults, with one having eye damage, suggests that these two turtles were most likely wild-caught. Nevertheless, it should be said that eye damage is not exclusive to wild-caught specimens. The high price asked for this species raises concerns that the trade is very lucrative and is clearly a driver of the continual poaching of this species. Rare and endemic species often fetch high prices, making this trade highly profitable for smugglers and illegal traders.

For Critically Endangered species, even the smallest numbers can have an impact on the wild population. Previous studies have estimated densities of 4.4 to 121.7 individuals per hectare (Schoppe et al. 2010). In the wild this species faces challenges of habitat destruction, exploitation for the illegal pet trade, and (national and international) human consumption. The rediscovery has catalysed poaching efforts, culminating in the species' largest recorded seizure to date in Palawan in June 2015. This seizure involved 3,921 individuals, supposedly representing a large proportion of the species'

estimated population size, which is thought to be highly restricted (Fidenci and Maran 2009). No permits have been issued by Philippine authorities to collect and/or trade in live native wild-caught reptiles since 2001, yet, trade in captive-bred individuals is allowed. Evidence of this species breeding in captivity is very scarce and limited to two documented events (Katala Foundation Incorporated 2018; Formoso 2019). Furthermore, evidences of laundering (fraudulently declaring wild-caught animals as bred in captivity) involving this species by zoos and wildlife breeding farms have been reported (Diesmos et al. 2012; Sy 2018). This species was documented for sale in Japan, Malaysia, Europe, and United States prior to the first legal export of supposedly captive bred individuals from the Philippines in 2011 (Diesmos et al. 2012; Schoppe and Shepherd 2013; Sy 2018). It cannot be denied that these animals have been within the country for a longer time, as this species has been observed for sale in Japan in the past. Despite the fact that only two animals were observed during this study, it provides evidence that this species is still available outside the Philippines and trade appears to continue. Although outside our study period, an additional specimen was observed for sale in Hong Kong on 24th October 2019. In addition, Sung and Fong (2018) found 10 specimens in seven advertisements between September 2013 and August 2016 in Hong Kong as well, but no price data were available for these advertisements.

The online sale of wildlife has received greater attention over the last few years, with several authors reporting high quantities of often protected species available on social media like Facebook and Instagram (Krishnasamy and Stoner 2016; Sy 2018). Recently, Facebook has started to increase efforts in removing platforms that allow the sale of wildlife, resulting in displacement of these advertisements to other platforms such as WeChat, WhatsApp, Telegram, and other classified websites which are increasingly difficult to monitor. It could therefore be possible that the sale of these Critically Endangered turtles is taking place on different platforms other than those included in this study. The removal of sale platforms by Facebook also influenced platforms monitored for this study and it is more likely that that trade levels are higher than observed under this study. In addition, trade might have moved to closed or secret groups, which can only be entered on invitation or approval of an administrator.

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Heavy oil degrading *Burkholderia* and *Pseudomonas* strains: insights on the degradation potential of isolates and microbial consortia

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ABSTRACT

Bacterial strains were isolated from heavy oil degrading microbial consortia enriched from mangrove sediment. Among the 60 distinct isolates that formed colonies on heavy oil as a sole carbon and energy source, *Pseudomonas* sp. T2B and *Burkholderia* sp. T2C showed the best growth in heavy oil and in various aliphatic and aromatic hydrocarbons. The two isolates were tested in their abilities to degrade heavy oil. At 1% oil concentration, T2B and T2C degraded 19.6% and 16.7% of heavy oil within 21 days, respectively. These values were significantly lower than that of the source consortium T2, which degraded 24.2% of the oil. This indicates that the consortium is more superior in degrading heavy oil than any of the isolates. The isolates can be used for biodegradation studies and can be utilized in producing an effective microbial consortium for bioremediation applications.

Keywords: *Pseudomonas, Burkholderia*, heavy oil, microbial consortium, hydrocarbons, biodegradation

INTRODUCTION

The increasing industrial production leads to significant damage to the environment by numerous contaminants. Due to human activities, organic pollutants that affect the quality of the environment and threaten public health are spilled often as a contaminant of soil, groundwater, and marine environments (Kaufmann et al. 2004; Murphy et al. 2016; Bacosa et al. 2017). Crude oil and petroleum hydrocarbons are among these contaminants. It is estimated that worldwide, about 800 million liters of petroleum enter the marine environment each year from the extraction, transportation, and consumption of crude oil and products from it (National Research Council USA 2003). An additional 700 million gallons is contributed by natural seepage. Past analysis of reported oil spills indicated that most of the oil comes from tankers, barges and other vessels as well from coastal oil refineries and land pipeline spills. Major oil spills in history such as the Exxon Valdez (1989), the Nakhodka oil spill (1997), the Erica spill (1999), the Prestige spill (2002)

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and Deepwater Horizon (2010), had brought extensive changes in marine ecosystem (Bacosa et al. 2015a; Gemmell et al. 2016; Murphy et al. 2016; Liu et al. 2017).

Heavy oil is one of the major products from crude oil and is defined by the U.S. Department of Energy as having API (American Petroleum Institute) gravities that fall between 10.0° and 22.3°. Like the conventional petroleum oils, it is a complex mixture of hydrocarbons and other compounds. Petroleum constituents represent saturates or aliphatic, aromatics, resins and asphaltenes (Bacosa et al. 2010; Evans et al. 2016, 2018). Heavy oil is deficient in simple alkanes and aromatics but enriched in long chain alkanes, high molecular weight aromatic hydrocarbons and polar components relative to conventional crude oil (Jack 1998; Bacosa et al. 2013). Polycyclic aromatic hydrocarbons (PAHs) are among the aromatic components of heavy oils that pose a serious threat to the environment (Bacosa et al. 2010). The United States Environmental Protection Agency named 16 PAHs in its priority list according to their toxicity and threat to the ecosystem. PAHs substances are known for their persistence, toxicity, carcinogenicity and mutagenicity (Bacosa and Inoue 2015). PAH-rich heavy oil is considered as resistant to biodegradation, recalcitrant and persistent in the environment. Thus, it is imperative to remove heavy oil from polluted environment to minimize its adverse impacts.

Oil spill is deleterious to a wide array of marine plants, animals and microbial communities through oxygen stress and direct toxic effects (Severin et al. 2016; Gemmell et al. 2017; Williams et al. 2017). In tropical and subtropical regions, the ultimate recipient of marine oil spills is the mangrove ecosystems which is one of the important components of the coastal environment. Oil spills in mangrove areas impart potential damage to their physical and ecological integrity. Removal of spilled oil in the environment can be done in various ways. It could be achieved by either physicochemical or biological methods (Bacosa et al. 2015b, 2016). However, the negative consequences as a result of implementing physicochemical approaches are currently directing greater attention to explore for more sustainable and environmentally-amiable biological alternatives. Among these popular options is bioremediation using a community of bacteria or isolates (Okoh 2006). Mangroves are sensitive ecosystem, which implies that less intrusive, biodegradation-based remedial alternatives are the suitable option since they present minimum harm to these ecosystems. These methods are less expensive and do not introduce additional chemicals to the environment. Compared to physiochemical methods, bioremediation offers a very feasible alternative for an oil spill response.

In an attempt to characterize the community composition and evaluate the oil and hydrocarbon-degrading abilities of bacteria in mangrove

areas in Okinawa, Japan, six bacterial consortia were obtained by enrichment method and reported in our previous study (Bacosa et al. 2013). These enriched bacterial consortia showed different abilities to degrade heavy oil. Although bacteria in nature behave as a community similar to an enriched consortium, studies on the abilities of each strain to grow or degrade specific hydrocarbon compound could provide insights on the importance of the strains in bioremediation applications. This study aims to isolate bacteria from the enriched consortia, investigate the abilities of the isolates to grow in pure hydrocarbon substrates, and determine the abilities of select strains to degrade heavy oil in comparison with the source consortium.

METHODS

Bacterial Isolation

The bacteria were isolated from the microbial consortia capable of degrading heavy oil as a sole carbon and energy source. These six consortia namely, T1, T2, T3, O, K1 and K2, were enriched from sediment samples that were collected from the mangroves of Okinawa, Japan (Bacosa et al. 2013). To obtain heavy oil-degrading isolates, a serial dilution of the bacterial suspension from each enrichment culture was plated in Petri plates containing mineral salt medium (MSM), 1.5% agarose, and 20 µl of heavy oil. The composition of the MSM is indicated in Bacosa et al. (2013). The plates were incubated for two weeks at 30°C. The colonies formed were preliminary screened using morphological characteristics as criteria which include, color, elevation, margin, and surface of the colony. A portion of the colony was inoculated into a test tube containing 10 ml MSM and 1% (v/v) of heavy oil. Subsequently, incubation was done for two weeks at 30°C with shaking.

Growth in Heavy Oil and Hydrocarbons

The isolates were then screened for growth in heavy oil and pure hydrocarbon compounds namely, decane (C10), hexadecane (C16), eiocosane (C20), octacosane (C28), pristine (C19 branched), phenanthrene (3 rings) and pyrene (4 rings). Liquid hydrocarbons were filter sterilized and solid hydrocarbons were prepared in a stock solution with dichloromethane (10 mg ml^{-1}). Each hydrocarbon was added in 30 ml test tube containing 10 ml of MSM medium and 1% (v/v) of heavy oil or 1000 mg l^{-1} of each hydrocarbon substrate. After allowing dichloromethane to evaporate, solid hydrocarbons were applied with 10 cycles of ultrasonic wave (UP 200s Untraschall-prozessor). The isolates were grown at 30°C, with shaking (120 rpm) for 14 days. Growth was determined periodically using spectrophotometer at an optical density of 600 nm (Chaerun et al. 2004). Inoculated cultures were compared to uninoculated control. Growth criteria were as follows: No growth

(0)–absorbance difference of below 0.100; Low growth (+)–absorbance difference of 0.100-0.499; Medium growth (++)–absorbance difference of 0.500-0.999; High growth (+++)–absorbance difference of 1.000 above. These methods and criteria were adapted from the work of Chaerun et al. (2004). Isolates which showed high growth in heavy oil and/or hexadecane and at least two of the pure hydrocarbon substrates were selected for sequencing

DNA Extraction

Total DNA was extracted from the six isolates to determine their identities. The bacterial cells were gathered by centrifugation (TOMY-High speed refrigerated microcentrifuge) at 5,000 rpm for 10 min. The cells were resuspended in 40 µl of sterile distilled water followed by freezing and thawing two times. Proteinase K (10 µl) and TTNE buffer (50 µl) were added and vortexed. Enzyme reaction was allowed to take place at 20 min for 60°C and enzyme de-excitation at 95°C for 10 min. To precipitate the bacterial cells, the suspension was centrifuged at 4°C for 5 min at 14,000 rpm. Supernatant containing the extracted DNA was then collected.

DNA amplification and purification

Nearly full-length 16S rRNA gene was amplified by polymerase chain reaction (PCR) using primers Eu 10F (5'- AGAGTTTGATCCTGGCTCAG-3'), corresponding to *Escherichia coli* positions 8-27 as a forward primer, and Eu 1500R (5'- GGTTACCTTGTTACGACTT -3'), corresponding to *Escherichia coli* positions 1492-1510 as a reverse primer (Takami et al. 1999). These primers can amplify the entire 16S rRNA gene (~1500 bp). PCR was performed in a 50 µl reaction containing Promega PCR Master Mix, $MgCl_2$, DMSO, primers, DNA template and nuclease free water using a 9700 Thermal Cycle (Applied Biosystems) (Bacosa et al. 2013). The PCR conditions were as follows: 9 min at 95°C and 30 cycles of denaturation (30 s at 95°C), annealing (30 s at 50°C), and extension (1 min at 72°C), followed by a 7-min extension at 72°C (Bacosa et al. 2010, 2011). The PCR amplification products were verified by 1.5% agarose gel electrophoresis and visualized under UV transilluminator.

PCR products were purified using Gene Elute Mammalian Total RNA Miniprep Kit (Sigma Aldrich) according to the manufacturer's protocol. Briefly, Wash solution 2 was diluted with 99% ethanol. The volume of the PCR product was then adjusted to 100 µl using elution solution. PCR product was added with Lysis solution and 2-Me mixture. After that, the solution with DNA was applied to the binding column and cleaned-up twice with Wash solution 2. Finally, the purified DNA was eluted from the column with 50 µl of elution solution

DNA Sequencing

Purified DNA was sequenced following the Big Dye Terminator v1.1/3.1 Cycle sequencing chemistry. Four universal primers (10F, 530F, 800R, and 1400R) were used to sequence the nearly full-length fragment of 16S rRNA gene (~1500 bp) (Kawasaki et al. 1992; Takami et al. 1999). The 20 µl sequencing reaction contains 4 µl BigDye R Terminator v3.1 Cycle Sequencing Kit, 2 µl V3.1 sequencing buffer, 0.5 µl Primer, 2 µl DNA template, and sterile deionized water (Bacosa et al. 2010, 2013; Bacosa and Inoue 2015). Prior to sequencing, the solution was purified by sodium dodecyl sulfate (SDS). To attain a concentration of 0.2%, 2 µl of 2.2% SDS was added to the samples, heated at 98°C for 5 min, and allowed to cool at room temperature. Samples were transferred to spin columns and centrifuged at 800 g for 2 min. The liquid that passed through the spin column was dried for about 30 min using an aspirator (KUBOTA CHRIST Alpha 1-2, TAITEC VC-96 N). Twenty microliters of Hi-Di Formamide was added to dried samples, heated for 2 min at 95°C and cooled for 5 min at room temperature. The samples were then transferred to a 96-well plate and sequenced in Applied Biosystems Genetic Analyzer 3130. DNA sequences were assembled and compared to the sequences in the GenBank.

Degradation Experiment and Oil Analysis

Isolates T2B and T2C were evaluated in the degradation of heavy oil following established procedure (Bacosa et al. 2013). Briefly, the standard inoculum was cultured for 1 week in 1% (v/v) heavy oil in MSM. The cells were harvested by centrifugation, rinsed three times with phosphate saline buffer and resuspended in MSM. An aliquot (500 µl) was added to 125-ml culture bottles containing 9.5 ml of MSM and heavy oil at a final concentration of 1% v/v. The cells were added to attain an initial concentration of ~5 x 10^5 cell ml^{-1}. In order to compare the degradation abilities of the bacterial isolates with the enriched consortia, T1, T2, T3, O, K1 and K2 consortia were prepared in the similar manner. Triplicate bottles were prepared for each isolate, consortium, and uninoculated controls. The bottles were sealed and placed in an orbital shaker and incubated for 21 days at 30°C with shaking (120 rpm).

The residual oil was extracted after 21 days of incubation. The cultures were acidified to pH 2-3 using hydrochloric acid before extracting twice with the same volume of carbon tetrachloride. The extract was diluted at acceptable range and analyzed by Horiba-Oil Content Analyzer (Model OCMA-350). Quantification was based on a five-point calibration curve. Biodegradation potential was expressed as biodegradation efficiency relative to the control (Bacosa et al. 2013).

Statistical Analysis

To determine the significant differences among the means of biodegradation efficiency, one-way analysis of variance (ANOVA) was performed using PAST software package V2.17 (Hammer et al. 2001). The differences between treatment means were further tested using Tukey's honestly significance difference (HSD).

RESULTS

Growth on Heavy Oil and Hydrocarbon Substrates

The screening of the isolates using colony morphology resulted in 60 distinct isolates. These isolates were investigated in their abilities to grow on heavy oil resulting in 25 good degraders. Moreover, these 25 strains were grown in various hydrocarbon compounds as sole carbon and energy sources. The results of the growth experiment are summarized in Table 1. Isolates T2B, T2C, T2E, T2F, and T2G grew well in heavy oil as a carbon and energy source. When investigated in the presence of hydrocarbon compounds, different patterns of degradation as indicated by bacterial growth were observed. Most of these strains did not grow in the presence of decane. Surprisingly, T2F which showed good growth in heavy oil was not able to grow in hexadecane. Only T2B and T2C were able to utilize both phenanthrene and pyrene for growth. It suggests that T2B and T2C isolates have capabilities to degrade both the aromatic and aliphatic components of heavy oil. T2B and T2C cultures in test tube in the presence of different hydrocarbon substrates are presented in Figures 1a and b. Their respective colonies that formed on plates with heavy oil are shown in Figures 2a and b.

Table 1 also shows that isolates with moderate or low growth in heavy oil have different capabilities to utilize a wide range of pure hydrocarbon substrates. Some of these strains utilized decane as a carbon and energy source. Heavy oil is abundant in long-alkane chains, not in short chain alkanes like decane. OC and K1D isolates demonstrated low growth in several hydrocarbon substrates, including heavy oil. It is worthy to note that most of the isolates, which showed favorable growth in heavy oil and pure hydrocarbon compounds, were isolated from T2 consortium.

Table 1. Growth characteristics of isolates in heavy oil and hydrocarbon compounds (1% v/v) cultured in MSM for 14 days. Legend: 0 – no growth: + - low growth: ++ - medium growth: +++ - high growth.

Isolates	Heavy oil	Decane	Hexadecane	Eiocosane	Octacosane	Pristane	Phenanthrene	Pyrene
T2B	+++	0	+++	++	++	0	+	+
T2C	+++	0	+++	++	0	+	+	+
T2E	+++	0	+++	+	++	0	0	0
T2F	+++	0	0	+	+	+	0	0
T2G	+++	+	+++	+	0	+	0	0
T1A	++	0	+++	++	+	+	0	0
T1B	+	+	+	+	+	0	+	0
T1C	++	++	++	++	0	++	0	0
T1F	++	+	++	+	+	+	0	0
T1G	+	+	++	0	0	+	0	0
T1H	++	0	++	++	+	++	0	0
T2A	++	+	++	++	+	++	0	0
T3A	++	0	+++	+	0	++	0	0
T3B	+	0	++	++	0	+	+	0
T3C	+	+	+	+	0	+	0	0
T3D	+	+	++	0	0	+	0	0
T3E	+	++	++	+	0	+	0	+
T3G	+	0	++	+	0	+	0	0
OC	+	+	+	0	+	+	0	+
OD	+	+	+	+	+	+	0	0
K1B	++	+	+	0	+	0	0	+
K1D	+	0	++	+	++	+	0	+
K1F	+	+	+	0	+	+	0	0
K2B	++	+	+	++	0	+	0	+
K2C	++	+	+	+	+	0	+	0
K2D	++	0	++	+	+	+	0	0

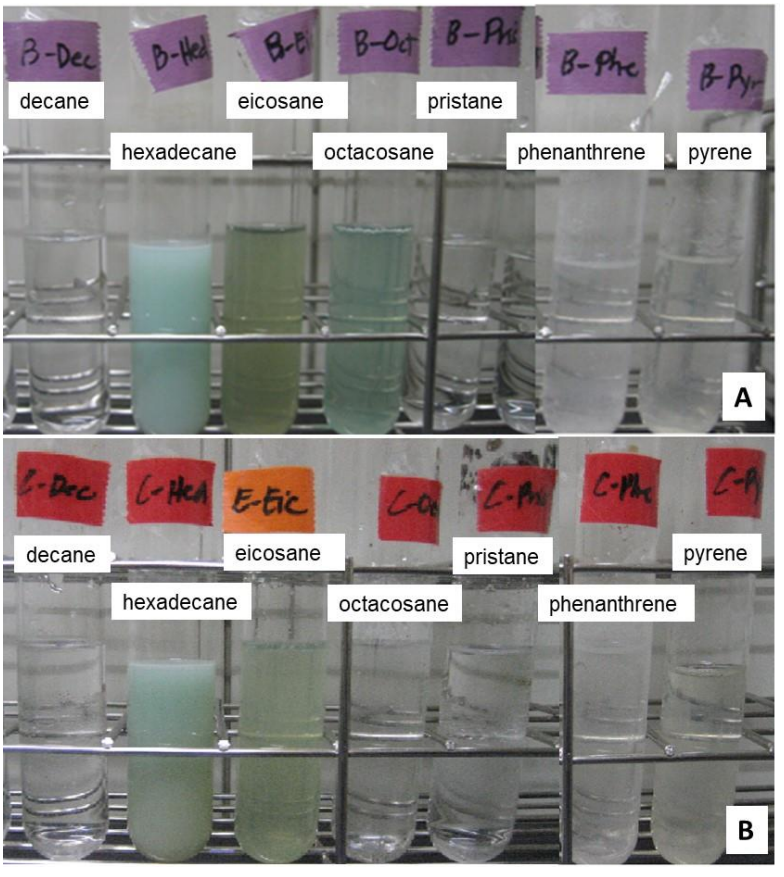

Figure 1. Growth of T2B (A) and T2C (B) isolates on different hydrocarbon substrates after 14 days of incubation. Increasing liquid turbidity indicates higher growth.

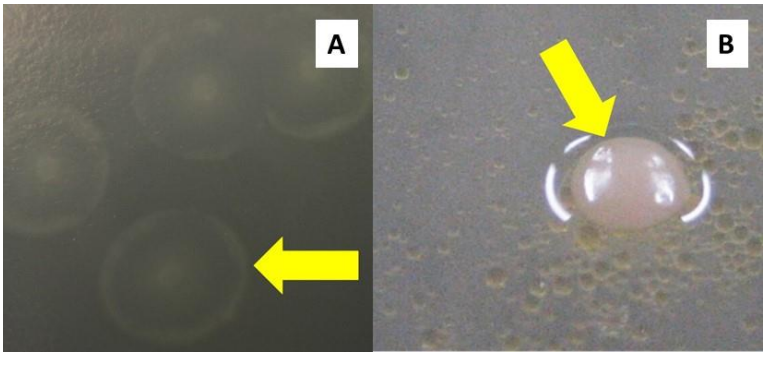

Figure 2. Colonies of T2B (A) and T2C (B) isolates on agar plate with mineral salt medium (MSM) and heavy oil.

Identification of the Isolates

Six isolates were selected as superior heavy oil degraders and were identified to species level based on 16S rRNA gene. The identities of these isolates are presented in Table 2. Sequencing results revealed that the bacterial isolates were closely related to *Pseudomonas aeruginosa* and *Burkholderia cepacia*. T2B, T2G, and T1A were related to *Pseudomonas aeruginosa*, while T2C, T2E, and T2F were affiliated to *Burkholderia cepacia*.

Table 2. Identities of the bacterial isolates based on 16S rRNA gene sequencing.

Strain	Closest relative sequence	Accession No.	Similarity
T2B	*Pseudomonas aeruginosa* K3	EF064786	1319/1323 (99.7%)
T2C	*Burkholderia cepacia* RRE5	AY946011	1322/1326 (99.7%)
T2E	*Burkholderia cepacia* WK	EF031062	1256/1257 (99.9%)
T2F	*Burkholderia cepacia* WK	EF031062	1346/1346 (100%)
T2G	*Pseudomonas aeruginosa* S25	DQ095913	1287/1287 (100%)
T1A	*Pseudomonas aeruginosa* S25	DQ095913	1285/1285 (100%)

Results showed that even though they belong to the same species, these strains formed distinct colonies, and have different capabilities to degrade petroleum hydrocarbons. It is interesting that T2F, which belonged to genus *Burkholderia* was not able to utilize hexadecane, a medium-chain hydrocarbon. As previously reported, although the enriched bacterial consortia were dominated by bacteria closely related to *Pseudomonas* and *Burkholderia,* they have different oil degradation abilities (Bacosa et al. 2013). The remaining isolates were not sequenced because they have low degradation abilities and were likely closely related to *Pseudomonas* or *Burkholderia*.

Heavy oil degradation by T2B and T2C isolates

Because T2B and T2C strains showed superior abilities to grow on heavy oil and utilized a wide range of aliphatic hydrocarbons and PAHs substrates, their potential to biodegrade heavy oil was further tested and compared to that of the bacterial consortia. After 21 days of incubation, *Pseudomonas* sp. T2B degraded 19.7% of the heavy oil, which was greater than *Burkholderia* sp. T2C (17.6%) (Figure 3). However, the heavy oil degradation abilities of both isolates were significantly lower than T2 consortium (24.2%, $p<0.05$). This shows that the consortium, which is a combination of both

strains and other bacteria that we were not able to isolate, was more effective in degrading hydrocarbons in heavy oil. The rest of the consortia degraded more heavy oil than any of the two isolates. K1, T1, O consortia utilized 31.6%, 30.2%, and 27.1% of the oil, respectively. T3 and K2 consumed about 23% of oil for growth within 21 days.

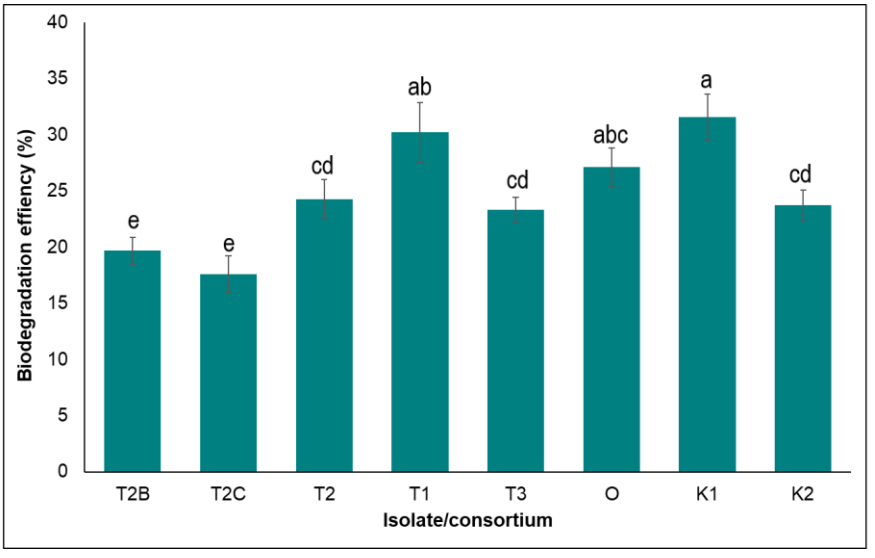

Figure 3. Heavy oil degradation efficiencies of T2B and T2C isolates compared to enriched bacterial consortia after 21 days of incubation. Error bars represent one standard deviation from the mean of three replicates. Different letters indicate significant difference based on Tukey's HSD test. P-value lesser than 0.05 was considered significant.

DISCUSSION

Pseudomonas sp. T2B and *Burkholderia* sp. T2C were successfully isolated from T2 consortium that degraded heavy oil. Both isolates were able to utilize various aliphatic and aromatic hydrocarbons as sole carbon and energy sources. The genus *Pseudomonas* is arguably the most diverse and ecologically significant bacteria which is ubiquitous in many environments (Spiers et al. 2000). They are known to have simple metabolic systems, encode genes for catabolic enzymes and harbor degradative plasmids, which make them capable of using several organic compounds including hydrocarbons for growth (Mukherjee et al. 2010; Salazar et al. 2012). *Burkholderia* is a genus with remarkable nutritional and physiological versatility. Many *Burkholderia* possess plasmid that contributes to its versatility and produce oxygenases-the enzymes that are essential for the initial oxidation of hydrocarbon chain and

aromatic ring (Okoh et al. 2001; Coenye and Vandamme 2003; Suto et al. 2007).

Pseudomonas and *Burkholderia* in bacterial consortia had been implicated in the degradation of hydrocarbons and petroleum products. *Pseudomonas* dominated the bacterial consortium from leaf soil that preferably degraded alkanes (Bacosa et al. 2011). However, *Burkholderia* was the key player in the faster disappearance of aromatic hydrocarbon over aliphatic hydrocarbons in kerosene degrading consortium (Bacosa et al. 2010, 2011, 2012). Aromatic hydrocarbons, particularly polycylic aromatic hydrocarbons (PAHs), are more toxic that aliphatic hydrocarbons and known as mutagenic and carcinogenic (Bacosa and Inoue 2015). Crude oil is a complex mixture of hundreds to a thousand of aliphatic and aromatic hydrocarbon. Aliphatic hydrocarbons are more abundant but less toxic, while and aromatic hydrocarbons are less abundance but more toxic and are mutagenic and carcinogenic (Bacosa et al. 2015a, 2016; Evans et al. 2016; Evans et al. 2018). Risk-based bioremediation takes into account the relative toxicity of complex pollutants like oil and its products. If aromatic hydrocarbons were being degraded faster, the risk level will be reduced significantly. This will eventually result in an efficient and cost-effective remediation of contaminated environments.

T2 consortium is primarily composed of *Pseudomonas* and *Burkholderia* as shown in its profile using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) (Bacosa et al. 2013). The current study revealed that both T2B and T2C did not surpass the degradation ability of T2 source consortium. Moreover, all bacterial consortia tested have greater degradation than both isolate suggesting that mixed bacterial consortia are more efficient in the degradation of heavy oil. Notably, T2 consortium has one of the lowest biodegradation efficiencies among the eight enriched consortia. This observation could be attributed to the competition of other bacteria in T2 consortium with T2B and T2C, or non-isolation of superior bacteria from other consortia. Biodegradation is a community and collective effort leading to greater results. Rahman et al. (2002) showed that individual bacterial cultures showed less growth and lower degradation of crude oil than the mixed bacterial consortium. The same pattern was also observed by Sathishkumar et al. (2008), in which a consortium composed of *Pseudomonas, Bacillus* and *Corynebacterium* degraded oil more effectively than any of the isolate. Another advantage of a bacterial consortium is its ability to metabolize completely the parent compound into carbon dioxide and water something that is not possible using chemical and physical remediation methods (Khehra et al. 2005; Bacosa et al. 2016). While a single strain can be simply incubated with a substrate, the relationship is quite complex in a microbial consortium. Some members of the consortium produce the oxygenases and dioxygenase to oxidize the hydrocarbon chain, while other

members produce the necessary micronutrients necessary for the main degraders (Dominguez et al. 2019). Other bacteria may produce biosurfactant to make the oil more soluble in water and biodegradable (Bacosa et al. 2015b, 2018a,b). Moreover, other bacteria consume the metabolic products from the degradation of parent compound. Some of the metabolites have been reported to be inhibitory and toxic to the degrader of parent compound (Kazunga and Aitken 2000). These toxicants have to be removed by other bacteria in the consortium to enhance the degradation of the parent compound. It is worth mentioning here that the isolates from the most effective consortia, T1 and K1, did not show remarkable growth in heavy oil and pure hydrocarbons substrates. The high degradation of these consortia was not due to superbacteria but can be attributed to the synergistic relationships of bacteria in the consortium.

Although the degradation potential was not as high as any of the consortia, isolating these strains from the mixed culture offers numerous advantages. The potential of the strains to degrade different hydrocarbons and their combinations, crude oil, and oil products can be ascertained. The full-length of the DNA sequence of these strains are already known, and the whole genome analysis to unravel the full potential of these strains can be carried out. Individual isolates can be also used in combination with other microorganisms to produce an effective and robust bacterial consortium for bioremediation applications.

Overall, *Pseudomonas* sp. T2B and *Burkholderia* sp. T2C were successfully isolated from T2 consortium. These strains were effective in the degradation of heavy oil and several aliphatic and aromatic hydrocarbons. Although their degradation was lower compared to the source consortia, these isolates could be useful in making a more effective synthetic consortium for the remediation of contaminated sites. The combination of these isolates with other consortium or strains towards applications in the degradation of petroleum hydrocarbons warrants further investigation.

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Growth and lipid levels of *Tetraselmis tetrahele* and *Nannochloropsis* sp. cultured under commercial fertilizers

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ABSTRACT

Microalgae are aquatic photosynthetic organisms that contain high amounts of lipid and are potential sources of biofuels as well as feed additives for aquaculture. This study analyzed the growth and algal lipid content of two microalgae species (*Tetraselmis tetrahele* and *Nannochloropsis* sp.) using commercial fertilizers and nutrient enrichment. The samples were cultured for 5 days in 1 L dextrose bottles fertilized using Tongkang Marine Research Laboratory (TMRL) enrichment media, inorganic fertilizers such as 14-14-14, and a combination of 14-14-14 and 21-0-0. The relative growth rate of two algae were measured by computing the *k* value while the lipid components were extracted using the Bligh and Dyer Method, and the lipid content of each sample was determined using the gravimetric method. The use of 14-14-14 fertilizer produced the highest growth rates (k=1.810) and lipid composition (14.789%) for *T. tetrahele*. By contrast, *Nannochloropsis* sp., grew well under TMRL enrichment media (k=9.708), and the use of 14-14-14 fertilizer resulted to high lipid content (5.000%).

Keywords: lipid content, microalgae, biofuel, nitrogen, aquaculture

INTRODUCTION

Microalgae are aquatic photosynthetic organisms with different sizes ranging between 1 μm and 2 mm (Baharuddin et al. 2016). Studies on microalgae gained global attention recently due to its numerous usage and application in the area of energy production, pharmaceuticals, food production, aquaculture, and waste recycling. Some studies published include the important role of microalgae in solubilizing high amount of CO_2 (Takagi et al. 2006), bioaccumulation of heavy metals (Naorbe and Serrano 2018), and feed ingredients in aquaculture (Seong et al. 2019).

Microalgal biomass is mostly composed of lipids, carbohydrates, and proteins (Choudhary et al. 2015). An increase in the lipid content of microalgae has an effect on the amount of protein and carbohydrates present. The quantities of microalgal lipids vary with the species, growth conditions, and the culture environment. Furthermore, the biomass of microalgae is correlated to the amount of lipid that could be extracted (Mata et al. 2010).

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The high amount of lipid in microalgae is one of the reasons why they are potential sources of biofuels (Singh and Gu 2010; Maizatul et al. 2017). Land-based crops are also considered as alternative sources but they are also used as primary sources of food and require large areas of land for production hence the preference on microalgae for biofuel production (Bajpai and Tyagi 2006; Maruyama et al. 2009; Maizatul et al. 2017).

Microalgae are more efficient converters of solar energy due to their simpler cellular structure compared to land-based crops (Mata et al. 2010; Griffiths et al. 2011). Other favorable characteristics include the relatively low cost associated with harvesting and transportation compared to those of other biomass materials such as trees and other crops. Also, by virtue of their relatively small sizes, microalgae can be chemically treated easily and can be grown under conditions which are deemed unsuitable for conventional crop production. These characteristics of microalgae enable the production of up to 30 times the amount of oil per unit area of land compared to land-based oilseed crops (Chisti 2007; Mata et al. 2010; Griffiths et al. 2011).

In the field of aquaculture, microalgae serve as food and feed additives during the early larval stages of shrimps, mollusk, echinoderms and other fishes, thus considered essential in aquaculture (Han et al. 2019).

The problems associated with the production of biofuels from algae and algal production for aquaculture in general is that it cannot compete in the global market due to high production cost, labor-intensive requirement, algal crashes, and contamination (Alabi et al. 2009; Leite et al. 2013). Algae-based biofuel and feed additives can be commercialized and be produced in a much larger scale with the development of a suitable cost-effective growth medium, low-energy intensive harvesting method, and an effective lipid extraction method.

Several studies on increasing the production of lipids in microalgae and suitable lipid extraction methods have been published in the past few years. The focus is on minimizing production cost through alternative culture systems such as ponds, raceways, and canals as well as using cheap and readily-available sources of nutrients such as wastewater (Nzayisenga et al. 2020). The findings suggest that environmental condition and nutrient starvation can alter the lipid content in microalgae (Zhu et al. 2016), and that there is a need to identify suitable algal strains to be used for the production of lipids (Venteris et al. 2014).

To contribute to the research for possible strains of microalgae with potential for mass production, this study analyzed the growth and lipid content of *T. tetrahele* and *Nannochloropsis* sp. when cultured using

commercial fertilizer in an indoor setup. This study gives information on growth and the amount of lipid present in the two microalgae species.

METHODS

Experimental Design

Two experiments were simultaneously conducted and each experiment was subjected to three treatments with three replications (Table 1). There were two variables in this study namely: independent - the two microalgae cultured using nutrient enrichment and commercial fertilizers; dependent - the growth rate and lipid content of the microalgae.

In these experiments, two microalgal species namely: *T. tetrahele* and *Nannochloropsis* sp. were selected. Both of these species are widely used in aquaculture and were reported to contain high amount of lipids which could potentially be used in the production of biodiesel. These algal species were sourced from pure starter cultures of the Phycology Laboratory of the College of Fisheries and Ocean Sciences (CFOS) at the University of the Philippines in the Visayas (UPV), Miagao, Iloilo. The experiment was conducted at the Wet Laboratory of the aforementioned college.

Table 1. Experimental design with estimated nitrogen-phosphorus-potassium (N-P-K %) and initial cell density. R – replications. *in the form of potassium nitrite and sodium ortho phosphate.

Treatments	R	Percent Nutrients			Experiment Initial Density (cells per ml)	
		N	*P*	*K*	Experiment 1 *Tetraselmis tetrahele*	Experiment 2 *Nannochloropsis* sp.
TMRL enrichment media	3	*	*	*	1,070,000	8,500,000
14-14-14	3	14	14	14	1,070,000	8,500,000
Combination of 14-14-14 and 21-0-0 fertilizer (1:2)	3	35	14	14	1,070,000	8,500,000

Algal Cultures

The 18 dextrose bottles were sterilized prior to use in the experiment in order to eliminate potential bio-contaminants. Likewise, the hoses and pipes used for aerating the cultures were also sterilized. The seawater used in

the culture has a salinity of 35 ppt and this was chlorinated, dechlorinated, and stocked for 24 hours before using. The initial density of the algae used in both experiments are stated in Table 1. The initial and final densities of *T. tetrahele* and *Nannochloropsis* sp. were determined using a haemacytometer (corner blocks for *T. tetrahele* and center squares and corner blocks for *Nannochloropsis* sp.) under an electron microscope at 10x magnification. Samples of *T. tetrahele* were treated with Lugol's iodine solution in order to immobilize the motile cells prior to counting.

The enrichment media Tongkang Marine Research Laboratory (TMRL) and treatments using commercial fertilizers were prepared as follows; TMRL was prepared based on the standard composition of TMRL medium containing 100 g of potassium nitrite, 10 g of sodium orthophosphate, 3 g of ferric chloride and 2 g of sodium silicate dissolved in 1000 ml of distilled water; 14-14-14 – 50 g of 14-14-14 dissolved in 1000 ml of distilled water and combination – 50 g of 14-14-14 and 100 g of 21-0-0 with 1:2 ration dissolved in 1,000 ml distilled water.

All of the 1 L dextrose bottles were at first filled with 800 ml of treated seawater followed by the addition 1 ml prepared fertilizers/nutrient media and finally with the 200 ml of inoculum. The dextrose bottles were distributed randomly in an indoor shelf equipped with fluorescent lights to support the growth of microalgae. Water temperature ranges from 26-28^{o}C and each bottle is provided with constant aeration for six days (Figure 1).

Growth Measurement

To determine the relative growth rate *(k* value*)* was calculated using Guillard's (1973) formula as described by Ronquillo et al. (1997):

$$k^1 = \frac{log_2\left(\frac{N_1}{N_0}\right)}{t_1 - t_2}$$

Where k = number of divisions per day; N_1 = concentration of cells in the culture at t_1; N_o = concentration of cells at t_o; t_1 = the final culture time in days; and t_o = the time initial cultur, e time in days. The mean *k* values and standard errors of the mean (SEM) were computed.

Figure 1. Indoor algal production in 1 L dextrose bottles at the Phycology Laboratory of the College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo.

Lipid Analysis and Lipid Extraction

Dry method and Bligh and Dyer method of extracting lipids were performed for lipid analysis. The dry method was done by washing the 10 ml *T. tetrahele* and *Nannochloropsis* sp. with distilled water twice after the centrifuge process (200 rpm). Prior to the experiment for dry weight and Bligh and Dyer method, the crucibles were oven dried and placed inside the desiccator to remove moisture. Lipid extraction was carried out by using 2:1 MeOH (methanol:chloroform). The extracted lipids from the algae were placed in the crucible and was oven dried for 3 h at 50°C. After drying, the samples were placed in the desiccator for moisture removal. After an hour, the final weight of the crucibles was measured using an analytical balance.

Data Analyses

The lipid content of the two microalgae species was analyzed using the approximate gravimetric method by Bligh and Dyer (1959). The extraction and partitioning methods were simultaneous and the precipitated proteins were isolated between the two liquid phases (Bligh and Dyer 1959). This method is particularly suitable for the lipid extraction of incubation medium, tissue homogenates, or cell suspensions. Each replicate was sampled five times for a more accurate statistical evaluation.

The one-way ANOVA with Tukey's test for multiple comparison at $p<0.01$ were carried out to compute for statistical difference in terms of growth rate and extracted lipids within the same species of microalgae.

RESULTS

Growth Rate

The relative growth rates (*k*) of *T. tetrahele* and *Nannochloropsis* sp. are shown in Table 2. The growth rates of microalgae in each treatment were significantly different from each other depending on the species and treatments they were exposed with (Table 2). *Tetraselmis tetrahele*, cultured under 14-14-14 had the highest *k* value of 1.810±0.36 while *Nannochloropsis* sp. cultured under TMRL had a significantly high *k* value which is 9.708±0.44 (Table 2).

Table 2. Average (±SEM) *k* values of *Tetraselmis tetrahele* and *Nannochloropsis* sp. exposed to different nutrients (n=5).

Nutrients	***Tetraselmis tetrahele*** **Mean (±SEM) *k* value (cell/day^{-1})**	***Nannochloropsis* sp. Mean (±SEM) *k* value (cell/day^{-1})**
TMRL	0.143±0.21^{c}	9.708±0.44^{a}
14-14-14	1.810±0.36^{a}	0.721±0.23^{b}
Combination of 14-14-14 and 21-0-0 fertilizer	0.860±0.07^{b}	0.816±0.11^{b}

Average Lipid content

The results showed that *T. tetrahele* had higher average lipid content than *Nannochloropsis* sp. in all three types of nutrients (Figure 2). *Tetraselmis tetrahele* cultured under 14-14-14 (14.789%) had a significantly high ($p<0.01$) lipid content than those cultured under the combination and TMRL (Figure 2). Similar results for *Nannochloropsis* sp. were observed wherein those cultured under 14-14-14 had a significantly ($p<0.01$) high lipid content of (5%) compared to other two treatments (Figure 2).

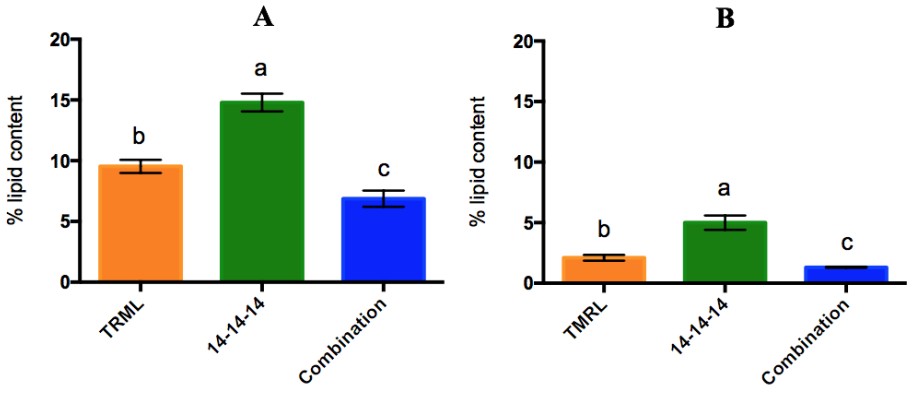

Figure 2. Average ±SEM percent dry weight lipid content of *Tetraselmis tetrahele* (A) and *Nannochloropsis* sp. (B) (n=5).

DISCUSSION

Growth Rate

Supply of nutrients plays a key role in algal growth both in artificial cultures and in the natural environment (Parrish el al. 1987; Pal et al. 2011; Khan et al. 2018). Nutrients such as nitrogen, phosphorus, and silicon are considered as periodically growth-limiting nutrients for phytoplankton in the marine environment (Tornabene et al. 1983; Parrish et al. 1987; Yu et al. 2009; Breuer et al. 2012; Recht et al. 2012). Although the effect of factors mentioned above were not studied in detail in the present experiment, the amount of nitrogen in each treatment can be estimated as lower in 14-14-14 fertilizer compared to the combination and TMRL based on their N-P-K values. The Nitrogen is 14% in 14-14-14 and 35% in combination. In TMRL, the nitrite in potassium nitrite is the source of nitrogen in the culture.

Based on the study conducted by Lim et al. (2012), among *Chaetoceros calcitrans*, *Chaetoceros muelleri*, *Isochrysis galbana*, *Nannochloropsis* sp., *Chlorella* sp., *Pavlova lutheri* and *Tetraselmis* sp., *Tetraselmis* sp. exhibited the second highest growth rate next only to *Tetraselmis chui*. In this study, our assumption is that the *T. tetrahele* cultured under combination and TMRL could have suffered from nitrogen toxicity based on the high initial amount of nitrogen in each fertilizer upon preparation of treatments thus exhibiting low growth rate compared to that grown in 14-14-14. Kim et al. (2016) mentioned

that the high growth rate in *Tetraselmis* cells is due to its ability to utilize other nitrogen and carbon sources efficiently.

Nannochloropsis sp. on the other hand exhibited good growth which can be attributed to the relatively high amount of nitrogen and other nutrients that are essential for their growth present in TMRL and not in commercial fertilizers. Ma et al. (2014) reported a specific growth rate for nine *Nannochloropsis* strains that ranged from 0.11 to 0.21 per day and this range is comparable to the results of the present study for the *Nannochloropsis* sp. cultured under TMRL.

Average Lipid content

Bligh-Dryer method, a cost-effective method for extracting lipid is normally used for extracting total lipids from microalgae (Maizatul et al. 2017). The amount of lipid extracted from microalgae varies depending on species, culture condition, nitrogen and carbon source, culture media used, and light intensity (Hu et al. 2008; Zienkiewicz et al. 2016; Alishah et al. 2019). Lipid accumulation takes place in many algae as a response to exhaustion of the nitrogen supply, several species of microalgae are found to accumulate large amounts of lipids in the cells under nitrogen limited growth (Martin et al. 2014; Suyono and Samudra 2015). This observation was relatively true for *T. tetrahele*, wherein *T. tetrahele* cultured under 14-14-14 fertilizer exhibited high growth rate and had the highest amount of lipid present. Interestingly on the other hand, in the case of *Nannochloropsis* sp. that were cultured in 14-14-14 fertilizers, low growth rate had yielded a high amount of lipid. This trend was also observed in *Nitzschia* sp. which despite its slow growth rate had a high lipid productivity (Duong et al. 2015).

In terms of lipid content, in Jena et al. (2012), 24% lipid per dry weight during the early stationary phase of *Scenedesmus* sp. was observed which was higher than the lipid content attained in the present study. Dayananda et al. (2005) also reported that the lipid content from *Botryococcus* sp. extracted using chloroform: methanol (1:2) reached 14% which was comparable to the extracted lipid content from *T. terahele* of this study but not for *Nannochloropsis* sp.

Detailed study on the lipid composition and metabolism in response to nitrogen deprivation is recommended in order to verify the difference in lipid production in microalgae. Further, strain improvement through improvement of genes could be used to enhance the lipid content of microalgae.

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Commonly gleaned macro-benthic invertebrates in a small offshore island of Cawili, Cagayancillo, Palawan, Philippines

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ABSTRACT

The inhabitants of small offshore islands are highly dependent on the health and availability of their resources. However, pieces of information about the commonly gleaned species in some remote areas are sparsely documented. In this study, we inventoried the species composition of the widely gleaned macro-benthic invertebrates such as gastropods, bivalves, and sea cucumbers in Cawili Island, a small inhabited island in the middle of the Sulu Sea in Palawan, Philippines. The samples obtained from fishers and snorkeling activities in shallow areas revealed a total of 85 species belonging to 27 families. Most of these were gastropods, composed of 68 species under 20 families. The list includes nine protected species (four gastropods, two bivalves, and three sea cucumbers). Gastropods and bivalves were mostly used for food and display in the house of the fishermen (souvenir), while the sea cucumbers were exclusively harvested for trade. Sustainable fishery activities are needed in this small offshore island where people heavily rely on their marine resources.

Keywords: bivalves, coastal communities, echinoderms, gastropods, reef-walking

INTRODUCTION

There are around 22,000 species of mollusks in the Philippines. The marine species constitute the largest number with the gastropods representing about 68% (Cabrera 1987). Intertidal mollusks such as macro-benthic marine gastropods and bivalves play major roles in promoting a balanced ecosystem. Coastal communities heavily rely on these resources as food source of livelihood and revenues, which include the multi-million-dollar shell craft industry (Gallardo et al. 1995; Floren 2003; Whittingham et al. 2003; Dolorosa et al. 2016). The shells of *Rochia nilotica* or topshell form part of the lucrative pearl button industry (Floren 2003; Bell et al. 2005). On the other hand, the large shells of giant clams (*Tridacna gigas* and *T. derasa*) are used as carving materials (Larson 2016; Neo 2017), or polished into fake giant clam pearls (Krzemnicki and Cartier 2017). Consequently, mollusk populations are in decline in spite of their huge social, economic, and cultural values (Floren 2003; Rogers-Bennett et al. 2013; Neo et al. 2015, 2017).

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The sea cucumbers are also popular target species (Choo 2008; Hasan and Abd El-Rady 2012) because of their high market value (Purcell 2014), and as sources of substances with pharmacological importance (Choo 2008). They also play an essential role in enhancing the ocean's productivity by recycling of sediments (Uthicke 2001; Conand 2006). Worldwide, there are over 1,400 sea cucumber species (Kerr and Kim 2001), and medium-high valued species had been overexploited in most countries (Lovatelli et al. 2004). In Palawan, Philippines, there are more than one hundred sea cucumber species (Jontila et al. 2017), and overharvesting has also been reported for some species in small islands proximate to the mainland Palawan (Dolorosa et al. 2017; Jontila et al. 2018).

In offshore islands with limited land areas for agriculture, the lives of the people are highly dependent on the health and availability of its marine resources. Unregulated fishing which includes gleaning or reef walking can cause habitat degradation, reduction in the abundance of target species (Ashworth et al. 2004; Cardinale et al. 2011; Al-Wazzan et al. 2020), localized extinction (Neo and Todd 2013), and massive impact on the lives of those highly dependent on fishing. While there were studies about the mollusks (Dolorosa et al. 2015; Hombre et al. 2016), and sea cucumbers (Jontila et al. 2014, 2017; Dolorosa et al. 2017) in some areas of Palawan, many small island communities are understudied. Identifying the commonly gleaned species in Cawili Island can, therefore, provide information on what species are available, what are its potential uses, which species are protected, and offer insights in crafting effective management and conservation policies for the island.

METHODS

Cawili Island (8°16'42"N, 120°48'57"E) in the middle of the Sulu Sea, is about 232 km southeast of Puerto Princesa City, Palawan (www.GoogleEarth.com). It is one of the major islands of Cagayancillo, a 6th class municipality in the Province of Palawan. Covering about 0.75 km^2, the generally flat (2 m above sea level) sandy-rocky island support only a limited variety of crops, including corn and coconut palm. The 150 families living on the island mostly depend on fishing, seaweed farming, gleaning, and harvesting of sea cucumbers (Dygico 2016).

Commonly gleaned species were photo-documented on several occasions from April to May 2018. Shells of gastropods and bivalves displayed in the houses of the residents were photo-documented. Snorkeling activities on shallow seagrass beds and walking on rocky areas during day low tides were conducted to record the occurrence of the species in their natural habitats. The sizes of photographed species were noted. The locals were also asked about the vernacular names and the uses of the species, categorized into three: for

food, souvenir (display only in the house of fishers), and trade. The species were grouped by family and arranged alphabetically. The photos were arranged in the same order of their occurrence on the table. The figure caption included the scientific name and size of each species in the picture.

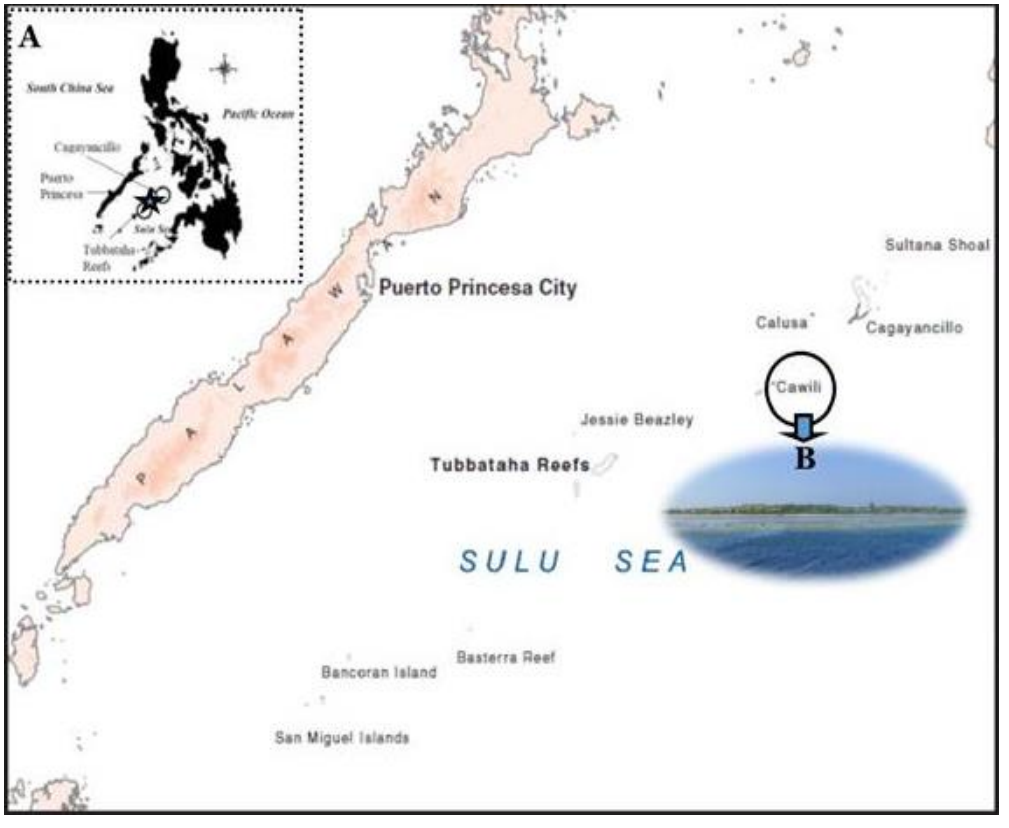

Figure 1. Map of the Philippines (A) and the location of Cawili Island (B) where the study was conducted.

The works of Springsteen and Leobrera (1986), Carpenter and Niem (1998), Kerr et al. (2006), and Jontila et al. (2014) aided the identification of the different species. The updated scientific names were obtained from the World Register of Marine Species (WoRMS 2020).

RESULTS

A total of 85 species belonging to 27 families were recorded. Gastropods had the most number (68 species), followed by sea cucumbers (10 species) and bivalves (7 species) (Table 1-3; Figure 2-7). Among the

gastropods, family Cypraeidae had the highest number (21%) represented by 14 species, followed by family Strombidae with 11 (16%) species (Figure 8a). Bivalves were mainly composed of families Cardiidae and Tellinidae, each represented by two species (29%) (Figure 8b). Family Holothuriidae had the most number (90%) of species among the sea cucumbers (Figure 8c). Thirty species of gastropods were used as food, while 20 species were for souvenir only (Table 1; Figure 9). Of the seven species of bivalves, six were exploited for food and the shells were mostly used for display or souvenir (Table 2; Figure 9). Majority (90%) of the sea cucumbers fall under the family Holothuriidae, and all species were collected for trade purposes (Table 3; Figure 9).

Table 1. List and usage of commonly gleaned species of gastropods in Cawili, Cagayancillo, Palawan. F- food, S – souvenir, T – trade.

Family Name	English Name	Scientific Name	Local Name (Cagayanen)	Usage		
				F	S	T
Astraeinae	Common Delphinula	*Angaria delphinus*	Bokotan		/	
Bullidae	Ampulle Bulla	*Bulla ampulla*	Bonbolan		/	
Cassidae	Vitex Bonnet	*Casmaria erinaceus*	Sosobla		/	
	Horned Helmet	*Cassis cornuta*	Tambuli	/	/	
	Bullmouth Helmet	*Cypraecassis rufa*	Tambuli	/	/	
	Few-wrinkled Bonnet	*Phalium glaucum*	Sosobla		/	
Cerithiidae	Necklace Cerith	*Clypeomorus batillariaeformis*	Gibaw gibaw		/	
	Rough Vertagus	*Rhinoclavis aspera*	Gang gasang		/	
	Banded Vertagus	*Rhinoclavis fasciata*	Tetendaw	/	/	
	Common Vertagus	*Rhinoclavis vertagus*	Pepindaw	/	/	
Conidae	Abbreviated Cone	*Conus abbreviatus*	Tambroso		/	/
	Hebrew Cone	*Conus ebraeus*	Tambroso		/	/
	False Virgin Cone	*Conus emaciatus*	Tambroso	/	/	
	Turtle Cone	*Conus ermineus*	Tambroso	/	/	
	Red Sea Cone	*Conus erythraeensis*	Tambroso	/	/	
	Lettered Cone	*Conus litteratus*	Tambroso	/	/	
	Magical Cone	*Conus magus*	Tambroso	/	/	
	Feathered Cone	*Conus pennaceus*	Uyok-uyok	/	/	
	Flea-Bite Cone	*Conus pulicarius*	Tambroso		/	/
	Virgin Cone	*Conus virgo*	Tambroso	/	/	

Family Name	English Name	Scientific Name	Local Name (Cagayanen)	Usage		
				F	S	T
Cymatiidae	Trumpet Triton	*Charonia tritonis*	Trumpet	/	/	
	Cosmopolitan hairy triton	*Monoplex aquatilis*	Lampas	/	/	
Cypraeidae	Turtle Cowrie	*Chelycypraea testudinaria*	Tamblilo		/	/
	Tiger Cowrie	*Cypraea tigris*	Tamblilo		/	/
	Wandering Cowrie	*Erronea errones*	Tamblilo		/	/
	Map Cowrie	*Leporicypraea mappa*	Mapa-mapa		/	
	Pacific Deer Cowrie	*Lyncina vitellus*	Tamblilo		/	/
	Arabian Cowrie	*Mauritia arabica*	Baboy-baboy	/	/	
	Depressed Cowrie	*Mauritia depressa*	Mangyan		/	/
	Eglantine Cowrie	*Mauritia eglantina*	Baboy-baboy	/	/	
	Gold-ringer Cowrie	*Monetaria annulus*	Sigay mama	/	/	/
	Money Cowrie	*Monetaria moneta*	Sigay		/	/
	King's Cowrie	*Naria bernardi*	Baboy-baboy		/	
	Erosa Cowrie	*Naria erosa*	Tamblilo	/	/	
	Graceful Cowrie	*Purpuradusta gracilis*	Tamblilo		/	/
	Teres Cowrie	*Talostolida teres*	Tamblilo		/	/
Fasciolariidae	Threaded Band Shell	*Filifusus filamentosus*	Tabaco		/	/
Mitridae	Episcopal Miter	*Mitra mitra*	Mitra-mitra		/	/
	Papal Miter	*Mitra papalis*	Mitra-mitra		/	/
Nassariidae	Whitesh Nassa	*Nassarius albescens*	Suso		/	/
		Nassarius limnaeiformis	Tangad-tangad		/	
		Nassarius velatus	Suso		/	
	Lovely Nassa	*Nassarius venustus*	Suso		/	
Naticidae	Black-mouth Moon snail	*Mammilla melanostoma*	Bonbolan		/	
	Pear-shaped Moon Snail	*Polinices mammilla*	Bonbolan		/	
Neritidae	Horny Nerite	*Neritodryas cornea*	Lebeng-lebeng		/	
Strombidae	Little Bear Conch	*Canarium urceus*	Siksikad	/		
	Strawberry Conch	*Conomurex luhuanus*	Silan	/		
	Diana Conch	*Euprotomus aurisdianae*	Langa-langa	/		

Family Name	English Name	Scientific Name	Local Name (Cagayanen)	Usage		
				F	S	T
	Bubble Conch	*Euprotomus bulla*	Langa-langa	/		
	White Hump Conch	*Gibberulos gibberulus*	Siksikad	/		
	White hump-back Conch	*Gibberulos gibberulus albus*	Siksikad	/		
	Gibbo Conch	*Gibberulos gibberulus gibbosus*	Siksikad	/		
	Chiragra Spider Conch	*Harpago chiragra*	Salagra		/	/
	Orange Spider Conch	*Lambis chrocata*	Pangahan	/		
	Milled Spider Conch	*Lambis millepeda*	Pangahan	/	/	
	Silver Conch	*Lentigo lentiginosus*	Langa-langa	/		
Terebridae	Marlinspike	*Oxymeris maculata*	Onsoy-onsoy		/	
	Spotted Auger	*Terebra guttata*	Onsoy-onsoy		/	
Tonnidae	Pacific Grinning Tun	*Malea pomum*	Subla-subla		/	
	Pacific Partridge Tun	*Tonna perdix*	Subla-subla		/	
Trochidae	Commercial Trochus	*Rochia nilotica*	Samong		/	/
	Maculated Top	*Trochus maculatus*	Dipulos		/	
Turbinellidae	Common Pacific Vase	*Vasum turbinellus*	Lomboy-lomboy		/	
Turbinidae	Gold-mouth Turban	*Turbo chrysostomus*	Bugtungan	/	/	
	Tapestry Turban	*Turbo petholatus*	Bogtungan	/	/	
Turridae	Bayer's Turrid	*Glyphostoma bayeri*	Tangad-tangad		/	
Volutidae	Bat Volute	*Cymbiola vespertilio*	Uk-uyok	/	/	
TOTAL				30	59	18

Table 2. List and usage of commonly gleaned species of bivalves in Cawili, Cagayancillo, Palawan. F- food, S – souvenir, T – trade.

Family Name	English Name	Scientific Name	Local Name (Cagayanen)	Usage		
				F	S	T
Mactridae	Smooth Beach Clam	*Atactodea striata*	Basala	/	/	/
Ostreidae	Black-lip Pearl Oyster	*Pinctada margaritifera*	Tabah	/		
Psammobiidae	Agrutan	*Asaphis violascens*	Beggat-beggat		/	

Family Name	English Name	Scientific Name	Local Name (Cagayanen)	Usage		
				F	S	T
Tellinidae	Cat's Tongue Tellin	*Scutarcopagia linguafelis*	Tuway	/	/	
	Virgate Tellin	*Tellinella virgata*	Tuway	/	/	
Cardiidae	China Clam	*Hippopus hippopus*	Basa	/	/	/
	Floated/Scaly Clam	*Tridacna squamosa*	Manelet	/	/	/
TOTAL				6	6	3

Table 3. List and usage of commonly gleaned species of sea cucumber in Cawili, Cagayancillo, Palawan. F- food, S – souvenir, T – trade.

Family Name	English Name	Scientific Name	Local Name (Cagayanen)	Usage		
				F	S	T
Holothuriidae	Leopard fish	*Bohadschia argus*	Batik-batik			/
	Chalky sea cucumber	*Bohadschia marmorata*	Tagekan			/
		Bohadschia sp.	Tagekan			/
	Brown sandfish	*Bohadschia vitiensis*	Tagekan			/
	Black beauty	*Holothuria atra*	Sapatos			/
	Ashy pink sea cucumber	*Holothuria fuscocineria*	Batonan			/
	White teatfish	*Holothuria fuscogilva*	Susohan			/
	Sandfish	*Holothuria scabra*	Koltero			/
	Prickly redfish	*Thelenota ananas*	Talipan			/
Stichopodidae	Selenka's sea cucumber	*Stichopus horrens*	Hanginan			/
TOTAL						10

Figure 2. Commonly gleaned species of gastropods collected in Cawili, Cagayancillo, Palawan. A) *Angaria delphinus*, 3.8 cm; B) *Bulla ampulla*, 4.5 cm; C) *Casmaria erinaceus*, 4.6 cm; D) *Cassis cornuta*, 8.3 cm; E) *Cypraecassis rufa*, 7.9 cm; F) *Phalium glaucum*, 5.2 cm; G) *Clypeomorus batillariaeformis*, 2.2 cm; H) *Rhinoclavis aspera*, 3.2 cm; I) *Rhinoclavis fasciata*, 6.9 cm; J) *Rhinoclavis vertagus*, 5.1 cm; K) *Conus abbreviatus*, 2.9 cm; L) *Conus ebraeus*, 3.7 cm; M) *Conus emaciatus*, 6.8 cm; N) *Conus ermineus*, 3.6 cm; O) *Conus erythraeensis*, 3.8 cm; P) *Conus litteratus,* 7 cm; Q) *Conus magus*, 5.6 cm.

Figure 3. Commonly gleaned species of gastropods collected in Cawili, Cagayancillo, Palawan. A) *Conus pennaceus*, 10.9 cm; B) *Conus pulicarius*, 4.5 cm; C) *Conus virgo*, 5.8 cm; D) *Charonia tritonis*, 14.9 cm; E) *Monoplex aquatilis*, 8.5 cm; F) *Chelycypraea testudinaria*, 10 cm; G) *Cypraea tigris*, 7.2 cm; H) *Erronea errones*, 2.6 cm; I) *Leporicypraea mappa*, 8 cm; J) *Lyncina vitellus*, 5.2 cm; K) *Mauritia arabica*, 4.7 cm; L) *Mauritia depressa*, 2.6 cm; M*) Mauritia eglantina*, 5.7 cm; N) *Monetaria annulus*, 2.5 cm; O) *Monetaria moneta*, 2.8 cm; P) *Naria bernardi*, 4.4 cm; Q) *Naria erosa*, 3.4 cm.

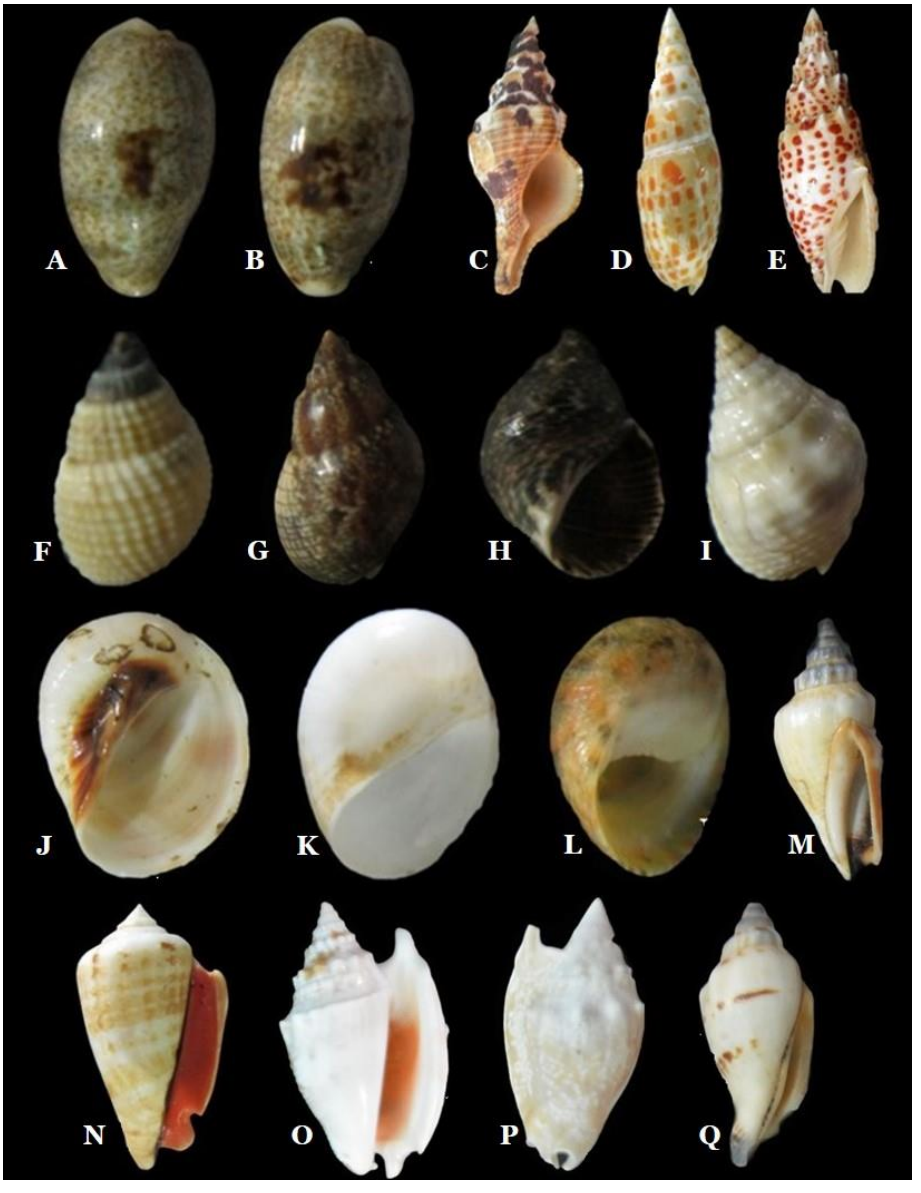

Figure 4. Commonly gleaned species of gastropods collected in Cawili, Cagayancillo, Palawan. A) *Purpuradusta gracilis*, 2.3 cm; B) *Talostolida teres*, 2.3 cm; C) *Filifusus filamentosus*, 9 cm; D) *Mitra mitra*, 9.6 cm; E) *Mitra papalis*, 11.1 cm; F) *Nassarius albescens*, 1.5 cm; G) *Nassarius limnaeiformis*, 1.8 cm; H) *Nassarius velatus*, 1.7 cm; I) *Nassarius venustus*, 2.7 cm; J) *Mammilla melanostoma*, 3.5 cm; K) *Polinices mammilla*, 4.2 cm; L) *Neritodryas cornea*, 2.7 cm; M) *Canarium urceus*, 4.8 cm; N) *Conomurex luhuanus*, 5.6 cm; O) *Euprotomus aurisdianae*, 7.1 cm; P) *Euprotomus bulla*, 7 cm; Q) *Gibberulus gibberulus*, 4.2 cm.

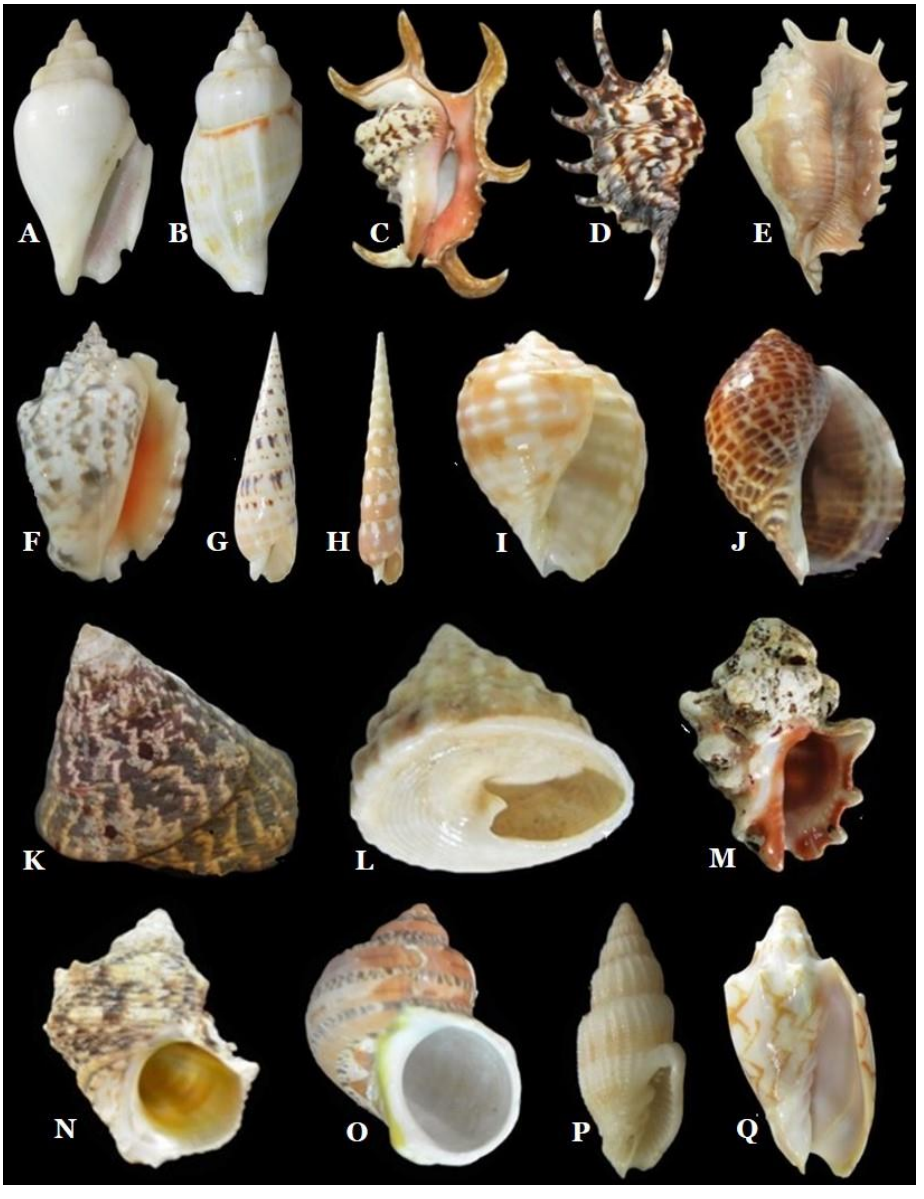

Figure 5. Commonly gleaned species of gastropods collected in Cawili, Cagayancillo, Palawan. A) *Gibberulus gibberulus albus*, 4.1 cm; B) *Gibberulus gibberulus gibbosus*, 7 cm; C) *Harpago chiragra*, 7.3 cm; D) *Lambis chrocata*, 6.1 cm; E) *Lambis millepeda*, 9.2 cm; F) *Lentigo lentiginosus*, 6.9 cm; G) *Oxymeris maculata*, 13.6 cm; H) *Terebra guttata*, 10.8 cm; I) *Malea pomum*, 4.8 cm; J) *Tonna perdix*, 6.9 cm; K) *Rochia nilotica*, 10 cm; L) *Trochus maculatus*, 3.6 cm; M) *Vasum turbinellus*, 5.2 cm; N) *Turbo chrysostomus*, 5.2 cm; O) *Turbo petholatus*, 6 cm; P) *Glyphostoma bayeri*, 2.6 cm; Q) *Cymbiola vespertilio,* 7.5 cm.

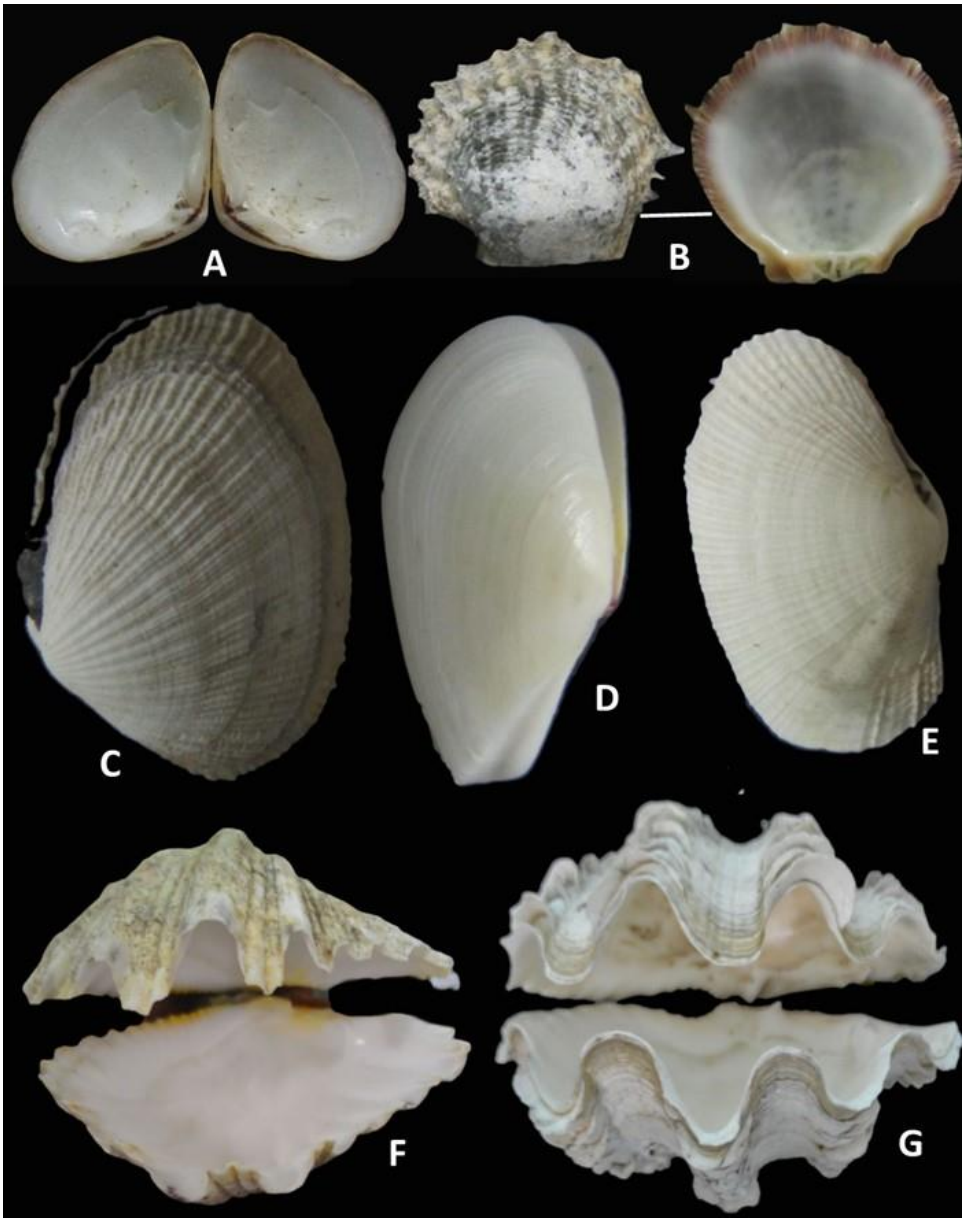

Figure 6. Commonly gleaned species of bivalves collected in Cawili, Cagayancillo, Palawan. A) *Atactodea striata*, 2.5 cm; B) *Pinctada margaritifera*, 4.9 cm; C) *Asaphis violascens*, 6.1 cm; D) *Tellinella virgata*, 4.1 cm; E) *Scutarcopagia linguafelis*, 4.3 cm; F) *Hippopus hippopus*, 14.3 cm; G) *Tridacna squamosa*, 13.2 cm.

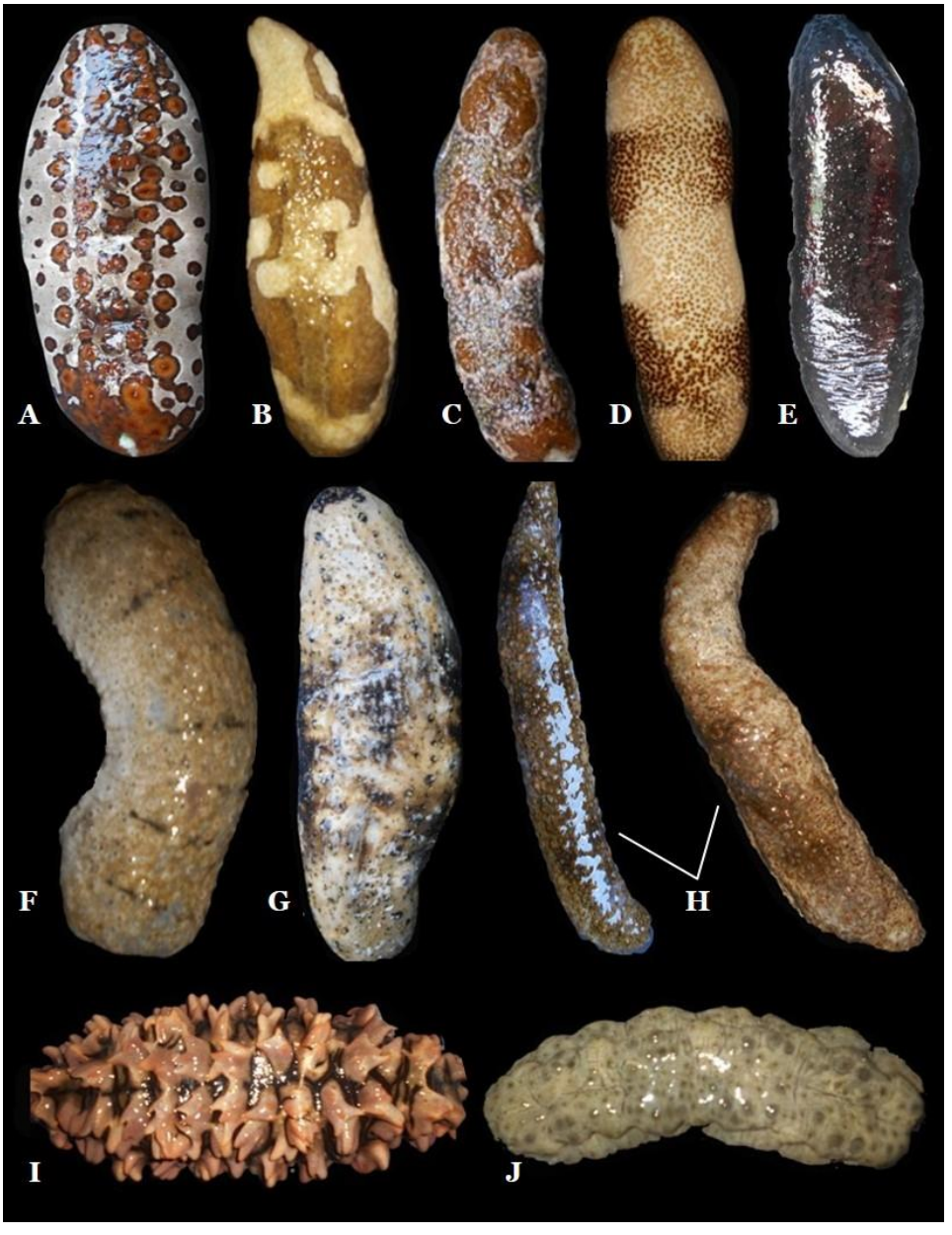

Figure 7. Commonly gleaned species of sea cucumbers collected in Cawili, Cagayancillo, Palawan. A) *Bohadschia argus,* 16.5 cm; B) *Bohadschia marmorata,* 15.2 cm; C) *Bohadschia* sp., 19.3 cm; D) *Bohadschia vitiensis,* 20.1 cm; E) *Holothuria atra,* 18.2 cm; F) *Holothuria scabra,* 13.5 cm; G) *Holothuria fuscogilva,* 22.5 cm; H) *Holothuria fuscocineria,* 24.3 cm; I) *Thelenota ananas*, 18 cm; J) *Stichopus horrens,* 17.7 cm.

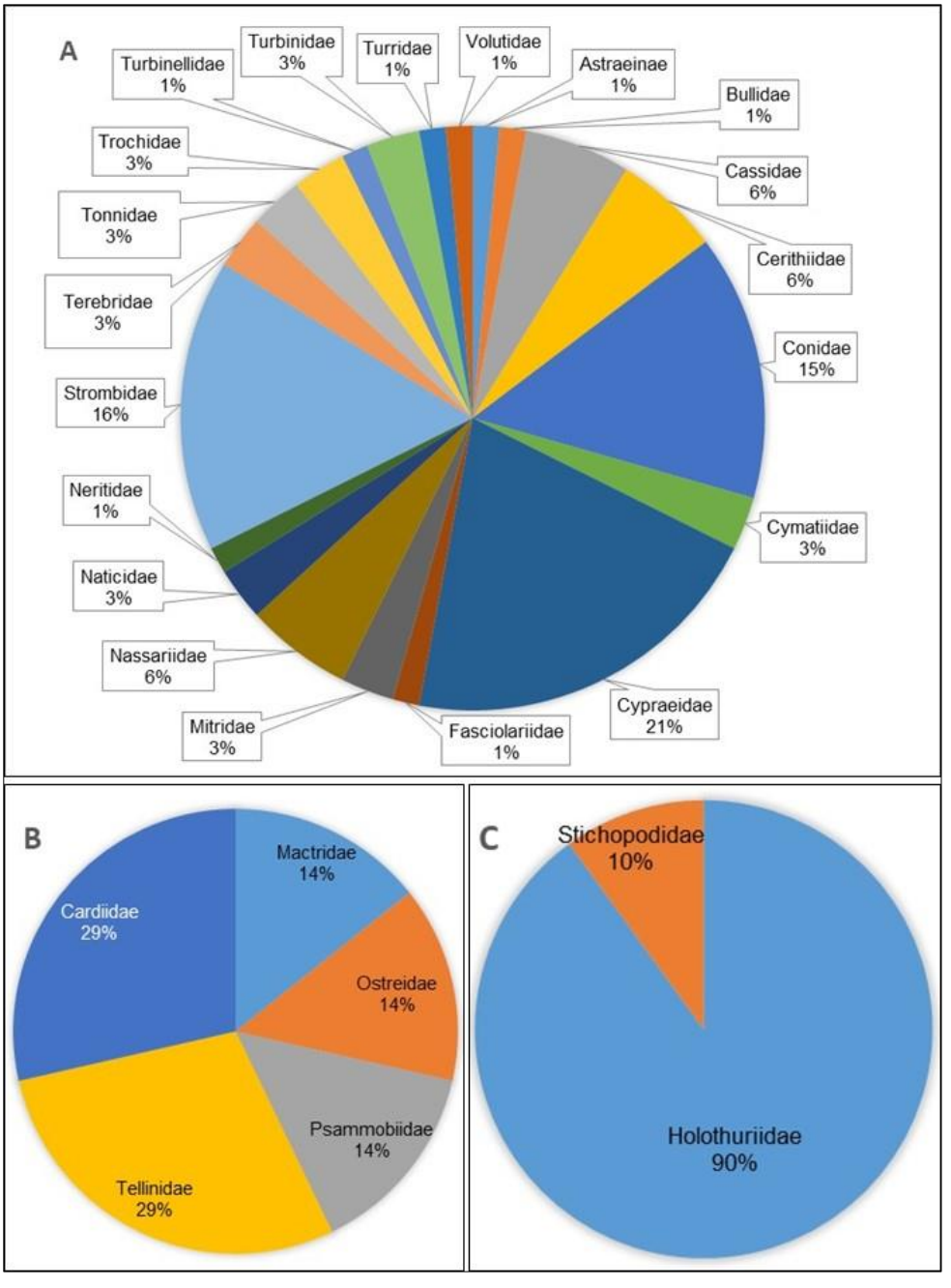

Figure 8. Percent composition of recorded species under the class Gastropoda (A), Bivalvia (B) and Holothuroidea (C).

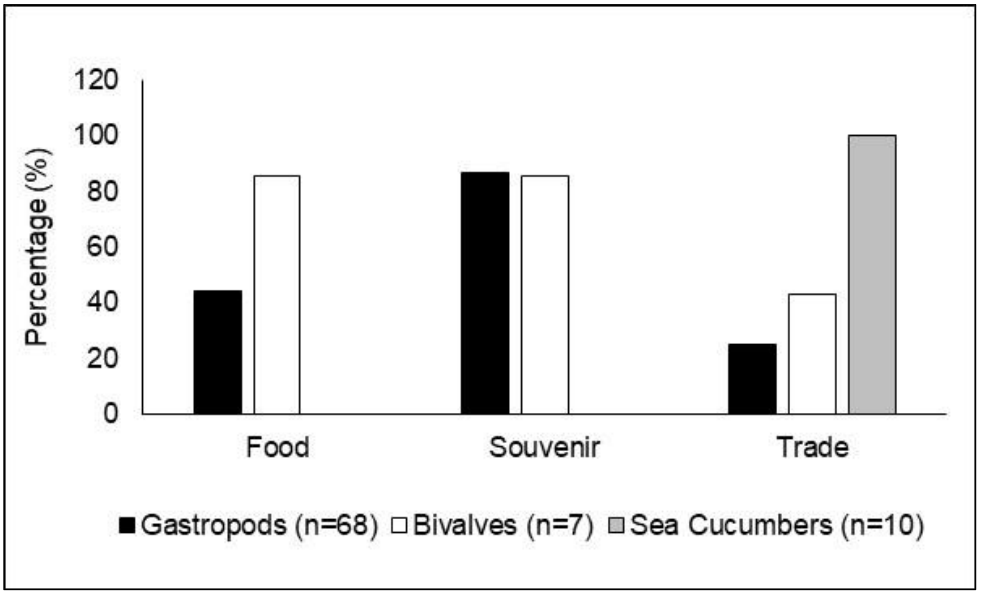

Figure 9. Percent usage of the commonly gleaned macro-benthic invertebrates in Cawili Island, Cagayancillo, Palawan, Philippines.

DISCUSSION

The number of recorded gastropod species in Cawili was comparable to other areas in Palawan, but not for bivalves and sea cucumbers (Table 4). Habitat complexity and level of disturbance (e.g., gleaning) are known to influence the number of marine species (Beauchamp and Gowing 1982; Dissanayake and Stefansson 2010; Pawar and Al-Tawaha 2017). Gleaning is a traditional unregulated method of collecting edible, and high valued marine macro-benthic invertebrates by walking on exposed habitats and shallow waters during the day and night low tides (Whittingham et al. 2003; Palomares et al. 2014; De Guzman et al. 2016). It is an essential source of food and an alternative livelihood (Whittingham et al. 2003; Nieves et al. 2010; De Guzman et al. 2016), but these activities can bring damage on the reef (Woodland and Hooper 1977) and overharvesting (Jontila et al. 2018). Habitat alteration and decline in abundance of target species could therefore, affect the health and well-being of coastal communities, especially for a remote island like Cawili where land resources for agriculture are limited.

Table 4. Comparison in the number of commonly gleaned gastropods, bivalves, and sea cucumber from Cawili, Cagayancillo, Palawan to different studies, and localities in Palawan. (*) asterisk sign in the right column (Sources) is for the sea cucumber citation paper.

Area	Number of species recorded			Sources
	Gastropods	Bivalves	Sea Cucumbers	
Iwahig River-Estuary, PPC	50	15	-	Dolorosa and Dangan-Galon 2014
Tubbataha Reefs, Cagayancillo	79	17	16	Dolorosa et al. 2015; Dolorosa 2015*
Kalayaan Group of Island	69	9	-	Hombre et al. 2016
Turtle and Binunsalian Bay, PPC	89	19	-	Picardal and Dolorosa 2014
WPU-BMRS, Puerto Princesa City	64	8	-	Hombre 2015
Arreciffe Island, Honda Bay, PPC	-	-	14	Jontila et al. 2017*
Roxas, Palawan	-	-	16	Saclet 2013*
Balabac, Palawan	-	-	21	Idlan 2013*
Rasa Island, Narra, Palawan	-	-	24	Dolorosa et al. 2017*
Cawili, Cagayancillo	**68**	**7**	**11**	**This study**

Many of the gastropods and bivalve shells are used for display, while the sea cucumbers are exclusively harvested for trade (Tables 1-3; Figure 8). Many of the displayed shells are of low value but these are used in the shell craft industry (Floren 2003). Included in the list of commonly gleaned marine resources in Cawili are nine protected species. Two gastropods: *C. cornuta* and *C. tritonis* are protected under the Fisheries Administrative Order (FAO) No. 158 of 1986 (Floren 2003; BFAR 2019), while the other two (*R. nilotica* and *C. rufa)* are protected under the Fisheries Administrative Order (FAO) No. 208 (DA 2001). The giant clams *Tridacna* spp. are listed in Appendix II of CITES, which means that the species are not necessarily threatened with extinction, but in which trade must be controlled, in order to avoid utilization

incompatible with their survival (CITES 2020). All giant clams species in the Philippines are also protected under FAO No. 208 (DA 2001). On the other hand, the International Union for the Conservation of Nature (IUCN) classified the sea cucumber *Holothuria fuscogilva* as vulnerable. In addition, *Holothuria scabra* and *Thelenota ananas* are considered endangered (IUCN 2020). The presence of nine threatened species from the harvest of local fishers makes Cawili an important ecological refuge.

The prevalent harvest and consumption of protected species could further reduce their abundance (Newton et al. 1993; Dolorosa et al. 2016; Jontila et al. 2018). In spite of penalties for violators as stipulated in the Philippine law, the collection, consumption, and trade of threatened species remained a common problem in the Philippines (Floren 2003; Dolorosa et al. 2016; PCSD 2020) and elsewhere (Deines 2018; Patankar 2019; Gamboa-Alvarez et al. 2020). The inclusion of threatened species as part of the catch in Cawili Island might be associated with the remoteness of the area, weak law implementation, lack of awareness on the part of the fisherfolks, and the tradition of having these creatures as part of their diets.

Many studies have shown that unregulated exploitation has resulted in a population decline of mollusks (e.g., Rogers-Bennett et al. 2013; Dolorosa et al. 2016) and sea cucumbers (Purcell 2010; Jontila et al. 2018; Gamboa-Alvarez et al. 2020). These species are less mobile and highly visible in their shallow habitats, which make them vulnerable to overharvesting. The overharvesting of threatened species like *C. cornuta* and *C. tritonis* is considered a contributing factor in the outbreak of their prey like the Crown-of-Thorn starfish *Acanthaster planci* (Tewfik and Scheuer 2013), which fed on coral polyps and created significant damage on the reef (De'ath et al. 2012). It is, therefore, essential to conserve these vanishing species to avoid the unprecedented impact of ecological imbalance.

Of the eight giant clam species found in the country (Dolorosa et al. 2015; Ecube et al. 2019), only two species have been encountered in Cawili. The absence of other giant clam species may suggest localized extinction as also been reported in many areas within Palawan (Mecha and Dolorosa 2020), the Philippines (Gomez and Mingoa-Licuanan 2006), and other countries (Neo and Todd 2013; Neo et al. 2019). Only in well-managed reserves/resorts where populations of giant clams remained high (Conales et al. 2015; Daño et al. 2020). The giant clams are ecologically important as reef builders, shelter, and food source to many reef organisms (Neo et al. 2015). Hence, their presence in high numbers could enhance fish abundance (Cabaitan et al. 2008).

Sea cucumber fishery provides an essential source of income for the unemployed (Choo 2008; Dolorosa et al. 2017), especially for the inhabitants

of Cawili Island. This study did not record the sizes of dried sea cucumbers, but judging from the observed sizes of dried sea cucumbers in Cawili, many were already undersized based on the dried size limits from other countries. Size limits vary per species and country (see Purcell et al. 2012), which could have been based on sizes at sexual maturity. For example, New Caledonia implements the following size limits for *H. scabra* (30 cm live, 11 cm dried), *H. fuscogilva* (25 cm live, 16 cm dried), and *T. ananas* (45 cm live and 20 cm dried). These size limits could be adapted in Cawili, pending studies on size at sexual maturity of commercially harvested sea cucumber species in the country.

The inclusion of juvenile sea cucumbers in the fishery may lead to overfishing and disappearance of species. Sea cucumber overfishing can significantly reduce species diversity and density (Jontila et al. 2018) and may cause ecosystem imbalance. If this situation continues, recruitment failure may occur (Purcell et al. 2013), affecting not only the ecosystem but also the income of local fishers and the country's export revenue.

According to Purcell (2014), the prices of dried sea cucumbers in Chinese stores varied up to ten-fold and were mostly influenced by species, body size, and quality. For example, the price of large (12 cm dried length) *H. scabra* is about seven times higher than the small ones (5 cm dried length). High earnings from sea cucumber gathering could be, therefore, sustained by implementing size limits. When effectively practiced, size limits could ensure steady supplies of recruits (because the species are allowed to breed at least once before being captured and processed) and sustained the sea cucumber fishery on the island. An education campaign about the ecological values of the species and the consequences of overharvesting may help change the attitudes of the people and win their full support to conservation initiatives.

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Water lettuce and water spinach as potential feed ingredients for Nile tilapia *Oreochromis niloticus*

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ABSTRACT

Nile tilapia (*Oreochromis niloticus*) is an omnivore and is considered as one of the important aquaculture commodities. Different plant-based ingredients like corn, "ipil-ipil" leaves and even micro and macroalgae were already tested to reduce the cost on the use of animal-based protein source. This preliminary study therefore, is an attempt to assess the potential of water lettuce and water spinach as feed ingredients through average body weight (ABW), specific growth rate (SGR), survival rate and feed conversion ratio (FCR) of juvenile *O. niloticus*. Three treatments [water lettuce (WL), water spinach (WS), water lettuce and water spinach (WL+WS)] were prepared in the form of three different diets [Diet 1 (1:1 water lettuce: fish meal), Diet 2 (1:1 water spinach: fish meal) and Diet 3 (1:0.5:0.5) (fish meal: water lettuce: water spinach)] each replicated thrice and fed to tilapia for 60 days. Tilapia growth showed no significant differences in all diets. The diet with water spinach (*Ipomoea aquatica*) significantly improved the SGR. Based on the result, the *I. aquatica* could be included both in a simple or complex diet together with water lettuce (*Pistia stratiotes*). It took 30 days for *O. niloticus* to adjust to the introduced diets as reflected to their survival rate but the FCR was higher when fed with the test diets compared to the recorded commercially-fed tilapia. Overall, *I. aquatica* has an excellent performance for *O. niloticus* culture.

Keywords: Cichlid*, Pistia stratiotes, Ipomoea aquatica,* ABW, SGR, FCR

INTRODUCTION

There is a rising demand of fish for human consumption worldwide leading to the intensive culture and search for cheaper feed formulas for aquaculture (Bhosale et al. 2010). The commonly used ingredients for tilapia culture include fish meal and soybean meal. These ingredients have higher costs and fish meals add pressure to the marine resources, hence, there is an increasing effort to seek for alternative sources of protein from the local plant resources. At present, various studies are being conducted on the evaluation of protein from plants. It was found that plant sources like leucaena leaves, wheat and corn promote growth performance, feed utilization efficiency and

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amino acid composition equal or greater than those obtained with fish meal (Tacon 1989; Borgeson 2005; Song et al. 2014).

Tilapia (*Oreochromis niloticus*), an omnivorous fish, is popular and mass cultivated worldwide for having rapid growth, good feed acceptance, adaptation to captivity and white meat with few intramuscular bones (Montoya-Camacho et al. 2018). In the Philippines, tilapia culture had 0.4% contribution to the fisheries sector as of 2019. Furthermore, the total tilapia production reached up to 56,177.85 metric tons in the country last year (Troell et al. 2014; PSA 2019).

The use of less-expensive plant protein sources such as soybean, maize, pea, canola, sunflower seed and "ipil-ipil" as partial or total replacements for fish meal in the diet of tilapia and other cultured organisms is an international research priority at present (Llanes and Toledo 2011; Gonzalez-Salas et al. 2014; Plaipetch and Yakupitiyage 2014; Figueiredo-Silva et al. 2015; Hassaan et al. 2015; Khalifa et al. 2017). Other possible local fish feed plant ingredients with low economic values include water lettuce (*Pistia stratiotes*) and water spinach (*Ipomoea aquatica*). The leaves and stems of water lettuce contain 92.9% moisture, 1.4% protein, 0.3% fat, 2.6% carbohydrate, 0.9% fibers, 1.9% ash, 0.2% calcium and 0.06% phosphorus (Tripathi and Mishra 2007). Another potential plant as tilapia feed ingredient is the water spinach which contains 72.8±0.29% moisture, 11.0±0.50% crude lipid, 17.7±0.35% crude fibers, 54.2±0.68% carbohydrates and a crude protein of 6.30±0.27% (Umar et al. 2007). It also has a good source of minerals like potassium, manganese, iron and magnesium thus, it is being recommended to be used for nutritional purposes both for humans and animals (Umar et al. 2007).

This study therefore aims to find out the potential use of these plants as feed ingredients in the diet of Nile tilapia specifically on its specific growth rate (SGR), survival rate and feed conversion ratio (FCR).

METHODS

Study Site

This study was conducted at the Hatchery of the Fishery Department in Capiz State University-Dayao Satellite College, Dayao, Roxas City, Capiz, Philippines. The area is situated at the middle of the University ponds and the tanks used were shaded and located outside the hatchery infrastructure.

Feed Preparations

For the feed formulation, there are three treatments containing water spinach (WS), water lettuce (WL) and a combination of WL and WS. The ratio of incorporation were adjusted to Diet 1 (1:1 water lettuce:fish meal), Diet 2 (1:1 water spinach:fish meal) and Diet 3 (1:0.5:0.5) (fish meal:water lettuce:water spinach). The WL and WS were sourced from the private fish ponds in Barangay Barra and Barangay Dayao, Roxas City, Capiz respectively (Table 1).

Table 1. Amounts and ingredients of feeds used in the study.

Treatment	Ingredients	Amount (%)
Diet 1	Water lettuce	50
	Fish meal	50
Diet 2	Water spinach	50
	Fish meal	50
Diet 3	Fish meal	50
	Water lettuce	25
	Water spinach	25

All plant ingredients were sun-dried for 3 days, ground into fine particles, sieved or sifted, weighed and mixed by hand for at least 5 min or until well blended before adding the fish meal and cassava flour with water (4:1) as binder. These were further mixed with a wooden ladle and were cooked for 10-15 min under moderate heat. The pre-cooled doughs were then passed through an extruder and the feeds formed were cut into 2-3 mm length prior to one-day sun drying. The pellets were cooled down at room temperature for 30-60 min before storage.

Rearing and Monitoring of *O. niloticus*

Forty-five pieces of juvenile tilapia with an average (±SEM) weight and length of 22.8±1.4 and 9.7±0.6 cm respectively were obtained from the hatchery in Dayao, Roxas City, Capiz. The fish were conditioned for 3 days by feeding them with commercial diet containing 39% crude protein. After conditioning, the fish were stocked in each compartment of a 3-ton-rectangular fiber glass tank (in a shaded area) at 5 fish per 0.3 m^3 with continuous supply of aeration.

The initial feed rate was based on 7% of the fish biomass in all treatments. The daily feeding rate (DFR) was also computed (Table 2).

Table 2. Daily feeding ration (g) of Nile tilapia using diets with water lettuce, water spinach and the combination of water lettuce and water spinach throughout the culture period. WL – water lettuce; WS – water spinach.

Days of Culture / Treatment	0-15	16-30	31-45	46-60
Diet 1 (WL)	123.9	156.5	158.6	168.0
	72.5	83.0	63.0	68.4
	87.2	110.3	118.7	98.7
Diet 2 (WS)	131.3	149.1	121.8	127.1
	125.0	159.6	127.1	66.2
	105.0	101.9	109.2	112.4
Diet 3 (WL + WS)	131.3	123.9	69.3	70.4
	95.6	158.6	88.2	89.1
	115.5	138.8	99.9	105.0

Statistical Analysis

Average ± standard error of mean (SEM) of the SGR, % survival and feed conversion ratio (FCR) were computed using Microsoft Excel 2013. Statistical Package for Social Sciences (SPSS) version 20 was used to compare the treatments. The growth and feed efficiency data were tested for homogeneity of variance using Levene's tests and then subjected to one-way analysis of variance (ANOVA) at 0.05 level of significance to determine the differences among the three treatments. Furthermore, post-hoc tests using Duncan's Multiple Range Test (DMRT) were carried out to confirm where the differences among groups occurred.

RESULTS

The average body weight (ABW) of tilapia with regard to time apparently increased from day 0 up to day 60 in all treatments. The average body weight of *O. niloticus* fed with diets containing WL, WS and WL + WS had no significant differences except during 30th and 60th day of the culture

(Figure 1). At day 30, the ABW of those fed with WL + WS (36.1±1.9 g) were significantly higher compared to the other treatments. At the end of the culture period, WL + WS (44.2±1.8 g) only differed significantly with those fed with WL (34.2±3.1 g).

The specific growth rates of *O. niloticus* were statistically similar among all treatments throughout the culture period, nevertheless, sharp increase for each treatment was observed at the end of the culture period (Figure 2). On the 15th day, SGR reached up to 1.8±0.3% day^{-1}, 1.3±0.2% day^{-1} and 1.5±0.2% day^{-1} in diets containing WL, WS and WL + WS respectively (Figure 2). Those fed with WL had statistically similar SGR from day 15 to day 30 (0.9±0.3% day^{-1}) but significant increase was apparent at the end of the culture period. Similar trends were observed in the SGR of *O. niloticus* fed with WS and WS + WL in which the significant increases ($p<0.05$) were found after 60-day culture at 2.7±0.1 and 3.0±0.1% day^{-1} respectively.

The survival rate of tilapia decreased after 30-day culture and was maintained up to the end of the culture period (Table 3). No significant differences were found among the survival rates of *O. niloticus* fed with the different diets except at 30-day culture when those fed with WL (86.7±13.3%) were significantly higher than those fed with WL + WS (53.3±6.7%) and WS (80.0±0.0%).

The FCR of the fish also significantly increased from day 30 onwards though the performances of every diet did not vary from each other throughout the culture period. The final FCR of tilapia fed with WL, WS and WL + WS reached up to 7.6±0.8, 7.9±1.0 and 8.1±0.8 respectively.

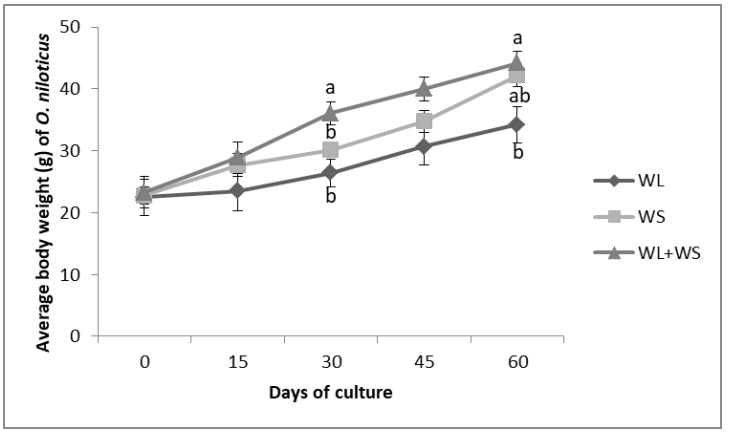

Figure 1. *Oreochromis niloticus* average body weight (ABW) (g) fed diets containing water lettuce (WL), water spinach (WS) and combination of water lettuce and water spinach (WL + WS). Data presented as Mean ± SEM ($p<0.05$), n=3.

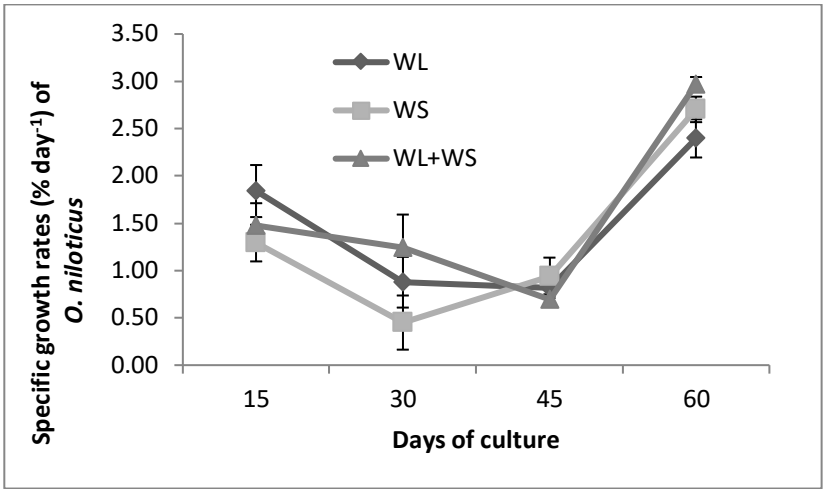

Figure 2. Specific growth rate (SGR) (% day^{-1}) of *O. niloticus* fed diets containing water lettuce (WL), water spinach (WS) and combination of water lettuce and water spinach (WL + WS). Data presented as Mean ± SEM. All treatments showed no significant differences in every period ($p>0.05$; n=3) although SGR values on the 60th day were significantly higher than those obtained in other sampling events. ($p<0.05$), n=4.

Table 3. Average (± SEM) Survival rate and feed conversion ratio (FCR) of Nile tilapia fed with different organic feeds every 15 days of culture. Values with the same superscripts in every treatment at the same culture period are not significantly different ($p>0.05$).

Days of Culture/ Treatment	**Parameters**	
	Average (± SEM) % Survival	**Average (± SEM) FCR**
Day 15		
Diet 1 (WL)	93.3±6.7	3.7±0.3
Diet 2 (WS)	93.3±6.7	5.6±0.9
Diet 3 (WL + WS)	86.7±6.7	5.0±1.2
Day 30		
Diet 1 (WL)	86.7±13.3^{a}	7.4±0.7
Diet 2 (WS)	80.0±0.0^{b}	3.8±4.5
Diet 3 (WL + WS)	53.3±6.7^{b}	7.6±0.6

Days of Culture/ Treatment	Parameters	
	Average (± SEM) % Survival	Average (± SEM) FCR
Day 45		
Diet 1 (WL)	80.0±11.6	8.0±1.1
Diet 2 (WS)	66.7±13.3	8.6±0.4
Diet 3 (WL + WS)	53.3±6.7	8.3±0.8
Day 60		
Diet 1 (WL)	80.0±11.6	7.6±0.8
Diet 2 (WS)	66.7±13.3	7.9±1.0
Diet 3 (WL + WS)	53.3±6.7	8.1±0.8

DISCUSSION

Tilapia species are generally herbivores with longer coiled intestines compared to carnivores and mouth characteristics necessary for plant shredding (Trewavas 1982). For this reason, several studies had been conducted to replace feed ingredients in the diet of cultured tilapia in consideration to the increasing costs. Studies including protein replacements and additives from different plant sources like duckweed, *Moringa olifera*, jute, maize and soybeans were already tested with promising results (Ritcher et al. 2003; Borgeson et al. 2006; Nnaji et al. 2010; Magouz et al. 2016; Singh et al. 2016; Caipang et al. 2019). Furthermore, macro and microalgae were already evaluated to replace protein in the diets of different cultured aquatic organisms like shrimps, carp and tilapia (Naorbe et al. 2015; Putri et al. 2017; Montoya-Camacho et al. 2018; Puello-Cruz et al. 2018; Sarker et al. 2018; Singh et al. 2018).

In this study, low-valued plants including water lettuce and water spinach were evaluated as potential feed ingredient for *O. niloticus*. The differences in the ABW of *O. niloticus* fed with three different diets were observed at day 30 and day 60 of culture. Those fed with WL alone had significantly lower ABW compared to those with combined WL + WS. This could be attributed by the characteristic of WL (*P. stratiotes*) as an accumulator of heavy metals in its environment (Varun et al. 2017). Aquatic environment, even those that are unpolluted contains toxic heavy metals primarily from discharges of industrial effluents and from atmospheric precipitation (Chavez et al. 2006). These metals in the form of organic and inorganic compounds can undergo methylation through microorganisms in

the sediments and the end-product can enter the food chain up to the trophic levels (Chavez et al. 2006). These toxic metals can be taken up by fish via the alimentary tract, gills and skin and these were found to cause damage to some vital tissues and organs affecting the fish growth and survival (Schreckenbach 1982; Svobodova et al. 1993). Examples of heavy metals that were found to accumulate in the tissues of *O. niloticus* include Ni and Pb (Nakkina 2016). Water lettuce, like microalgae, is commonly used as phytoremediation agent in heavy metal-contaminated waters despite its promising nutritional contents (Naorbe and Serrano 2018). It was recorded to contain 8.6-20.5% crude protein, 19.1-21.9% crude fibre, 3.8% crude fat and other trace elements like calcium, magnesium, phosphorus, etc. (Ayoade et al. 1982; Banerjee and Matai 1990; Rodriguez et al. 2000; Jimoh et al. 2016). This species however, has varying nutritional contents depending on its environmental source (Varun et al. 2017).

The plants used in this study were collected from a nearby vacant private fish pond (Brgy. Barra, Roxas City, Capiz) that were once used for aquaculture. The location of this fish pond source was adjacent to the community wastewater outlet, thus it is projected that the water and the plant contain heavy metals from the discharged effluents, previous aquaculture inputs and even from the atmospheric precipitation. Furthermore, the analysis conducted by Rodriguez et al. (2000) showed that *P. stratiotes* had low macro-element like phosphorus (0.26%) which is supposedly beneficial in the digestibility of the dietary fibers and better nutrient utilization (Song et al. 2015). These are the probable cause of the lower ABW of the fish compared to those fed in combination with WS (*I. aquatica*) in this study. The ABW of *O. niloticus* nevertheless, continued to increase regardless of the diet being fed through time. This result was further supported by the SGR of *O. niloticus*. Those fed with WS and WL + WS reached up to 2.7±0.1 and 3.0±0.1% day^{-1} at the end of the culture period respectively. This is higher compared to the SGR of *O. niloticus* fed with diet containing green algae *Caulerpa lentillifera* in the study conducted by Putri et al. (2017) which only reached 2.32±0.07 at 10% inclusion but relatively lower than those fed with commercial diet (40% crude protein) with an SGR of 3.72% (Tavares et al. 2008). The slow growth of *O. niloticus* fed with WS (*I. aquatica*) was probably due to its high carbohydrate and mineral contents and its further combination with the nutrients from *P. stratiotes* (Umar et al. 2007). All treatments decreased in survival rate after 30 days and were maintained up to the end of the culture period. Analysis with respect to time, however, revealed that those fed with WL had higher survival rate than those fed with WL + WS also at 30-day culture. In general, the survival rate of tilapia in this study is lower compared to those conducted by Workagegn et al. (2014) on *O. niloticus* fed different types of formulated diets that attained 100% survival rate and those conducted by Ahmad et al. (2009) on the fish fed *Origanium vulgare* extract which also gave 80-100% survival rate. This could be pointed to the absence of other feed ingredients which led

to the lack of other nutrients that could improve the immune system of *O. niloticus*. However, *O. niloticus* fed Diet 1 (WL) had comparable survival rate with those fed fermented and with activator *Lemna minor* and those fed commercial feeds and raised in an earthen pond (Bahnasawy et al. 2003; Pinandoyo et al. 2019). To further improve the survival rate, it is recommended that the protein contents of *P. stratiotes* and *I. aquatic* be concentrated and mixed with other ingredients for definite balanced and complete diet.

Feed conversion ratio (FCR) is a measure of the amount of feeds used to grow each kilogram of a farmed organism. Low values of FCR indicate efficient feeds, low production cost and sustainable process (Martinez-Cordova et al. 2016). In the present study, the FCR of the fish significantly increased from day 30 onwards though the performances of every diet did not vary from each other. Results in this study are higher compared to those fed with commercial feeds at 2.0±0.2 (Bolivar et al. 2011). This is probably due to the high fiber and ash contents of *P. stratiotes* and *I. aquatica*. Fibers are group of complex organic substances mostly found in plants and cannot be digested by enzymes in the intestinal tract of fish and other nonruminant animals, thereby cannot be converted into body mass (Li et al. 2012). The microbial activities in the lower gut of the fish which could aid in the fermentation of this fiber are also limited resulting to the lower dietary nutrient digestibility for growth and metabolism and higher FCR (Glencross 2009). The fibers in the plants used in this study probably reduced nutrient digestibility and absorption and resulted to higher ratio of the converted feeds to flesh (Hilton et al. 1983). The result of FCR in this study is similar to that conducted by Shiau et al. (1989) on Nile tilapia fed a diet containing 10% cellulose that reduced the feed efficiency and glucose absorption of the fish relative to the diet without cellulose. Similarly, high level of ash in the diet could significantly affect the nutrient utilization by fish (Ogunji et al. 2008). Specifically, it could affect the fish dietary apparent digestibility coefficients (ADCs) since these two have inverse relationship or negative correlations (Hajen et al. 1993; Robiana et al. 1997). Apparent digestibility coefficient directs the FCR and another study by Koprucu and Ozdemir (2005) further showed that high content of ash in the diet of tilapia resulted to lower ADCs of dry matter, protein, average amino acid, lipid and energy. This impractical FCR could be addressed by concentrating the protein of both ingredients prior to feed inclusion using either conventional or chemical extraction methods that extract and concentrate the protein from the plants, thereby eliminating other components like fiber and consequently ash (Bleakly and Hayes 2017).

It took 30 days for *O. niloticus* to adjust to the introduced diets as reflected to their survival rate. The experimental diet used in this study had probably poor palatability resulting to feed rejection on the first 30 days of the culture which also reflected on the low weight gain of the fish. Another

prospective factor is again, the high amount of fibers that are usually present in the plants used. Usually, fish like tilapia has low cellulose activity (Saha et al. 2006). This also affects the digestibility and gastrointestinal transit rate in the fish although some amount of fiber is required for optimal utilization of diet and overall growth of the fish (Dioundick and Stom 1990; Lanna et al. 2004). *Oreochromis niloticus* used in this study showed similar survival rate with that of *Tilapia rendalli* in the study of Hlophe and Moyo (2011) which adapted to utilizing higher plants by breaking down the fiber, specifically cell wall and later on to the exposure of the cell contents to the digestive processes. In this study, it probably took 30 days for the culture to develop the digestive enzymes and adapt to the new diets. This is in contrast to the study conducted by Bowen et al. (1998) wherein the food intake of the fish has rapid passage and took shorter time for digestion and ingestion resulting to higher survival rate. Overall, the tilapia accepted all the different diets and showed normal behavior and growth.

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Speaking and listening proficiency of AB English students: basis for instructional material development

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ABSTRACT

This descriptive study sought to find the listening and speaking proficiency levels of the selected AB English students when their scores were taken as a whole and when categorized according to specific listening and speaking microskills. It also aimed to find out particular microskills the students needed to improve on. The research made use of listening and speaking tests patterned from Test of English as a Foreign Language (TOEFL) and Test of English for International Communication (TOEIC). Results showed that when taken as a whole, the students had *Advanced* speaking skills. When categorized according to specific microskill, their proficiency level in recognizing and producing vowel and consonant sounds was *High Advanced*, and *Low Advanced* in controlled and free speaking tests. Improvements may focus on specific microskills: (1) recognizing and producing consonant sounds, (2) employing appropriate vocal variety in rate, pitch and intensity, (3) selecting appropriate organizational pattern according to the topic, context, and purpose, (4) formulating substantial thesis statements supported by well-thought details, and (5) delivering the message in a clear, fluent manner using appropriate nonverbal behavior. In their listening skills, students exhibited *Advanced Level of Proficiency* when their scores were taken as a whole. When categorized according to specific microskills, their proficiency levels are as follows: Literal level- *High Advanced,* Inferential level– *Low Advanced,* and Evaluative level–*Low Advanced.* These results imply that review, planning, implementation of classroom activities and seminars must be carefully re-examined to polish the students' listening and speaking skills. A well-designed instructional material should be planned out for this purpose.

Keywords: English language, microskills, competencies

INTRODUCTION

The important contributions of the English language to the Filipinos cannot be underestimated. As a medium for intellectual pursuits and international communication (Regala 2017), English opens opportunities in overseas employment, outsourcing industry, and enrolment in international schools (UKEssays 2013). This importance is reflected in the Philippine

Constitution where Article XIV Section 7 states "for purposes of communication and instruction, the official languages of the Philippines are Filipino and, until otherwise provided by law, English" (De Leon 2008).

Named as one of the largest English-speaking nations in the world, the Philippines has 90% literacy rate (Hernandez 2015) with most of its population proficient in speaking basic English. However, recent data in Common European Framework of Reference of Language (CEFR) show Filipino university graduates having an average of B1 in language proficiency (Enerio 2018), and having an average of 631.4 in Test of English for International Communication as reported by Hopkin's International Partners (Morallo 2018). These scores attained by Filipino graduates are comparable to 5th or 6th grade students in the United States and United Kingdom.

Because of these alarming reports, the Senate Committee on Education prompted by Resolution 622 filed by Senator Grace Poe looked into the matter as the decline in English proficiency may pose problems in the country's labor force amidst the competitive global market (Romero 2018). Some reasons for the decline include: quality of teachers and English textbooks (Wilson 2009), bad attitude toward the language, lack of practice, (Diaz 2018), and even the use of Filipino and other local languages in the English classroom (Mclean 2010).

On the other hand, Philippine languages have less number of consonants and vowels compared to English. This results to Filipinos having difficulty in producing sounds not present in the inventory of their first language such as schwa /ə/ and voiced consonants /z/, /b/, /v/ and /ð/ (Ryan 2009). Errors because of this discrepancy in segmental inventory are common among second language learners of English as found by Hassan (2014) among students in Sudan.

Aside from the differences in segmental inventory, learning a second language echoes further the suprasegmental features of the first language. These features include stress, intonation, juncture, blending, and even kinesic elements. For example, aside from Filipinos pronouncing words as they are spelled, they also follow a syllable-timed rather than stress-timed rhythm of English (Bautista and Bolton 2009) and rely heavily on the intonation of their first language (Beltran 2015).

This phenomenon where speakers apply knowledge from one language to another language is the core of language transfer theory (Weinreich 1974; Jarvis and Pavlenko 2008). Language transfer may be positive or negative. When relevant unit or structure of both languages is the same, the linguistic interference can result to correct language production called positive transfer. When linguistic interference results to errors, there is negative transfer.

Among Filipinos, the negative transfer often stems from fossilization of the linguistic features of the speaker's first language and sociolinguistic factors especially on the educational background of the speaker.

To reduce errors and improve English language production, Filipinos may begin honing their speaking and listening skills. These skills according to Celik and Yavuz (2015) should always be kept in coordination with one another to guide students' learning process more effectively.

In this study, speaking and listening microskills of university students were described so that the areas of difficulty may be identified and remediated through appropriate classroom activities and instructional materials. Specifically, this study aimed to determine the level of speaking and listening proficiency of the selected university students as a whole and categorized as to specific microskills; and to identify particular microskills where students display difficulty.

METHODS

Research Design

This descriptive study sought to find the level of speaking and listening proficiency of selected university students. According to Best and Kahn (1989), descriptive research involves the descriptive analysis and interpretation of existing conditions.

Profile of the Respondents

The respondents of this study were 31 first year English major students selected through purposive sampling. According to Fraenkel and Wallen (2009), purposive sampling is used when the researchers use personal judgment to select a sample depending upon the researchers' previous knowledge of the population and the purpose of the study.

In this study, respondents were chosen using these criteria: (1) year level and major being pursued (respondent must be a First Year AB English student for the academic year 2015-2016), (2) agreement to be a part of the study, and (3) availability and willingness to undergo speaking and listening tests.

Instruments Used in the Study

This study utilized listening and speaking tests patterned from Test of English as a Foreign Language (TOEFL) and Test of English for International

Communication (TOEIC). Both tests use the General American English which is in consonance with the English used as a standard in almost all Philippine schools and workplaces. The 50-item test was designed considering the listening and speaking microskills from the list of Brown (2001), Bloom's taxonomy of cognitive domain (Anderson and Krathwohl 2001), and competencies set in the AB English program (CHED 2012) (Tables 1 and 2). Both tests were validated by a pool of experts composed of three English teachers who are reviewers of TOEFL and TOEIC.

Table 1. Table of specifications for speaking competency test.

Speaking Competencies	**Bloom's Taxonomy of Cognitive Domain**					
	Remembering	Understanding	Applying	Analyzing	Evaluating	Creating
Vowel and Consonant Sounds 1. Demonstrating knowledge of the vowels and diphthongs 2. Demonstrating knowledge of the consonant sounds 3. Using consonant and vowel sounds correctly and clearly	1-10 11-20		21-30			
Controlled Speaking Task 4. Employing appropriate vocal variety in rate, pitch and intensity 5. Using appropriate paralanguage (pause, emphasis, tone, etc.) 6. Using appropriate kinesic elements (posture, gesture and facial expressions) that achieve congruence and enhance the verbal intent			31-34 35-37 38-40			
Free Speaking Task 7. Selecting appropriate organizational pattern according to the topic, context and purpose 8. Formulating a substantial thesis statement supported by well-thought details 9. Delivering the message in a clear, fluent manner using appropriate nonverbal behavior that supports the verbal message			47-50	41-43 44-46		

Table 2. Table of specifications for listening competency test.

Listening Competencies	Bloom's Taxonomy of Cognitive Domain					
	Remembering	Understanding	Applying	Analyzing	Evaluating	Creating
Literal Level 1. Retaining chunks of information 2. Understanding stated facts	1-10	11-20				
Inferential Level 3. Predicting outcomes 4. Inferring situations		21-30 31-40				
Evaluative Level 5. Drawing logical conclusions and judgments					41-50	

Data Gathering Procedure

After a short orientation session with the respondents, the listening and speaking tests were conducted. For the speaking test in vowel and consonant sounds, students were given 10 minutes to answer the paper-and-pencil test which included choosing words with different underlined vowel and consonant sounds. After this, they were called individually in a separate room to test their vocal production by making them read words containing critical vowel and consonant sounds. For the controlled speaking test, the students read paragraphs to test the appropriateness of vocal variety, paralanguage, and kinesic elements. Lastly, for the free speaking test, students chose one from a set of given questions.

For the listening test in the literal level, the respondents listened to an audio clip once and responded to a four-item multiple-choice test. Because there were five audio-clips with four questions each, the students answered 20 questions. For the inferential listening test, they listened to two audio clips where they predicted outcomes and made inferences by completing sentences based on the text. Lastly, for evaluative level, they listened to an audio clip after which they write conclusions and judgments in a paragraph format.

The students' objective answers in the paper-and-pencil tests for both speaking (20 items) and listening (20 items) were checked. Their videotaped

reading of words with critical vowel and consonant sounds, controlled speaking and free speaking were stored in a flash drive. Paragraphs used as responses to listening tests were photocopied. Both tests were scored by the three evaluators who are English teachers.

Data Analysis

To find out the general picture of the students' listening and speaking proficiency, the researchers subjected the data to statistical analysis using mean, percentage and standard deviation. The mean (±sd) scores were converted to percentages interpreted using the following ten-point scale adapted from the one designed by the American Council on the Teaching of Foreign Languages (Brown 2001):

Percentage		**Description**
1-10	-	Low Beginner
11-20	-	Beginner
21-30	-	High Beginner
31-40	-	Low Intermediate
41-50	-	Intermediate
51-60	-	High Intermediate
61-70	-	Low Advanced
71-80	-	Advanced
81-90	-	High Advanced
91-100	-	Superior

RESULTS

Students' Speaking Proficiency

As a whole, the speaking proficiency of the students is *Advanced*. When the scores were categorized according to the specific microskills, their proficiency in the vowel and consonant sounds is *High Advanced.* However, in controlled speaking and free speaking, students' level of proficiency is *Low Advanced* (Table 3).

As to specific microskills in vowel and consonant sounds, the students fare better in recognizing vowels and diphthongs (High Advanced) than in recognizing consonants (Advanced). Students may have used spelling as a clue in recognizing similar vowel sounds such as /i/ in *wheat, beat,* and *meet*, /a/ in *shock* and *stock,* and /o/ in *oat* and *oath* (Table 4).

In producing critical vowel and consonant sounds, the students have *High Advanced* level of proficiency with most (86.9%) of them having no

problems pronouncing sounds found also in their first language such as vowels /ɛ/, /a/, /i/, and /u/, and consonants /b/, /p/, /k/, /g/ and /ŋ/ (Table 4).

Table 3. The percent mean (±sd) speaking proficiency of AB English students. Scales and Description: 1-10—Low Beginner, 11-20—Beginner, 21-30—High Beginner, 31-40—Low Intermediate, 41-50—Intermediate, 51-60—High Intermediate, 61-70—Low Advanced, 71-80—Advanced, 81-90—High Advanced, 91-100—Superior.

Categories	Percent Mean (±sd)	Description
Whole	72.4 (±5.60)	Advanced
Vowel and Consonant Sounds	83.3 (±2.2)	High Advanced
Controlled Speaking	70.3 (±1.4)	Low Advanced
Free Speaking	69.5 (±0.8)	Low Advanced

Table 4. The percent mean (±sd) speaking proficiency of the AB English students when scores are categorized as to specific microskills. Scales and Description: 1-10—Low Beginner, 11-20—Beginner, 21-30—High Beginner, 31-40—Low Intermediate, 41-50—Intermediate, 51-60—High Intermediate, 61-70—Low Advanced, 71-80—Advanced, 81-90—High Advanced, 91-100 – Superior.

Categories	Percent Mean (±sd)	Description
Vowel and Consonant Sounds		
1. Demonstrating knowledge of the vowels and diphthongs	83.1 (±1.3)	High Advanced
2. Demonstrating knowledge of the consonant sounds	80.0 (±1.1)	Advanced
3. Using consonant and vowel sounds correctly and clearly	86.9 (±1.5)	High Advanced
Controlled Speaking Task		
4. Employing appropriate vocal variety in rate, pitch and intensity	69.0 (±0.5)	Low Advanced
5. Using appropriate paralanguage (pause, emphasis, tone, etc.)	72.6 (±0.5)	Advanced
6. Using appropriate kinesic elements (posture, gesture and facial expressions) that achieve congruence and enhance the verbal intent	70.9 (±0.5)	Advanced

Free Speaking Task		
7. Selecting appropriate organizational pattern according to the topic, context and purpose	68.8 (±0.3)	Low Advanced
8. Formulating a substantial Thesis statement supported by well-thought details	69.9 (±0.4)	Low Advanced
9. Delivering the message in a clear, fluent manner using appropriate nonverbal behavior that supports the verbal message	69.8 (±0.8)	Low Advanced

In controlled speaking test, the students' proficiency in microskill 4 is *Low Advanced* with some (69.0%) of them attempting to approximate the American stress-timed rhythm with some notable vocal variety. In microskills 5 and 6, students' proficiency is *Advanced* with some (72.6%) of them employing some appropriate paralanguage and a few (70.9%) using appropriate kinesic elements (Table 4).

Lastly, in free speaking test, the students' proficiency in microskills 7, 8, and 9 is *Low Advanced* which shows that they need more improvement in organization, formulation of substantial thesis and details and delivery (Table 4).

Students' Listening Proficiency

As a whole, the listening proficiency of the students is *Advanced*. When the scores were categorized as to the specific microskills, their proficiency in the literal level is *High Advanced* while in inferential and evaluative levels, their proficiency is *Low Advanced* (Table 5).

Table 5. The percent mean (±sd) listening proficiency of AB English students. Scales and Description: 1-10—Low Beginner, 11-20—Beginner, 21-30—High Beginner, 31-40—Low Intermediate, 41-50—Intermediate, 51-60—High Intermediate, 61-70—Low Advanced, 71-80—Advanced, 81-90—High Advanced, 91-100-Superior.

Categories	Percent Mean (±sd)	Description
Whole	77.9 (±3.4)	Advanced
Literal Level	85.4 (±2.9)	High Advanced
Inferential Level	63.9 (±3.2)	Low Advanced
Evaluative Level	64.8 (±1.0)	Low Advanced

The students have *High Advanced* proficiency in all specific microskills in the literal level of listening skill. This shows that students can retain information listened to and understand stated facts. Their exposure to movies, advertisements, and other audiovisual materials at home, in school and even online must have scaffolded their familiarity of American English (Table 6).

In inferential and evaluative levels, however, the students' level of proficiency ranges from *Low Advanced* to *Advanced*. Lastly, in the evaluative level, the students' responses show they still need to improve making conclusions and sound judgments based on audio materials listened to, and to minimize the urge to simply write down and condense the information (Table 6).

Table 6. The percent mean (±sd) listening proficiency of the AB English students when scores are categorized as to specific microskills. Note: Scales and Description—1-10—Low Beginner, 11-20—Beginner, 21-30—High Beginner, 31-40—Low Intermediate, 41-50—Intermediate, 51-60—High Intermediate, 61-70—Low Advanced, 71-80—Advanced, 81-90—High Advanced, 91-100 – Superior.

Categories	Percent Mean (±sd)	Description
Literal Level		
Retaining chunks of information	84.1 (±1.8)	High Advanced
Understanding stated facts	86.7 (±1.6)	High Advanced
Inferential level		
Predicting outcomes	73.7 (±1.9)	Advanced
Inferring situations	67.9 (±1.7)	Low Advanced
Evaluative Level		
Drawing logical conclusions and sound judgments	64.8 (1.9)	Low Advanced

Microskills where Students Display Difficulty

For controlled speaking task. Among the microskills under the controlled speaking task, in employing appropriate vocal variety in rate, pitch and intensity, the students' speaking task is *Low Advanced*. Most (69.0%) of the students read the paragraph word for word, a discrepancy most attributed to the syllable-timed rhythm of their first language. They also gave equal weight to each word which makes their manner of speaking to take the staccato effect.

For free speaking task. All microskills under free speaking task have *Low Advanced* levels which may not pass in global standards. In microskill 7, most (68.8%) of the students did not establish distinct organizational pattern in their answers. They simply began by reading the questions, then transforming the questions into statements while others used transition words such as *first, second, third, then, finally*.

In microskill 8, most (69.9%) of the students failed to advance a convincing, persuasive, and substantial thesis statement that shows their argument. By not having a strong thesis statement, their discussions have become fragmented.

In microskill 9, a number (69.8%) of students jumped from one idea to the next, and sometimes got lost in the middle of their answers as shown in very long pauses and fillers such as *ah, uhm* and *you know*. Further, very few of them employed nonverbal behavior like appropriate hand gestures and facial expressions that go with the verbal message.

For listening. In inferring situations, 67.9% of the students showed difficulty in using real-world knowledge to make sound inferences. This may be due to their heavy reliance on a multiple-choice type of test, lack of exposure to the listening task and a limited number of listening strategies.

In drawing logical conclusions and sound judgments, 64.8% of the students showed a tendency to simply copy stated facts rather than provide a substantial explanation or description.

DISCUSSION

Students' Speaking Proficiency

As a whole, the students speaking proficiency is *Advanced*. This finding concurs with the findings reported in the study of Hernandez (2015) which posits that Filipinos can speak, read and write at a rudimentary level. However, this level at 72.4% proficiency rate is barely passing in international standards such as in TOEIC, a result which is similar to the findings of other studies like Enerio (2018) and Morallo (2018).

In the vowel and consonant sounds, students' speaking task is *High Advanced*. At 83.3% proficiency rate, the students have successfully identified critical American vowel and consonant sounds. This may be attributed to the similarities in vowel and consonant inventories between English and

Philippine languages (Tayao 2011) and the positive transfer of these in speaking English.

In controlled speaking and free speaking, however, the result is *Low Advanced*. At just 70.3% for controlled speaking and 69.5% for free speaking. This may be due to the students' lack of self-confidence (Pangket 2019), limited vocabulary (Leong and Ahmadi 2017), and less opportunities to speak in front of an audience.

Shen (2013) and Wang (2014) highly encouraged the exposure of the students to public speaking exercises through workshops and trainings.

Students' Listening Proficiency

As a whole, students listening proficiency is *Advanced*. At 77.9% proficiency rate, this result is in consonance with what Tendero (2008) found among Filipino college students whose listening skill is merely passing.

For the literal level, students have *High Advanced* listening proficiency. Remembering information listened to and retaining this to short-term memory (Talwar et al. 2018), having adequate vocabulary, and exposure to American English are some possible factors for the 85.4% proficiency rate.

On the other hand, students have *Low Advanced* level of proficiency in both Inferential and Evaluative Levels. This result may be due to the rate of delivery and unfamiliar vocabulary (Stepanoviene 2012), speaker's unfamiliar accent, listeners' lack of background knowledge on the topic, failure to concentrate, and lack of interest to listen (Hamouda 2013).

To help students improve their listening proficiency, teachers may need to expose students to a variety of listening exercises (Eken and Dilidüzgün 2014; Al-Nafisah 2019), teach them bottom-up, top-down, and interactive listening strategies (Field 2004; Yeldham 2018) and explore both traditional and latest instructional materials and methods in listening (Ma 2010).

Microskills where Students Display Difficulty

For controlled speaking task. While the students' utterances were still comprehensible, their speech did not show appropriate use of vocal variety in rate, pitch, and intensity which can affect emphasis in meaning or alter pragmatic functions. They read the paragraph word for word, a discrepancy most attributed to the syllable-timed rhythm of the students' first language which differs to the stress-timed rhythm of English (Bautista and Bolton 2009). Moreover, most students use Hiligaynon accent delivered

without using American English juncture and blending. This negative transfer of intonation from the first language to English is also observed by Beltran (2015) among Filipino student teachers.

For free speaking task. In microskill 7, the students' proficiency level shows that there is a lack of organizational pattern that highlights their important points and establishes a cohesive speech. Because of this, their speech seems to be beating around the bush. This result is similar to the findings of Lockwood et al. (2008) among Filipino call center agents whose speaking differ from the deductive structure and linear pattern used by Americans.

In microskill 8, the students' level of proficiency is at 69.9% only. The difficulty in making thesis statements is in contrast to what Dunbar et al. (2006) found among American students whose thesis statements in a public speaking class were above satisfactory standards.

In microskill 9, the students' speeches showed a need for improvement, a result which is similar to what Lasala (2014) found among Filipino secondary senior students.

Since wage disparity is another problem Filipino graduates might face in working abroad, there is a need for them to improve on these microskills. According to Wongsamuth (2015) even when a Filipino manages to secure a job, rarely does their pay come close to that of a white candidate. In the study of Furnham and Wilson (2011) over half of participants believe wage disparities exist between men and women; whites and blacks.

Thus, to improve students' speaking proficiency and prepare them better to be globally competitive, they need to be given more opportunities to enhance these microskills through seminars on various speaking tasks (Albino 2017) and techniques (Moradi and Talebi 2014), fluency training (de Jong and Perfetti 2011) and speaking workshop (Emandi 2015).

For listening. Filipinos' problem in listening comprehension specifically on predicting outcomes and inferring situations may be due to unfamiliar vocabularies and difficult grammar structure (Cubalit 2016). As second language learners, they struggle with getting a general understanding of what has been said. Capan and Karaca (2013) concluded that the inability of the learners to comprehend is a factor aggravating learners' listening comprehension level.

In drawing logical conclusions and sound judgments, the students' responses showed that they focus more on copying or summarizing facts from the audio material, and they have not bothered to make sound judgments to

make sense of the information. This may be due to lack of exposure to listening tasks that encourage evaluation which has a similar result found among Turkish students in the study of Saricoban and Karakurt (2016).

To improve their level of listening proficiency, students need to be exposed to varied listening tasks such as form-filling, sentence completion, summary completion, description and evaluation of spoken texts (Graham 2016). The most salient recommendation of the study is making an instructional material for speaking and listening (Tavil 2010; Yang et al. 2012). This material may include a variety of activities that are found to improve students' proficiency both in their speaking and listening skills (Gowhary et al. 2015; Aji 2017).

English has been perceived as related to one's economic status, intelligence, and employment (Bacon and Kim 2018) both locally (Beerepoot and Hendriks 2013) and internationally (TESDA 2011). Global competitiveness has also accelerated the importance of oral communication skill and is cited as one of the most desired graduates' employability skills (Jackson 2014). Having impeccable listening and speaking skills in English are among the most important skills that help graduates get high paying jobs (Pandey and Pandey 2014). Lastly, acquiring communicative and linguistic competences becomes a "must" in view of a more flexible and smoother insertion into the labor market worldwide (Greculesco et al. 2014).

In the 2011 tracer study (Yap et al. 2012) conducted among the AB English graduates, all 74 respondents were employed, but only 10% of them landing a job abroad and having a monthly salary of over PhP 25,000. Therefore, the researchers believed that to improve their students' chances of getting high-paying jobs both in the country and abroad, enhancing their communication skills in English should be in place.

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High density of *Tridacna crocea* in Rita Island, Puerto Princesa City, Palawan, Philippines

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ABSTRACT

In spite of being a protected species in the Philippines, the *Tridacna crocea* or crocus clam, the smallest among eight known giant clam species in the country, is getting rare in most reef areas of Palawan. However, a high density of this species has recently been noted in Rita Island, Ulugan Bay. A total of 44 photos with known dimensions having a total area of 13.26 m^2 suitable habitats with clams were analyzed for size structure and density of *T. crocea*. In total, 215 *T. crocea* were noted with 84.91 (±25.6) mm average shell length (±sd). The average density (±sd) was 16.22 (±15.75) individual (ind)·m^{-2} but the clams had occurred up to 17 individuals in 0.28 m^2 or 59.91 ind·m^{-2}. This high density of *T. crocea* could be due to suitable environment and the absence of exploitation within the vicinity of the island resort. The importance of other resorts in biodiversity conservation may be investigated.

Keywords: abundance, bivalves, giant clams, resort, shell length

Giant clams (Cardiidae: Tridacninae) are closely associated with the coral reefs and widely distributed throughout the Indo-Pacific region (bin Othman et al. 2010). These iconic invertebrates perform various important ecological roles such as food, shelter and capacity to promote a balanced reef ecosystem (Soo and Todd 2014; Neo et al. 2015). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the International Union for Conservation for Nature (IUCN) and several Philippine policies call for the protection of the giant clam species (DA 2001). However, several factors including intense anthropogenic pressure (Neo and Todd 2012), exploitation for human consumption, and illegal fishing (bin Othman et al. 2010) remained prevalent and had caused the depletion of giant clam populations (Neo et al 2017; Ramah et al. 2018).

Tridacna crocea or crocus clam, which could attain a maximum shell length of 15 cm, is the smallest among the eight known species of giant clams in the Philippines (Dolorosa et al. 2015c; Neo et al. 2015; 2017; Ecube et al. 2019;). It is highly polymorphic and cryptic in habit to avoid its mantle from being attacked by predators (Todd et al. 2009). Although, this species is the most abundant giant clam species (Juinio-Meñez et al. 2003) they are now seldom encountered in many reefs of Palawan (Dolorosa et al. 2015a,b). This

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notes provide information on the size structure and density of *T. crocea* in Rita Island, Puerto Princesa City, Palawan.

Rita Island (10 °4'27.42"N; 118 °46'49.32"E) in Ulugan Bay, Puerto Princesa City is one of the emerging tourist destinations in the western coast of Palawan. The island is surrounded by sloping sandy, rocky substrate with some patches of corals. It is also known as sea turtles nesting site (PCSDS 2006). During a visit on the island on 4 May 2019 to confirm the presence of the largest giant clam *Tridacna gigas*, photos of suitable habitats with *T. crocea* were taken while SCUBA diving. As a size reference, a polyvinyl chloride (PVC) rod was placed beside the clams before each photo was taken. The area of the habitat covered by the photo and the sizes of the clams present were determined using Coral Point Count with Excel extension ver. 4.1 (Kohler and Gill 2006; Figure 1). The density was determined by dividing the number of clams with the area covered in each photo.

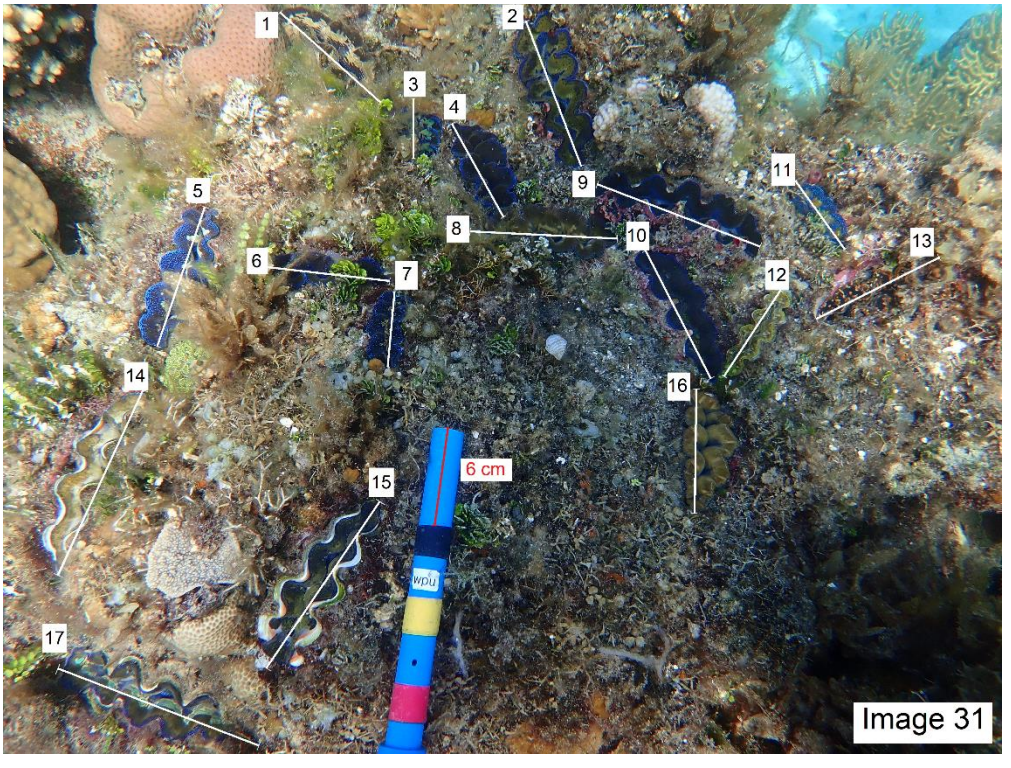

Figure 1. A photo containing 17 individuals of *Tridacna crocea* within 0.28 m², and a polyvinyl chloride (PVC) tubing used as size reference.

Out of 48 photos, 44 were selected based on the presence of *T. crocea*. In total, 215 individuals were recorded. The population is normally distributed as reflected in Figure 2. The size range (21.4 mm to 147.6 mm) and average

shell length (±sd) of 84.91 (±25.6) mm in Rita Island was larger compared to Tubbataha Reefs Natural Park where *T. crocea* measured between 10 mm and 135 mm with an average shell length (±sd) of 67.67 (±32.26) mm (see Conales et al. 2015).

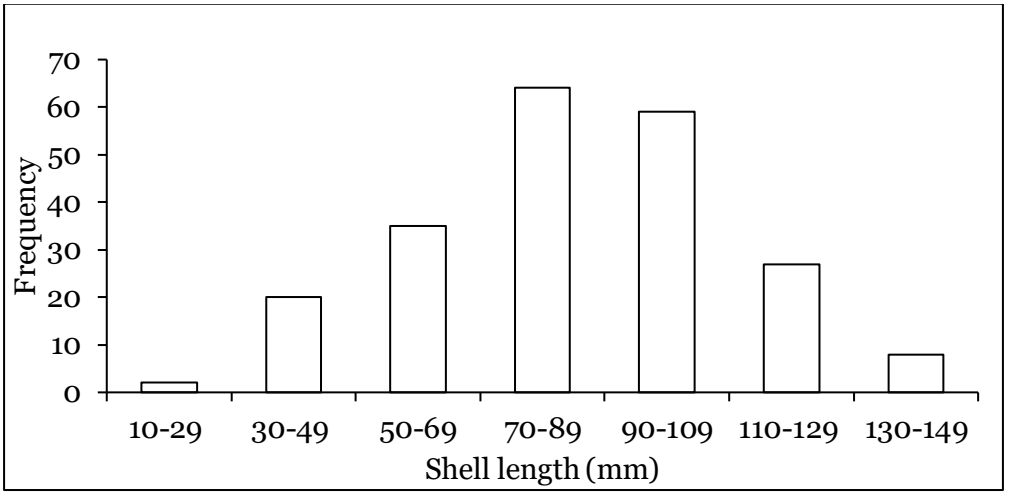

Figure 2. Size structure of *T. crocea* (n=215) in Rita Island, Puerto Princesa City, Palawan, Philippines.

The evaluated photos had a total area of 13.26 m^2, thus representing an average density (±sd) of 16.22 (±15.75) ind·m^{-2}. Highest density was recorded in image 31 with 17 individuals in 0.28 m^2 or 59.91 ind·m^{-2} while lowest density was recorded in image 21 with only 1 individual in 0.37 m^2 or 2.69 ind·m^{-2}.

Tridacna crocea are known to occur at high densities as also been reported in Tubbataha Reefs Natural Park where the clams near the ranger station had an average (±sd) density of 7.67 (±5.59) ind·m^{-2} (Conales et al. 2015). In the coastal area of Bay Canh Island and Cau Island, South China Sea, the highest densities of *T. crocea* could occur up to 23 ind·m^{-2} and 25 ind·m^{-2} respectively (Selin and Latypov 2011).

The high density of *T. crocea* in Rita Island could be due to the suitability of environmental factors and the absence of harvesting within the vicinity of the resort. In the past, the adjacent oyster inlet was observed to host substantially high densities of various species of giant clams (BJ Gonzales pers. comm.). Continued protection of the area is recommended to ensure the safety of these marine bivalves, and for them to serve as natural sources of recruits for nearby areas. Studies on genetic structures of *T. crocea* population and the potential of other resorts in protecting these vanishing species may be investigated.

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Guide for Authors

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Punctuations

1. Unfamiliar terms, abbreviations, and symbols must be defined/spelled out at first mention.
2. Mathematical equations should be clearly presented so that they can be interpreted properly. Equation must be numbered sequentially in Arabic numerals in parentheses on the right-hand side of the equations.

3. Numbers lesser than 10 should be spelled out (for example: eight trees, 10 fish) except when followed by a unit of measure (for example: 9 cm, not nine cm). Numbers should be spelled-out when starting in a sentence (example: Nine fishermen were…).
4. No apostrophes in years (example: 2014s not 2014's)
5. No periods in acronyms (example: UNESCO not U.N.E.S.C.O.; CITES not C.I.T.E.S.)
6. Write dates in this manner: day-month-year (example: 20 October 2012 or 20 Oct 2012).
7. Use the International System of Units of measurements. Separate the value and the unit of measure (example: 5 mm, 25 g, 30 m^3, 100 µm, 9 ind ha^{-1}, 10 sacks ha^{-1}, 2 kg h^{-1} day^{-1}). To fix a single space between the value and its unit of measure, use the MS word command "CTR+SHIFT+SPACE BAR" to provide a space between the value and its unit of measure.
8. Do not separate a percent sign with the number (example: 5%, 30%).
9. Use 24-h system for time (example: 13:00 instead of 1:00 pm). To express a measured length of time, abbreviations for hour (h), minutes (min) and seconds (sec) will be used (example: 2 h and 30 min; or 2.5 h).
10. Use a single capital letter when writing latitude and longitude (example: 9°44'27.80"N and 118°41'2.01"E).
11. Compass points (north, south, east, west) and their derivations (northern, southern, eastern, western) are lowercased (example: north of Palawan) except when they form part of the place name (example: South Cotabato; Eastern Samar).

References

1. References to the literature citations in the text should be by author and year; where there are two authors, both should be mentioned; with three or more authors, only the first author's family name plus "et al." need be given. References in the text should be cited as:
    - Single author: (Frietag 2005) or Frietag (2005)
    - Two authors: (De Guzman and Creencia 2014) or De Guzman and Creencia (2014)
    - More than two authors: (Sebido et al. 2004) or Sebido et al. (2004).
2. Use a semi-colon followed by a single space when citing more than two authors. Arrange by date of publication with the latest being the last in the list (example: Sebido et al. 2004; Frietag 2005; De Guzman and Creencia 2014).
3. Use a comma followed by a single space to separate citation of different references authored by the same author (example: Jontila 2005, 2010). If the same author and year are cited, use a "letter" to distinguish one paper over the other (example: Creencia 2010a,b).
4. Alphabetize authors with the same year of publications. Use semi colons to separate each publication (example: Balisco and Babaran 2014; Gonzales 2014; Smith 2014).
5. Write journal's name in full (examples: The Palawan Scientist, not Palawan Sci; Reviews in Fisheries Science, not Rev. Fish. Sci.).
6. The list of citation at the end of the paper should include only the works mentioned in the text and should be arranged alphabetically.
7. Citing journal articles– name(s) and initial(s) of author(s), year, full title of research article (in sentence form), name of the journal (not abbreviated),

volume number, issue number (if given), range of page numbers, DOI number (if available) and/or web link:

Dolorosa RG, Grant A and Gill JA. 2013. Translocation of wild *Trochus niloticus*: prospects for enhancing depleted Philippine reefs. Reviews in Fisheries Science, 21(3-4): 403-413. DOI: 10. 1080/ 10641262. 2013. 800773.

Jontila JBS, Balisco RAT and Matillano JA. 2014. The sea cucumbers (Holothuroidea) of Palawan, Philippines. AACL Bioflux, 7(3): 194-206. http://www.bioflux.com.ro/docs/2014.194-206.pdf

8. Citing of books – name(s) of author(s), year of publication, full title of the Book (capitalize each main word), publisher, place of publication and total number of pages.

Gonzales, BJ. 2013. Field Guide to Coastal Fishes of Palawan. Coral Triangle Initiative on Corals, Fisheries and Food Security, Quezon City, Philippines. 208pp.

9. Citing a chapter in a book – name(s) of author(s), year, full title of the chapter in a book (capitalize each main word), last name of editor and title of book, edition, publisher, place of publication and page range of that chapter:

Poutiers JM. 1998. Gastropods. In: Carpenter KE and Niem VH (eds). FAO Species Identification Guide for Fishery Purposes. The Living Marine Resources of the Western Central Pacific Seaweeds, Corals, Bivalves and Gastropods. Food and Agriculture Organization, Rome, pp. 364-686.

10. Citing a Webpage – names of the author (s), year, Title of the article, webpage address and date accessed.

Morrison H and Pfuetzner S. 2011. Australia Shells. http://www.seashells.net.au/Lists/ TEREBRIDAE. html. Accessed on 4 September 2011.

CITES (Convention on International Trade of Endangered Species. 2014. The CITES Appendices. Convention on International Trade in Endangered Species of Wild Flora and Fauna. www.cites.org. Accessed on 5 January 2014.

11. Citing a thesis or dissertation – author's family name, initial names of the author, year, title of the thesis, degree, name of institution, address of the institution, total number of pages (pp).

Guion SL. 2006. Captive breeding performance of *Crocodylus porosus* (Schneider 1901) breeders at the Palawan Wildlife Rescue and Conservation Center. BS in Fisheries. Western Philippines University-Puerto Princesa Campus, Palawan, Philippines. 28pp.

Lerom RR. 2008. Biosystematics study of Palawan landraces of rice (*Oryza sativa* L.). Doctor of Philosophy, Institute of Biological

Sciences, University of the Philippines-Los Baños College, Laguna, Philippines. 197pp.

12. Citing a Report

    Picardal RM and Dolorosa RG. 2014. Gastropods and bivalves of Tubbataha Reefs Natural Park, Cagayancillo, Palawan, Philippines. Tubbataha Management Office and Western Philippines University. 25pp.

13. In Press articles when cited must include the name of the journal that has accepted the paper.

    Alcantara LB and Noro T. In press. Growth of the abalone *Haliotis diversicolor* (Reeve) fed with macroalgae in floating net cage and plastic tank. Aquaculture Research.

14. Citing an article from an online newspaper.

    Cuyos JM. 2011. Endangered deep-sea shells seized from Mandaue firm. Inquirer Global Nation, Cebu. http://globalnation.inquirer.net/cebudailynews/news/view/20110325-327558/Endangered-deep-sea-shells-seized-from-Mandaue-firm. Accessed on 31 May 2012.

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