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Cover Photo

A school of Yellowbanded sweetlips *Plectorhinchus lineatus* in Black Rock, Tubbataha Reefs Natural Park, Cagayancillo, Palawan, Philippines. The biomass of this species could be estimated using established parameters from length-weight relationship studies. The study of Palla et al. in this volume presents data from a closely related species, *Plectorhinchus pictus* where parameter estimates for *P. lineatus* could be based from (Photo by: RG Dolorosa).

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EDITORIAL

The Palawan Scientist Journal, a recipient of the CHED Journal Incubation Grant, and listed in the Asian Citation Index, is gaining the respect of researchers within and outside the Philippines. This is manifested by the diversity of papers and author's affiliations in this 10th volume of the journal.

The paper of Lawrence M. Lao of Hiroshima University on marine algae of the Cuyo Islands found that there are untapped marine resources particularly the red algae that could be exploited commercially. An article by Herminie P. Palla and co-authors provided the first comprehensive information on length-weight relationships for 87 fish species in the province of Palawan. Researchers can now, therefore, compute fish biomass in Palawan without using parameter estimates from other countries.

Abegail L. Gonzales of Batangas State University found no significant relationships between self-efficacy and technological, pedagogical, and content knowledge of senior high school biology teachers in Batangas City. She suggested specific training designs for faculty development programs including the conduct of similar studies in other fields.

Jhonamie Mabuhay-Omar and co-authors determined the effects of two combinations of forest management practices applied on replacement pine plantations after the occurrence of pine wilt disease in Hiroshima, Japan. The variations among sites in terms of soil chemical properties, relative light intensity difference, microbial biomass and abundance, and the high correlations among biological and physico-chemical properties of soil led to a conclusion that there is high interdependence among soil's characteristics.

Edgar D. Jose reported a developed protocol in propagation, reforestation and sustainable management of almaciga (*Agathis philippinensis*), a vulnerable resin-producing tree species valuable to the indigenous community as a source of income. An article on rapid assessment of mammalian fauna of Cleopatra's Needle Critical Habitat done by Paris N. Marler and co-authors found some rare and vulnerable species suggested the importance of Cleopatra's Needle as critical habitat in Palawan.

We are thankful to these authors for choosing the Palawan Scientist in making their works globally accessible. May their works be of great value to those working in similar fields.

Let's continue in making the knowledge work to influence and inspire others.

CONGRATULATIONS!!

Romeo R. Lerom, PhD
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Marine algae of the Sulu Sea islands, Philippines III. Taxonomic account of the Gracilariaceae (Rhodophyta) from the Cuyo Islands

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ABSTRACT

The Cuyo Islands lie on the northern fringes of the vast Sulu Sea straddling between the westernmost Philippine island of Palawan and the central portion of the archipelago. A recent study revealed a high diversity of the benthic brown algae (Phaeophyceae) there. However, records of other algal groups like the green and red algae have been sporadic and few. This study is a survey of the representatives of the Gracilariaceae, a group of economically-important red algal species valued for its agar content. Collections were made by snorkeling and Scuba diving from shallow-water and subtidal habitats from 16 stations in six islands. Results revealed a total of five species of the Gracilariaceae distributed throughout the Cuyo Islands. As these species constitute a significant but untapped marine resource for commercial exploitation, the results of this survey will serve to provide useful baseline information for resource management, eventually contributing towards better livelihood generation and options in coastal villages.

Keywords: *Gracilaria*, *Gracilariocolax*, *Hydropuntia*, Palawan, seaweed, taxonomy

INTRODUCTION

Numerous islands, atolls and reefs are scattered throughout the vast expanses of the Sulu Sea on the western fringes of the Philippine archipelago. The most well-known among these are the uninhabited Tubbataha Reefs, a pair of pristine atolls lying close to the geographic center of the Sulu Sea. Their biological importance as habitats for diverse marine life led to their inscription as a UNESCO World Heritage Site in 1993 and their inclusion in the Ramsar List of Wetlands of International Importance in 1999. On the other hand, the other island groups in the Sulu Sea are populated by people of varied ethnolinguistic backgrounds who mainly depend on the sea for daily subsistence through such activities as seaweed farming and fishing.

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A historical account of seaweed taxonomic studies in the Sulu Sea islands covering the latter half of the 20th century was provided by Liao and Young (2002) who also compiled a comprehensive synoptic list of seaweeds from the Tubbataha Reefs. Since that first survey, two latter studies have been added. Liao et al. (2013) studied 17 species of benthic brown algae (Phaeophyceae) from the Cuyo Islands, many of them typical reef-dwelling species of the Dictyotaceae and the massive tropical species of *Sargassum* that frequently form extensive beds. Later, Santiañez et al. (2015) reported 176 species of seaweeds from another group of islands collectively called the Balabac Marine Biodiversity Conservation Corridor (BMBCC) located on the westernmost flanks of the Sulu Sea just off the southern tip of Palawan Island. Adjacent to the BMBCC is Bugsuk Island where an earlier seaweed survey was conducted and published by Trono and Ang (1982).

The Gracilariaceae is a large cosmopolitan group of marine red algae that is predominantly tropical in distribution. It consists traditionally of eight genera including both free-living and parasitic representatives, with the total number fluctuating and bloating to 13 genera in recent times due to continuing revisions based on morphological and molecular grounds (Liao and Hommersand 2003; Iyer et al. 2005; Yang and Kim 2015). Agar is the main commercial polysaccharide extracted from the cell wall of species of the Gracilariaceae and finds its use in the food and biotechnology industry. This has resulted in extensive efforts to harvest raw materials from natural stocks or its cultivation particularly near estuarine habitats around Southeast Asia (Guanzon and De Castro 1992).

This study serves to document the diversity and distribution of species of the Gracilariaceae found in the Cuyo Islands by providing morphological information that will help with species identification. The information represents a contribution towards producing a list of species with potential economic importance that can guide resource management planning. The same information will also contribute data to the knowledge of the taxonomy and phytogeography of the Gracilariaceae in the Philippines.

METHODS

Study Sites

The Cuyo Islands form part of the political province of Palawan and is comprised of three municipalities namely, Agutaya, Cuyo and Magsaysay. Six islets were visited, and 16 sampling stations were established. The details of the collecting sites and dates can be found in Liao et al. (2013).

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Collection and Identification

Samples were collected by snorkeling and Scuba diving and taken to the laboratory for processing. Part of the samples were pressed to make herbarium specimens while some were preserved in 5% formalin-seawater solution for anatomical studies. Hand sections with double-edged razor blades were made to observe internal anatomy under a compound microscope. All prepared voucher specimens (herbarium materials) were distributed for permanent deposition in ten Philippine and foreign herbaria. For the complete list, refer to Liao et al. (2013). Various taxonomic references and authentic herbarium materials were examined for comparison. Specimens are assigned distinctive code numbers following the designated collecting site numbers as described earlier in Liao et al. (2013). For example, 78EM-43 (68-73) means materials were obtained from station 78EM-43 and consisted of six samples numbered serially from 68 to 73.

RESULTS AND DISCUSSION

Taxonomic Account

Artificial key to the species of the Gracilariaceae of the Cuyo Islands

1A Plants pustulate, occurring on surfaces of larger algae,
parasitic..***Gracilariocolax* sp**.
1B Plants erect, attached to substrate by holdfast, free living 2
2A Thalli bearing distinct constrictions along the main axis 3
2B Thalli smooth throughout, without distinct constrictions 4
3A Thalli filiform, with faint constrictions at the base of
branches..***Hydropuntia edulis***
3B Thalli robust, with prominent constrictions throughout the thalli, issued at
relatively uniform intervals ....***Gracilaria salicornia***
4A Branching prominently dichotomous, diameter of segments relatively uniform
throughout, up to 3 mm in diameter ... ***Gracilaria canaliculata***
4B Branching irregular, becoming secund distally, diameter at the base
up to 5 mm, tapering into acute and bent tips...***Gracilaria arcuata***

Gracilaria arcuata Zanardini

Zanardini 1858: 265, pl. 5, fig. 2 (type locality: Mari Rubro, or the Red Sea); Weber-van Bosse 1928: 429, fig. 173; Cordero 1977: 124, pl. XX, B, fig. 101; Cordero 1981: 55, fig. 18A; Trono et al. 1983: 32, fig. 7; Trono 1997: 208, fig. 133; Hurtado et al. 2006: 10, middle figure.

Illustrative materials: Tsutsui et al. 2005: 194, figs. 1-3; Lin 2009: 10, figs. 2a-c; Wang et al. 2015: 92.

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Plants forming low tufts, to 7 cm tall, with some parts repent, attached to rocks and reef debris at several points; fronds terete, 4-5 mm in diameter at the base where faint constrictions may be observed occasionally, becoming slightly narrowed distally and markedly arcuate towards the tips; branching dichotomous to irregular, sometimes secund near the acute apices, intricate; medulla made up of loose cells, cuboidal, thin-walled, to 600 µm in diameter; cortical cells two layers, oblong, arranged side by side on their longer sides.

Specimens examined: 78EM-14 (98-104); 78EM-16 (101-104); 78EM-18 (283-290, 517-523); 78EM-20 (277-279); 78EM-43 (68-73); 78EM-45 (183, 184)

Remarks: Materials examined are of two forms. One is the more common form that is well-branched, with distinctly arcuate and intricate tips like 78EM-14 (98-104) and 78EM-20 (277-279). This is the var. *snackeyi* Weber-van Bosse first described from Indonesia. The other form is less branched at the distal portions, with acute or round tips and inconspicuously arcuate like 78EM-16 (101-104) and 78EM-43 (68-73), thus approaching var. *arcuata*. The latter form was originally described from the Red Sea, and recorded mainly from the western Indian Ocean and the Mediterranean. It is much slender and elongated (as observed in authentic materials from Tunisia) than the Cuyo materials under study. It appears then that var. *snackeyi* is the most common form encountered in the East Indies.

Chang and Xia (1976) observed both forms in materials from the South China Sea. However, their text-fig. 13-1 labelled as var. *typica* (=*arcuata*) is no different from the var. *snackeyi* as illustrated by Weber-van Bosse (1928), while text-fig. 13-2 identified as var. *snackeyi* f. *rhizophora* Børgesen resembles closely var. *arcuata* except for its thicker diameter.

Gracilaria canaliculata **Sonder**

Sonder 1871: 56 (type locality: Wagap, New Caledonia); Calumpong and Meñez 1997: 179, middle figure; Trono 2004: 81, fig. 134.

Synonym: *Gracilaria crassa* Harvey ex J. Agardh 1876: 417; Cordero 1977: 127, figs. 108-110; Cordero 1981: 56, fig. 20A

Illustrative materials: Hurtado et al. 2006: 10, bottom figure; Coppejans et al. 2009: 168, figs. 10D, 23D, 135; Lin 2009: 14, figs. 4a, b.

Thalli consists of erect and prostrate portions, the latter appressed to small rocks and coral fragments and closely attached by hapters at many points; fronds terete, to 3 mm in diameter, marked by many faint

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constrictions here and there, segments between constrictions smooth, segment tips tend to be slightly swollen and blunt; branching di- to trichotomous and irregular, angle of branching usually wide, between 60-90°with point of branching rarely marked off by constrictions; medullary cells polygonal in cross-section, to 500 µm across, diminishing in size towards the peripheries; cortical layer composed of small cells, ovoid, tightly packed, to 10 µm in diameter.

Specimens examined: 78EM-18 (291-296, 425, 515, 516)

Remarks: In the Philippines, this plant has almost always been reported under the name *Gracilaria crassa* Harvey ex J. Agardh, a name now considered as a junior synonym of *Gracilaria canaliculata* Sonder. The latter name, however, is not the earliest name given to this plant. Kützing (1868: 29) had first applied the name *Sphaerococcus canaliculatus* Kützing to it which unfortunately turned out to be a latter homonym of *S. canaliculatus* C. Agardh (1822: 260). Realizing this, Sonder (1871) proposed the present name which rendered the specific name of Kützing invalid and unusable.

Dawson (1954: 438) remarked about the constrictions in *G. canaliculata* (as *G. crassa*) saying that "younger stages" are "without regular constrictions, but develop as the plant matures, and these become more pronounced and the segments more elongated…" approaching the form of *Gracilaria salicornia* (C. Agardh) Dawson.

The first published report of *G. canaliculata* from the Philippines was made by Cantoria et al. (1951) in connection with a study on the pharmacopoeial properties of some marine algae. Upon examining the unpublished thesis of the first author on which this report was based, the specimens referred to as *G. canaliculata* are actually materials of *Hydropuntia eucheumatoides* (Harvey) Gurgel and Fredericq as shown in the photographs.

***Gracilaria salicornia* (C. Agardh) Dawson**

Dawson 1954: 4, fig. 3 (type locality: Manila Bay); Galutira and Velasquez 1964: 506, pl. 5, figs. 15a, b, pl. 8, figs. 32a, b; Velasquez et al. 1971: 30, pl. 13, fig. 62; Cordero 1977: 132, pl. XIX, C, figs. 116-118; Vannajan and Trono 1978: 22, fig. 24; Cordero 1981: 57: pl. 38, fig. 22A; Trono et al. 1983: 23, figs. 3, 4b; Hurtado et al. 2006: 12, middle figure.

Basionym: *Sphaerococcus salicornia* C. Agardh 1820: pl. 8, figs. 1-4.

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Synonyms: *Corallopsis salicornia* (C. Agardh) Greville 1830: 53. *Gracilaria cacalia* (J. Agardh) Dawson 1954: 2. *Corallopsis cacalia* J. Agardh 1852: 583.

Illustrative materials: Tsutsui et al. 2005: 197, top figures; Ohba et al. 2007: 93, top figures; Lee et al. 2015: 170, middle figures; Wang et al. 2015: 96, bottom figure.

Plants erect, up to 6 cm tall, attached by a single holdfast; fronds generally terete throughout, to 1.6 mm in diameter, the basal parts show inconspicuous constrictions at random portions, reclined, parts touching substrate developing short disc-tipped hapters, distal portions markedly constricted, composed of almost uniformly sized segments, clavate, with sharply tapered bases and inflated distal portions, to 4 mm in diameter, the broad apices depressed or lacunate, issuing 1-3 new branches from there, dichotomous or irregular; medulla composed of uniformly thin-walled cells, ovoid, 250-300 µm in diameter, progressively smaller distally; cortical cells composed of 2-3 layers, ovoid, 7-10 µm in diameter.

Specimens examined: 78EM-12 (105-112); 78EM-13 (170-176); 78EM-20 (272-276); 78EM-45 (185-195)

Remarks: This species is distinctive among species of *Gracilaria* in having well-defined deep constrictions spaced apart along the main axis, producing a series of clavate segments. This unique feature was used to erect a segregate genus *Corallopsis* within the Gracilariaceae with *C. salicornia* as its type species. However, upon examination of vegetative and reproductive structures of *C. salicornia*, it was concluded that they are similar with those found in the generitype of *Gracilaria*, *G. compressa* and thus should remain within the genus (Liao and Hommersand 2003). *G. cacalia* is often regarded as an intermediate form between *G. canaliculata* and *G. salicornia*, distinguished only by the degree of constrictions on the thallus, i.e., faint constrictions in the former while deep and well-defined constrictions in the latter. Lin (2009: 24, figs. 8a-b) studied a sample from southern Taiwan identified as *G. salicornia* but which resembled the form of *G. cacalia* as did Coppejans et al. (2009: 172, fig. 139) in samples from Sri Lanka. Another species *G. minor* (Sonder) Durairatnam is characterized by fronds that are "stalked below soon becoming constricted and articulated above" (Durairatnam 1961: 64). Some phycologists have recognized *G. minor* as a variety under *G. salicornia*, while others have suggested the conspecificity of the two (Womersley and Bailey 1970). Xia (1986) listed several species as synonyms of *G. salicornia* including *G. cacalia*, *G. minor*, among others.

Trono and Ganzon-Fortes (1980) described two ecological forms of *G. salicornia* from Batangas: one form inhabiting areas with strong water

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movement shows less prominent constrictions approaching the morphology of *G. cacalia*, while another one growing in calmer waters show strong constrictions typically seen in *G. salicornia*. It is clear that this species shows morphological plasticity contributing to its complicated taxonomic history.

***Gracilariocolax* sp.**

Illustrative materials: Yamamoto 1986: 281 (as *Congracilaria babae*); Yamamoto 1991: 381 (as *Congracilaria babae*); Lin 2009: 6, figs. 1a-c (as *Congracilaria babae*).

Plants minute, parasitic, attached to the surface of host alga (*Gracilaria salicornia*) in random places, but more commonly on older parts; irregularly globose, mushroom-shaped, often in loose aggregates, pigmented, subtended by short stalks which are up to 700 µm in diameter.

Specimens examined: 78EM-13 (170-176)

Remarks: This adelphoparasitic species is widely reported around Southeast Asia as *Congracilaria babae* Yamamoto (Yamamoto 1991; Yamamoto and Phang 1997; Kongkittayapun and Chirapart 2011) growing on a specific host alga, *Gracilaria salicornia*. It has also been reported to occur on *Hydropuntia edulis* (S.G. Gmelin) Gurgel and Fredericq (=*Gracilaria edulis*) collected from Indonesian waters (Gerung et al. 1999) and on *Hydropuntia* sp. from East Malaysia (Ng et al. 2014).

Weber-van Bosse (1928) established the new genus *Gracilariocolax* to accommodate a parasitic species associated with *Gracilaria* collected from Java. In addition, she described a few other parasitic species under the genus *Gracilariophila*. Very little is known about parasitic species on *Gracilaria* until Yamamoto (1986) described another species under his newly proposed genus *Congracilaria* which he recognized as being distinct because of the production of bisporangia as opposed to tetrasporangia in *Gracilariocolax*, among other characters. Later, Ng et al. (2013) noted that the distinction between bisporangia and tetrasporangia in *Congracilaria* and *Gracilariocolax* was superfluous. Furthermore, molecular analysis revealed that *Congracilaria* was nestled within the *Gracilaria sensu stricto* clade, and supported its recognition as a species of *Gracilaria* by Ng et al. (2014), effectively subsuming the genus *Congracilaria* under *Gracilaria*.

Gerung and Yamamoto (2002) opined that the genus *Gracilariophila* is restricted to the western coasts of North America and specifically growing on species of *Gracilariopsis*. They went on to transfer all previously described species of *Gracilariophila* from Indonesia into *Gracilariocolax*, a move that is supported here. As a result, all parasitic species on *Gracilaria* at least in

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Southeast Asia should be recognized as species of *Gracilariocolax*. The placement of the samples from Cuyo Islands cannot be ascertained within the genus *Gracilariocolax* and must await further morphological studies.

Hydropuntia edulis (S.G. Gmelin) Gurgel and Fredericq

Silva 1952: 293; Cordero 1977: 128, fig. 111; Trono 1997: 210, fig. 134; Hurtado et al. 2006: 11, bottom figure.

Basionym: *Fucus edulis* S.G. Gmelin 1768: 113 (type locality: East Indies, probably Ambon in Maluku province, Indonesia)

Synonyms: *Gracilaria edulis* (S.G. Gmelin) Silva 1952: 293.

Gracilaria coronopifolia of Filipino authors [not J. Agardh 1852: 592]; Galutira and Velasquez 1964: 508, pl. 5, fig. 14, pl. 9, fig. 34; Cordero 1977: 127, pl. XX, A, figs. 104, 104a; Trono et al. 1983: 20, figs. 2, 9b

Gracilaria verrucosa of Filipino authors [not (Hudson] Papenfuss 1950: 195, rejected name (=*Gracilariopsis longissima* (S.G. Gmelin) Steentoft, Irvine & Farnham)]; Galutira and Velasquez 1964: 507, pl. 4, fig. 13, pl. 8, fig. 33; Villones and Magdamo 1968: 29, fig. 29; Velasquez et al. 1971: 30, pl. 13, fig. 61; Cordero 1977: 135; Vannajan and Trono 1978: 22, fig. 18; Cordero 1981: 59, fig. 39; Trono et al. 1983: 17, figs. 1, 4a

Gracilaria confervoides (Linnaeus) Greville 1830: liv, 123; illegitimate name; Montagne 1844: 662

Illustrative materials: Calumpong and Meñez 1997: 178, bottom figure (as *Gracilaria verrucosa*); Lin 2009: 33, figs. 11a-d (as *Hydropuntia edulis*); Lee et al. 2015: 169, middle figures (as *Hydropuntia edulis*); Wang et al. 2015: 94.

Plants bushy, erect, sometimes entangled, 8-15 cm tall, attached by single disc-like holdfasts; fronds terete, to 1.5 mm in diameter, to 300 µm broad at the ultimate branchlets, branching irregular, often essentially dichotomous or secund, more pronounced and frequent towards the tips, often resulting in crowded corymbose apical portions, commonly of uniform diameter, tapering gently towards the apical parts, at times with inconspicuous and shallow constrictions at the base; branchlets fine and filiform issued randomly on main axis, widely scattered, more evident near the tips; medullary cells thin-walled, ovoid, 200-260 µm in diameter, decreasing in size abruptly towards the peripheries; cortical cells forming 2-3 layers, elongate to cuboidal, arranged side by side, to 10 µm across.

Specimens examined: 78EM-12 (101-104); 78EM-13 (150-165); 78EM-20 (280-295, 295A)

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Remarks: For the longest time, species of *Gracilaria* that are uniformly slender and terete and exhibiting hair-like thalli have been identified as *Gracilaria verrucosa* (Hudson) Papenfuss for lack of better diagnostic characters and for reasons of convenience. As a result, *G. verrucosa* was considered a cosmopolitan species that occurs in virtually all localities in the tropical and temperate regions, until Steentoft et al. (1995) examined historic materials as well as fresh materials obtained from the type locality in the U.K. Today *G. verrucosa* is a rejected name and materials formerly associated with this name are recognized under *Gracilariopsis longissima* (S.G. Gmelin) Steentoft, Irvine & Farnham with its distribution limited to western Europe. For a full discussion of this complicated nomenclatural history, see Steentoft et al. (1995). Species from East and Southeast Asia and the Pacific formerly known as *G. verrucosa* have now been renamed as *Hydropuntia edulis* (S.G. Gmelin) Gurgel and Fredericq or described as new species.

Gracilaria coronopifolia J. Agardh is a species first described from Hawaii and has been reported from many Philippine localities. These previous reports should be carefully re-examined. It resembles *G. edulis* in having uniformly slender main axes that are branching repeatedly into acute tips. *G. coronopifolia* branches more frequently in distal portions resulting to a corymbose shape as seen by Cordero (1977: 127, pl. XX, A) in one sample from Siquijor which needs to be re-studied. Yamamoto et al. (1999) observed spermatangial conceptacles from cultured samples of Philippine materials identified as *G. coronopifolia* and found them to be different from those in materials from Hawaii which is the type locality of *G. coronopifolia*. They concluded that *G. coronopifolia* does not occur in the western Pacific region and may be restricted only to the Hawaiian archipelago. Furthermore, authentic *G. coronopifolia* may not be present in the Philippines as it was not among the species recorded in the treatise of Trono (1997, 2004).

GENERAL DISCUSSION

Among the three previous records of the Gracilariaceae from the Cuyo Islands (Liao et al. 2013), only one species, *Gracilaria blodgettii* Harvey, was not encountered in the present study. *G. blodgettii* was first reported by Cordero (1977) based on samples collected from Catadman Sound on Cuyo Island by G.T. Velasquez. To the current list of three species of the Gracilariaceae are added three new records: *G. canaliculata*, *Hydropuntia edulis* and *Gracilariocolax* sp., bringing the total number of known species to six.

The Gracilariaceae marine flora of the Cuyo Islands is relatively poor compared to nearby areas. Hurtado et al. (2006) recorded 11 species of Gracilariaceae from 11 sites around Panay Island about 150 km directly east

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of the Cuyo Islands. Across the vast Sulu Sea about 650 km to the southwest of the Cuyo Islands lie Balabac island and a few islets around it. Santiañez et al. (2015) conducted a comprehensive survey there and found at least nine species of the Gracilariaceae. In another survey on two sites off the town of Bulusan, Sorsogon on mainland Luzon island, at least 14 species were documented by Kraft et al. (1999), one of the highest number recorded for a specific locality in the Philippines, and probably anywhere in Southeast Asia.

It seems that most species of the Gracilariaceae are euryhaline (Bird and McLachlan 1986) with many species better adapted to estuarine conditions where they have been found to produce high biomass. This euryhaline trait enabled some species like *Gracilaria vermiculophylla* (Ohmi) Papenfuss to become successful species for aquaculture and pollution management (Yokoya et al. 1999), and conferring invasive species traits to it. Furthermore, it was found to be highly resistant to desiccation, sand burial and herbivory further contributing to its colonizing and invading success (Thomsen and McGlathery 2007). As a matter of fact, *G. vermiculophylla* has dispersed into many habitats in Europe and on both the Pacific and Atlantic coasts of North America where it had colonized with much success.

While there are many euryhaline species of Gracilariaceae, it is not unlikely that there may also be stenohaline ones. This could partly explain the different species composition found in specific areas. For instance, *G. salicornia* may be characterized as a true marine species having been recorded from many truly marine habitats throughout its geographic range but conspicuously absent from areas subjected to wide salinity fluctuations, although Chirapart (2016) has observed that this species is remarkably tolerant to various environments. Many of the finer filiform species of *Gracilaria* like *G. tenuistipitata* Zhang and Xia, *G. fisheri* (Xia and Abbott) Abbott, Zhang and Xia and *Gracilariopsis heteroclada* Zhang and Xia, are more adapted to lower and fluctuating salinities commonly found in estuaries. The estuaries of the Asian continent, for example, in the Indo-Chinese region are particularly suitable for the growth of these species where there is a thriving industry based on harvesting biomass from natural stocks as well as from aquaculture. Such environments are not present in small islands like the Cuyo Islands where there are practically no large river systems that can create estuarine habitats. On larger islands like neighboring Panay and Luzon islands, some limited estuarine conditions might exist to allow the growth of these commercially-important species. The relatively low species number found in the Cuyo Islands may be partly attributed to the truly marine conditions occurring there that only allow stenohaline, marine species to grow. However, many species of the Gracilariaceae, just like other seaweed species, also show vulnerability to deteriorating environmental conditions. In Thailand, Chirapart (2016) noted that the diversity and biomass of some species of *Gracilaria* have declined as a result of environmental degradation.

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The waters around the Cuyo Islands provide a truly marine environment free of large salinity (and temperature) fluctuations due to their isolated geographic position that is far away from the diluting influence of river systems usually associated with large islands and continental masses. The six species of Gracilariaceae thus far recorded from the Cuyo Islands have been documented from similar island environments that support more stable salinity and temperature regimes conducive for the growth and survival of stenohaline marine species.

The present report dealt with historical collections from about four decades ago. In addition, the collections were made during a short visit during the dry season months. It is therefore recommended that a year-round or at least seasonal collection be made to compare species found during the dry and rainy months as local microclimate conditions affecting marine communities may play an important role in the occurrence of species. It will also be of interest in this era of rapid environmental change to compare species diversity over the past decades (Liao and Bataan 2016) with collections from the present times providing telling indicators of the combined impact of natural and anthropogenic effects. In the Cuyo Islands, however, it is expected that the former would exert greater impact as the level of human activity there has not increased appreciably over the years to significantly and adversely alter the relatively pristine state of the marine environment observed there four decades ago. The information generated in this study can also provide baseline information useful for the management of economically-important species of the Gracilariaceae which are potential sources of agar. Combined with biomass and seasonality data, the harvest and culture of these species may be proposed as a feasible economic activity.

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Length-weight relationship of marine fishes from Palawan, Philippines

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ABSTRACT

The parameters of the length-weight relationships (LWR) of fishes are the primary variables applied to estimate the biomass of reef fishes in situ. Estimates of reef fish biomass using fish visual census survey in the Philippines still utilized the values of LWR parameters derived from the results of studies conducted abroad due to paucity of local information. This paper presents the first comprehensive records of the LWR of marine fishes from the West Philippine Sea and the Sulu Sea, western Philippines. Data were collected between 1998 and 2014 using various artisanal and commercial fishing gears. A total of 11,539 specimens covering 33 families, 59 genera and 87 species were investigated. The allometric coefficient *b* varied between 2.140 (*Gnathanodon speciosus*) and 3.410 (*Taeniura lymma*) with the mean of 2.840±0.25. The values of r^2 ranged from 0.521 to 0.996. This paper provides the first comprehensive information on the LWR of marine fishes from the western Philippines consisting of 15 new LWR values and 12 higher maximum lengths for online database.

Keywords: Sulu Sea, West Philippine Sea, reef fish, Honda Bay

INTRODUCTION

The parameters of the length-weight relationships (LWR) of fishes are of primary importance in fishery assessment and management (Garcia et al. 1998). It provides estimates of total fish biomass even when length is only known and weight is practically not available. For instance, to evaluate the fish biomass in a coral reef as requirement in the establishment of marine protected area, the fish visual census (FCV) is the popular method being used. This method requires the length and number of individual fish in situ while the total biomass is determined empirically by applying the established

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parameters of the LWR. Length and weight measurements in conjunction with age data can give information on the stock composition, age at maturity, life span, mortality, growth and production (King 1996; Diaz et al. 2000).

The LWR of fishes is useful in assessing the relative well-being of the fish population. It is important in estimating the standing stock biomass, and comparing the ontogeny of fish population from different regions (Petrakis and Stergiou 1995). Length-weight relationship parameters are often used as an indicator of fatness and general well-being or of gonad development of fish and are useful for between region comparisons of life histories of a specific species (Le Cren 1951; Wotton 1990).

Palawan is one of the major fish producing provinces in the Philippines. It supplies fresh and processed fish to Metro Manila and other neighboring provinces. At present, it is also the top exporter of live reef fish products to mainland China and Hong Kong (PSA 2016). Nevertheless, basic information on the biology and ecology of many commercially important marine fishes are poorly documented.

In the Philippines, estimates of coral reef fish biomass mainly utilize the results of studies conducted in New Caledonia (Kulbicki et al. 1993) due to paucity of locally available information. Only limited number of studies have been reported so far in the country (De la Peña 1998; Gonzales et al. 2000; Palla and Wolff 2007). The most comprehensive study was recently reported from the southern Philippines comprising 139 fish species (Gumanao et al. 2016). In this study, the standard length-total length, standard length-fork length relationships and 15 new records of maximum fish length and weight were reported. Further, it has been established that growth of fish is largely influenced by its environmental conditions. Thus, information derived from other geographical regions may give inaccurate estimate. Hence, it is essential to establish the LWR of fishes in the locality. The objective of this study was to provide information on the LWR parameters (a, b, r^2) of 87 marine fish species from Palawan, Philippines.

METHODS

The province of Palawan is located within the coordinates of 7.951°N-12.428 °N and 115.904°E-120.704 °E (Figure 1). The data were obtained from the results of series of fish stock assessment studies conducted from 1998 until 2014. These include the studies in Aborlan coastal waters, Arreceffi Island in Honda Bay, Puerto Princesa Bay, Ulugan Bay, Green Island Bay and Taytay Bay. Specimens were derived from landing sites, fish markets and actual fishing surveys caught using artisanal and commercial fishing gears such as;

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the hook and line, gillnet, trammel net, fish corral, spear gun, otter trawl, ring net, fish pot and beach seine. To reduce bias in sizes, specimens were bought unsorted with prior agreement from fish vendors. Specimens were identified following Schroeder (1980), Carpenter and Niem (1999, 2001a,b), Gonzales (2013), and Motomura et al. (2017).

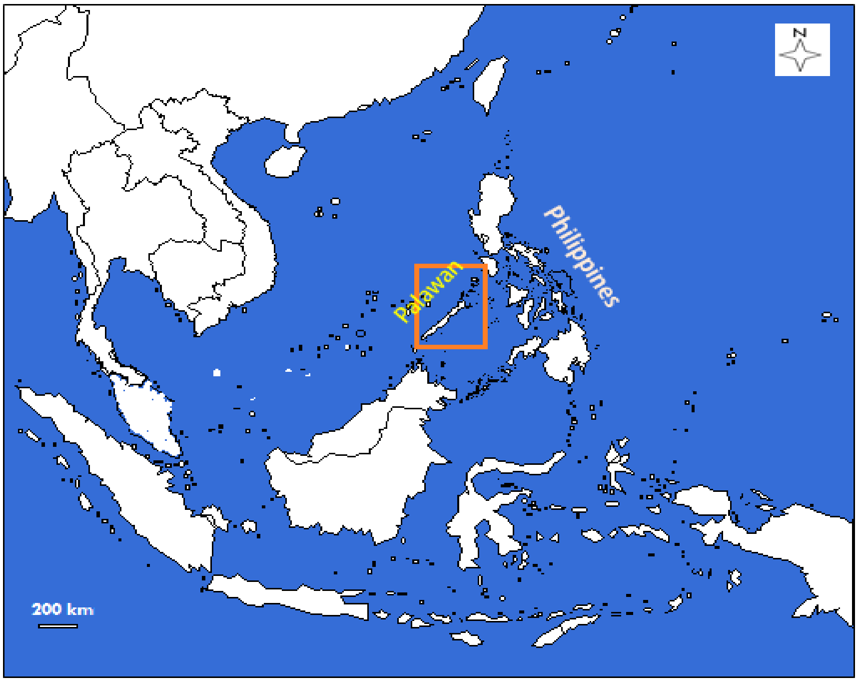

Figure. 1. Map of Southeast Asia, denoting the Philippines and the Island of Palawan (redrawn from www.google.com/search?q=outline+map+southeast +asia).

The total length (TL), fork length (FL) and standard length (SL) of individual fish were measured to the nearest 0.1 cm using measuring board and weighed to the nearest 0.5 g using top loading balance (500 g and 10 kg max). The length-weight relationship (LWR) of fishes was estimated using the equation:

$$W = a * L^b$$

where: W= weight (g), L= total length (cm), a = constant, b= growth exponent. A logarithmic transformation was used to make the relationship linear (Le Cren 1951):

$$\log W = \log a + b \log L$$

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The LWR parameters *a* and *b* as well as the coefficient of determination (r^2) were derived from least squares regression (Ricker 1973). The slope (*b*) was used to describe the three dimensional growth of fish in length, width, and depth. If $b = 3$, growth is isometric, if $b < 3$, it is negative allometric and if $b > 3$, it is positive allometric (Froese 2006). The systematic arrangement of families followed Nelson (2006), while the species were arranged alphabetically in each family.

RESULTS

A total of 11,539 individuals belonging to 33 families, 59 genera and 87 species were examined (Table 1). The allometric coefficient (*b)* varied between 2.140 (*Gnathanodon speciosus*) and 3.410 (*Taeniura lymma*) with the mean of 2.840±0.250. Four species showed isometric growth, 55 species with negative allometric and 28 species with positive allometric growth. The coefficient of determination (r^2) ranged from 0.521 (*Paramonacanthus japonicus*) to 0.996 (*Lutjanus argentimaculatus*). In addition, studies reported 11 species earlier from the province were also cited (De la Peña 1998; Gonzales et al. 2000; Palla and Wolff 2007) (Table 1).

DISCUSSION

The record of 87 species (3 cartilaginous, 84 bony) reported in this study represents the first comprehensive information on the LWR of marine fishes from the western Philippines. Among these species, 82 comprised the first record in the area in addition to the four (*Cephalopholis argus, C. boenak, C. miniata* and *C. sonnerati*) presented earlier by Gonzales et al. (2000) and one (*Pentaprion longimanus*) Palla and Wollf (2007), respectively (Table 1). In a recent study, 139 species were reported from the southern Philippines (Gumanao et al. 2016). In this paper, only 26 species recorded in the present study were listed, making an overall number of 200 species reported for the LWR parameters in the Philippines.

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Table 1. Parameters of the length-weight relationships of 87 marine fishes in Palawan, Philippines. 11 species cited from published literatures and 7 species recorded both in Puerto Princesa Bay and Honda Bay.

Family	Species	n	LWR			Length			Source
			a	b	r^2	min.	max.	type	
Carcharhinidae	*Carcharhinus melanopterus* (Quoy and Gaimard 1824)	55	0.015	2.750	0.832	44.3	60.3	TL	This study
Dasyatidae	*Neotrygon orientalis* (Last, White and Séret 2016)	44	0.022	3.100	0.930	17.0	34.0	DW	This study
	Taeniura lymma (Forrskål 1775)	54	0.023	3.410	0.965	14.5	31.5	DW	This study [fr]
Plotosidae	*Plotosus lineatus* (Thunberg 1787)	62	0.024	2.680	0.781	16.5	25.0	TL	This study
Synodontidae	*Saurida longimanus* Norman 1839	37	0.004	3.110	0.927	12.7	25.5	TL	This study [fr]
Mugilidae	*Crenimugil buchanani* (Bleeker 1853)	86	0.011	3.010	0.977	19.2	53.5	TL	This study
	Moolgarda seheli (Forrskål 1775)	31	0.025	2.760	0.988	14.8	46.7	TL	This study
Hemiramphidae	*Hemiramphus far* (Forrskål 1775)	177	0.018	2.690	0.917	21.2	35.6	TL	This study
	Hyporhamphus neglectus (Bleeker 1866)	41	0.007	2.860	0.824	26.7	38.7	TL	This study [fr]
Belonidae	*Tylosorus punctulatus* (Günther 1872)	15	0.002	2.900	0.867	44.0	59.5	TL [hm]	This study [fr]
Platycephalidae	*Cymbacephalus nematophthalmus* (Günther 1860)	35	0.021	2.780	0.944	9.2	42.0	TL	This study [fr]
Ambassidae	*Ambassis gymnocephalus* (Lacepède 1802)	303	0.130	2.200	0.563	4.5	11.0	TL	This study
Serranidae	*Anyperodon leucogrammicus* (Valenciennes 1828)	67	0.040	2.650	0.956	14.2	42.5	TL	This study
	Cephalopholis argus Schneider 1801	51	0.013	3.060	0.958	7.1	27.0	TL	This study
	Cephalopholis boenak (Bloch 1790)	30	0.027	2.840	0.969	11.0	22.5	TL	This study
	Cephalopholis cyanostigma (Valenciennes 1828)	184	0.019	2.960	0.938	14.0	55.0	TL [hm]	This study
	Cephalopholis miniata (Forsskål 1775)	110	0.028	2.850	0.933	17.0	35.0	TL	This study
	Cephalopholis sonnerati (Valenciennes 1828)	72	0.012	3.120	0.966	9.8	44.0	TL	This study
	Epinephelus fasciatus (Forsskål 1775)	182	0.027	2.840	0.887	5.8	32.0	TL	This study
	Epinephelus merra Bloch 1793	61	0.008	3.240	0.954	13.5	28.0	TL	This study

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Family	Species	n	LWR			Length			Source
			a	b	r^2	min.	max.	type	
	Epinephelus quoyanus (Valenciennes 1830)	15	0.014	3.040	0.977	4.5	31.5	TL	This study [fr]
	Variola louti (Forrskål 1775)	48	0.011	3.000	0.972	15.0	34.0	SL	This study
Priacanthidae	*Priacanthus macracanthus* Cuvier 1829	143	0.035	2.470	0.936	10.1	13.5	TL	This study
Apogonidae	*Apogonichthyoides melas* Bleeker 1848	168	0.020	3.000	0.620	7.8	12.1	TL [hm]	This study [fr]
	Cheilodipterus singapurensis Bleeker 1860	52	0.128	2.230	0.872	6.0	16.9	TL	This study
Centrogenyidae	*Centrogenys vaigiensis* (Quoy and Gaimard 1824)	143	0.034	2.910	0.890	6.0	17.9	TL	This study [fr]
Carangidae	*Atule mate* (Cuvier 1833)	560	0.009	3.066	0.988	11.4	29.9	TL	This study
	Carangoides ferdau (Forsskål 1775)	65	0.013	3.010	0.993	12.4	31.7	TL	This study
	Carangoides fulvoguttatus (Forsskål 1775)	68	0.012	2.820	0.990	9.0	71.0	SL	This study
	Caranx ignobilis (Forsskål 1775)	44	0.024	2.800	0.879	18.4	62.0	TL	This study
	Decapterus kurroides Bleeker 1855	24	0.056	2.350	0.903	13.9	21.4	TL	This study
	Decapterus macrosoma Bleeker 1851	132	0.010	2.950	0.772	14.4	22.2	TL	This study
	Decapterus russelli (Rüppell 1830)	52	0.008	3.070	0.960	13.1	23.2	TL	This study
	Gnathanodon speciosus (Forsskål 1775)	10	0.359	2.140	0.810	35.4	55.0	TL	This study
	Scomberoides tol (Cuvier 1832)	21	0.010	2.860	0.947	28.4	39.1	TL	This study
	Selaroides leptolepis (Cuvier 1833)	35	0.014	2.920	0.703	15.0	17.7	TL	This study
	Uraspis secunda (Poey 1860)	26	0.082	2.410	0.812	16.8	19.0	TL	This study
Leiognathidae	*Equulites oblongus* (Valenciennes 1835)	69	0.009	3.170	0.753	9.2	14.6	TL	This study [fr]
Lutjanidae	*Aphareus rutilans* Cuvier 1830	101	0.017	2.590	0.948	28.0	78.0	FL	This study
	Lutjanus argentimaculatus (Forsskål 1775)	13	0.029	2.810	0.996	16.5	42.6	TL	This study
	Lutjanus decussatus (Cuvier 1828)	12	0.014	3.040	0.948	13.7	20.6	TL	This study
	Lutjanus ehrenbergii (Peters 1869)	153	0.047	2.640	0.916	10.7	25.0	TL	This study
	Lutjanus fulviflamma (Forsskål 1775)	67	0.062	2.560	0.868	10.7	18.5	TL	This study

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Family	Species	n	LWR			Length			Source
			a	b	r^2	min.	max.	type	
	Lutjanus vitta (Quoy and Gaimard 1824)	716	0.030	3.040	0.938	11.3	28.0	TL	This study
Caesionidae	*Caesio lunaris* Cuvier 1830	54	0.018	2.410	0.533	12.5	17.0	FL	This study
Gerreidae	*Gerres abbreviatus* Bleeker 1850	16	0.011	3.180	0.995	10.2	25.6	TL	This study
	Gerres abbreviatus Bleeker 1850	57	0.015	2.730	0.910	9.0	21.5	FL	This study
	**Gerres oyena* (Forsskål 1775)	169	0.016	2.990	0.950	7.0	16.7	TL	This study
	***Gerres oyena* (Forsskål 1775	257	0.017	2.960	0.958	7.0	23.0	TL	This study
	Pentaprion longimanus (Cantor 1849)	300	0.032	2.670	0.828	9.1	15.6	TL [hm]	This study
Haemulidae	*Plectorhinchus pictus* (Tortonese 1936)	45	0.012	2.830	0.985	10.0	41.5	SL	This study
Nemipteridae	*Nemipterus aurora* Russell 1993	26	0.030	2.710	0.896	13.8	27.8	TL [hm]	This study [fr]
	Nemipterus bathybius Snyder 1911	102	0.029	2.650	0.906	9.7	32.0	TL [hm]	This study
	Nemipterus furcosus (Valenciennes 1830)	310	0.016	2.890	0.963	12.0	25.2	TL [hm]	This study
	Nemipterus hexodon (Quoy and Gaimard 1824)	200	0.015	2.940	0.907	12.0	23.5	TL [hm]	This study
	Nemipterus peronii (Valenciennes 1830)	192	0.013	2.770	0.994	8.0	48.5	FL [hm]	This study
	**Pentapodus caninus* (Cuvier 1830)	173	0.051	2.640	0.900	7.2	27.2	TL	This study
	***Pentapodus caninus* (Cuvier 1830)	109	0.021	2.910	0.860	7.2	15.3	TL	This study
	Pentapodus emeryii (Richardson 1843)	81	0.015	3.010	0.786	7.0	15.6	TL	This study [fr]
	Scolopsis ciliatus (Lacepède 1802)	381	0.009	3.300	0.797	6.2	16.5	TL	This study
Lethrinidae	*Lethrinus chrysostomus* Richardson 1848	68	0.010	3.010	0.978	9.5	25.5	SL	This study
	Lethrinus genivittatus Valenciennes 1830	133	0.014	3.020	0.952	11.0	22.7	TL	This study
	Lethrinus harak (Forsskål 1775)	273	0.015	3.000	0.976	11.6	36.5	TL	This study
	**Lethrinus lentjan* (Lacepède 1802)	200	0.067	2.510	0.904	7.0	18.8	TL	This study
	***Lethrinus lentjan* (Lacepède 1802)	423	0.012	2.840	0.812	7.0	38.5	SL	This study
	Lethrinus miniatus (Forster 1801)	82	0.010	2.900	0.982	14.0	73.5	SL	This study

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Family	Species	n	LWR			Length			Source
			a	b	r^2	min.	max.	type	
	Monotaxis grandoculis (Forsskål 1775)	69	0.013	2.800	0.986	14.0	57.0	FL	This study
Sciaenidae	_Otolithes ruber_ (Bloch and Schneider 1801)	39	0.015	2.730	0.910	10.0	20.0	FL	This study
Mullidae	_Mulloidichthys vanicolensis_ (Valenciennes 1831)	14	0.058	2.510	0.565	25.3	31.0	TL	This study
	Parupeneus indicus (Shaw 1803)	34	0.011	3.070	0.995	15.7	38.7	TL	This study
	Parupeneus macronema (Lacepède 1801)	33	0.010	3.110	0.885	14.3	21.3	TL	This study
	Upeneus tragula Richardson 1846	17	0.019	2.810	0.985	16.0	26.3	TL [hm]	This study
	Upeneus vittatus (Forsskål, 1775)	50	0.016	2.900	0.956	8.0	16.0	TL	This study
Ariommatidae	_Ariomma indicum_ (Day, 1871)	33	0.042	2.660	0.808	16.2	20.2	TL	This study
Labridae	_Choerodon anchorago_ (Bloch, 1791)	98	0.024	3.000	0.988	10.5	27.0	TL	This study
Gobiidae	_Amblygobius phalaena_ (Valenciennes 1837)	68	0.022	2.900	0.526	7.0	11.2	TL	This study
Siganidae	*_Siganus canaliculatus_ (Park 1797)	290	0.022	2.950	0.978	6.7	33.6	TL [hm]	This study
	**_Siganus canaliculatus_ (Park 1797)	275	0.015	2.650	0.964	6.0	24.0	SL	This study
	Siganus fuscescens (Houttuyn 1782)	192	0.037	2.510	0.907	6.7	22.5	TL	This study
	*_Siganus guttatus_ (Bloch 1787)	32	0.022	3.050	0.985	7.8	26.2	TL	This study
	**_Siganus guttatus_ (Bloch 1787)	488	0.025	2.950	0.938	7.8	39.0	TL	This study
Sphyraenidae	*_Sphyraena barracuda_ (Edwards 1771)	74	0.006	3.030	0.956	10.0	50.5	TL	This study
	**_Sphyraena barracuda_ (Edwards 1771)	179	0.063	2.570	0.973	10.0	54.2	TL	This study
	Sphyraena obtusata Cuvier 1829	182	0.010	2.570	0.969	13.0	28.5	FL	This study
Emmelichthyidae	_Emmelichthys struhsakeri_ Heemstra and Randall 1977	35	0.012	2.950	0.939	14.0	20.0	TL	This study [fr]
	Erythrocles schlegelii (Richardson 1846)	26	0.006	3.280	0.915	14.5	18.0	TL	This study [fr]
Scombridae	*_Rastrelliger kanagurta_ (Cuvier 1816)	114	0.005	3.270	0.940	20.7	31.5	TL	This study
	**_Rastrelliger kanagurta_ (Cuvier 1816)	43	0.021	2.160	0.888	10.0	16.0	FL	This study
Balistidae	_Rhinecanthus verrucosus_ (Linnaeus 1758)	18	0.012	3.080	0.986	11.5	21.0	TL	This study [fr]

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Family	Species	n	LWR			Length			Source
			a	b	r^2	min.	max.	type	
Monacanthidae	*Monacanthus chinensis* (Osbeck 1765)	400	0.027	2.920	0.778	6.6	16.6	TL	This study
	Pseodomonacanthus macrurus (Bleeker 1856)	111	0.012	2.830	0.968	8.5	25.5	SL [hm]	This study [fr]
	Paramonacanthus japonicus (Tilesius 1809)	158	0.032	2.840	0.521	6.1	12.3	TL	This study
Tetraodontidae	*Arothron immaculatus* (Bloch and Schneider 1801)	39	0.085	2.550	0.913	6.2	19.9	TL	This study
	Chelonodon patoca (Hamilton 1822)	200	0.019	3.180	0.842	6.0	19.7	TL	This study
Clupeidae	*Amblygaster sirm* (Walbaum 1792)	450	0.005	3.200	-	11.0	24.0	TL	De la Peña 1998
	Sardinella gibbosa (Bleeker 1849)	50	0.009	3.030	-	10.0	13.5	TL	De la Peña 1998
	Sardinella longiceps Valenciennes 1847	200	0.024	2.640	-	7.6	10.0	TL	De la Peña 1998
Serranidae	*Cephalopholis argus* Schneider, 1801	504	0.012	3.100	-	12.7	36.0	TL	Gonzales et al. 2000
	Cephalopholis boenak (Bloch, 1790)	456	0.011	3.100	-	9.6	26.0	TL	Gonzales et al. 2000
	Cephalopholis microprion (Bleeker, 1852)	213	0.011	3.160	-	10.2	19.5	TL	Gonzales et al. 2000
	Cephalopholis miniata (Forsskål, 1775)	275	0.017	2.990	-	13.9	39.0	TL	Gonzales et al. 2000
	Cephalopholis sonnerati (Valenciennes, 1828)	305	0.012	3.100	-	14.0	51.0	TL	Gonzales et al. 2000
Leiognathidae	*Photopectoralis bindus* (Valenciennes 1835)	943	0.032	2.740	-	7.5	9.1	TL	Palla and Wolff 2007
	Equulites elongatus (Günther, 1874)	1000	0.015	3.420	-	8.0	17.0	TL	Palla and Wolff 2007
Gerreidae	*Pentaprion longimanus* (Cantor, 1849)	956	0.021	3.160	-	10.0	16.5	TL	Palla and Wolff 2007

n = sample size; TL- total length; FL – fork length; SL – standard length; DW- disc width ; a, b – regression coefficients; r^2 – coefficient of determination, *Puerto Princesa Bay, **Honda Bay , fr- first record, hm – higher maximum length

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Temporally, the slopes (*b*) varied slightly for a span of 14 years among four groupers (*Cephalopholis* spp.). However, *P. longimanus* has considerably decreased in slope over the past decade which can be attributed to difference in sample size. In the context of growth type with reference to slope, 63% exhibited negative allometry, 32 % positive allometry and only 5% displayed isometric growth. This suggests that majority of fishes in Palawan exhibited low well-being.

Carlander (1969) pointed out that the coefficient *b* in the LWR of fishes usually ranged from 2.5 to 3.5. In this study only 8% of all species evaluated had the values beyond this range. The lowest value of *b* for *Gnathanodon speciosus* was due to low sample size and narrow size range which was only represented by medium size individuals. Whereas the highest *b* in *Taeniura lymma* remains unclear since the other species *N. orientalis* displayed *b* value close to 3.0 despite both species had relatively sufficient sample size and were measured in disc width. These species of ray have been landed without tail as common practice due to the danger posed by their venomous tail spine.

In terms of the coefficient of determination (r^2), majority (67%) of the total species examined attained the values of over 0.90. This indicates highly significant relationships of length to weight of fishes under study.

The LWR parameters of 87 species recorded in this study supplements the recently reported 139 species from southern Philippines making up a total of 200 species all over Philippines. Accordingly, this paper provides the first LWR values for 15 species and higher maximum length for 12 species (Table 1) to the online database of FishBase (Froese and Pauly 2017). Further studies of similar nature and involving some biological aspects of commercially important species are necessary to support the formulation of policies for sustainable utilization and appropriate management of fisheries resources in the country.

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Exploring Technological, Pedagogical, and Content Knowledge (TPACK) and Self Efficacy Belief of Senior High School Biology Teachers in Batangas City

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ABSTRACT

Teachers are considered as one of the key factors in the educational process, especially, in the implementation of a new curriculum. Thus, the introduction and implementation of the new K to 12 curricula, requires monitoring, assessment and evaluation of areas in teaching and learning, in order to ensure that the set goals and objectives were successfully attained. This study aimed to explore two important aspects of teaching such as self-efficacy belief and technological, pedagogical, and content knowledge (TPACK) of private and public senior high school biology teachers from Batangas City for AY 2017-2018. Surveys, focus group discussion and class observation methods were used to gather data. Using mean and standard deviation, the results revealed that biology teachers have moderate self-efficacy belief in both aspects of self-efficacy belief, the personal biology teaching efficacy and biology teaching outcomes expectancy. On the other hand, in TPACK, they were rated as moderately proficient. Furthermore, the study also implies that there is no significant relationship between these two variables when compared using Pearson's *r*, which means that the self-efficacy belief of senior high school (SHS) biology teachers of Batangas City has nothing to do with their TPACK. Designing of a training design framework for faculty development programs and similar studies for physics and chemistry teaching are suggested.

Keywords: Biology Teaching, Content Knowledge, Pedagogical Knowledge, Self-Efficacy Belief, Technological Knowledge, Senior High School

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INTRODUCTION

Having good teachers are very important. Schools and the communities have always sought out the best educators they could get in the belief that their students' success depends on it. Waseka et.al. (2016) emphasized that the most important factor affecting the quality of education is the quality of the individual teacher in the classroom. Tucker and Stronge (2017), on the other hand, stressed that effective teachers not only make students feel good about school and learning, but also, that their work actually results in increased student achievement. As Downes (2011) would say, they are the most important school-based determinant.

Research studies are continuously being conducted to further enhance the quality of educators, ultimately to produce quality graduates. Here in the Philippines, the evolution of the educational system, especially now in the implementation of the K to 12 Program, is really quite challenging. When former president Benigno Aquino signed into law Republic Act 10533 also known as the Enhanced Basic Education Act of 2013, major changes happened. Everybody is on the adjustment phase since it is newly implemented. Inevitably, there are consequences from this shift. Among teachers, there are deep-seated anxieties about the new duties expected of them. To address this issue on teachers, Department of Education (DepEd) conducted mass trainings. However, teachers noted that trainings they have undergone were rushed and not well thought of. France Castro, secretary general of the Alliance of Concerned Teachers (ACT) said the time spent for teachers' training is not enough. He further added that there are lots of new things for teachers to learn in order to implement the new curriculum, thus, one to two months training is not enough (Umil and Viray 2012). Some critics of K to 12 say that the curriculum is "imprecise" and "vague" and the teachers are not ready for their senior high school (SHS) responsibilities. Alliance of Concerned Teachers (ACT) president Antonia Lim said that most teachers are "not prepared" and "competent" enough to teach the SHS subjects after undergoing only five days of training (Quintas 2016). It is on this premise that this study looked into the SHS biology teachers' technological, pedagogical and content knowledge and self-efficacy belief.

Furthermore, the researcher also finds it important to explore these aspects since almost all General Biology teachers are either assigned to a new workplace or transferred from tertiary to SHS or otherwise and facing a new curriculum. It is also the initial stages of having SHS students, it is but relevant to conduct such study to further improve the teaching and learning process so as to ensure the realization of the set goals and objectives for the

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new educational system. It is also worthy to note that this is one of the highlighted topics included in the Philippines' National Integrated Basic Research Agenda (NIBRA) for 2017 – 2022. Therefore, this study was guided by the following research questions:

1. What is the biology teachers' perceived self-efficacy belief in terms of: a) Personal biology teaching efficacy and b) Biology teaching outcomes expectancy?

2. What is the biology teachers' pedagogical content knowledge in terms of: a) Pedagogical knowledge, b) Content knowledge and C) Technological knowledge?

3. Is there a significant relationship between self-efficacy belief and pedagogical content knowledge?

METHODS

This study utilized three data gathering methods: survey, focus group discussion and class observations. A modified Biology Teaching Efficacy Belief Instrument (BTEBI) was used to determine biology teachers' self-efficacy belief based on Savran and Cakiroglu (2001). On the other hand, to evaluate biology teachers' TPACK, researcher self-constructed questionnaire was used. Both questionnaires were validated by four experts in the field and subjected to dry run to selected schools in the province. The data gathered were tabulated and analyzed. Survey questionnaires for both pedagogical content knowledge and self-efficacy were found out to be reliable using Cronbach's alpha.

Class observations were conducted by the researcher and two experts in biology teaching. Fourteen out of 23 teacher-respondents were observed. They were rated using the same instrument used in the TPACK survey. To further substantiate the results, a focus group discussion was also conducted.

After collecting the data, mean and standard deviation were used in the identification of self-efficacy and TPACK, while Pearson's r test of correlation was used for the test of the relationship of self-efficacy belief and TPACK.

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RESULTS

In this study, self-efficacy belief of biology teachers have two subscales: personal biology teaching efficacy (PBTE) which is the teachers' belief in their ability to perform biology teaching; and, biology teaching outcome expectancy (BTOE) which reflects their belief that their students can learn biology.

Biology Teachers' Self-Efficacy Belief in terms of Personal Biology Teaching Efficacy (PBTE)

Table 1 shows the biology teachers' self-efficacy belief in terms of personal biology teaching efficacy. It can be gleaned from the table that the statement, *"I cannot effectively monitor my students during experiments because I don't have much experience in handling such activities"* has the lowest mean value of 2.96 followed by the statement *"I am not confident enough that I teach biology effectively because I haven't attended much training in biology teaching"* with mean value of 3.00. The statement, *"I believe I have the necessary skills to teach Biology because my principal and my colleagues say so"* has also a low mean value of 3.13 compared to the rest of the statements. However, these three mentioned indicators of personal biology teaching efficacy have verbal interpretation of moderate self-efficacy belief.

Conversely, the statements *"As much as possible, I don't want my principal to evaluate my teaching because he/she might find my weaknesses as teacher and it's very obvious with my demeanor especially when being observed", "I can continually find better ways to teach Biology through observation of seasoned colleagues", and, "I can identify the steps necessary to teach Biology concepts effectively because I had trainings when I was a pre-service teacher",* have the highest mean values of 3.43, 3.39 and 3.26, respectively, which are all interpreted also as moderate self-efficacy belief.

As a whole, SHS biology teachers were rated as having moderate self-efficacy belief in terms of personal biology teaching efficacy as implied by its composite mean value of 3.20.

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Table 1. Respondents' Personal Biology Teaching Efficacy (PBTE)

Statements	Mean	Standard Deviation	Verbal Interpretation
Mastery Experiences			
I can identify the steps necessary to teach Biology concepts effectively because I had trainings when I was a pre-service teacher.	3.26	0.62	Moderate
I cannot effectively monitor my students' during experiments because I don't have much experience in handling such activities.	2.96	0.64	Moderate
I am not confident enough that I teach biology effectively because I haven't attended much training in biology teaching.	3.00	0.80	Moderate
I can teach the subject effectively because I understand Biology concepts well enough from my undergraduate/graduate degrees.	3.22	0.50	Moderate
I can typically answer my students' questions in Biology with confidence because I know biology well enough since I constantly study the course.	3.22	0.50	Moderate
I usually welcome questions from students because I am confident that I can answer any question in Biology since I always update myself with the trends and topics related to it.	3.17	0.49	Moderate
Vicarious Experiences			
I can continually find better ways to teach Biology through observation of seasoned colleagues.	3.39	0.50	Moderate
When my students have difficulty understanding a biology concept, I can help the students understand it better because I learned variety of strategies from my mentors.	3.17	0.49	Moderate
Verbal Persuasion			
I believe I have the necessary skills to teach Biology because my principal and my colleagues say so.	3.13	0.50	Moderate
Somatic and Emotional States			
I can hardly explain to students why Biology experiments work because I am naturally not so good in explaining especially when I am depressed or has personal problems.	3.22	0.60	Moderate
As much as possible, I don't want my principal to evaluate my teaching because he/she might find my weaknesses as teacher and it's very obvious with my demeanor especially when being observed.	3.43	0.51	Moderate
Even I exert much efforts, I cannot turn my students to like Biology maybe because it's quite obvious with how I speak that I am still a newbie.	3.17	0.72	Moderate
Composite Mean	**3.20**	**0.59**	**Moderate**

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Biology Teachers' Self-Efficacy Belief in terms of Biology Teaching Outcomes Expectancy (BTOE)

Table 2 shows the self-efficacy belief of SHS biology teachers in terms of biology teaching outcomes expectancy. It is apparent from the table that teachers have moderate self-efficacy with a mean value of 2.35 in the statement, *"If my students are underachieving in Biology, it is most likely due to my shortcomings in teaching them because I, myself, am still learning since this is a new preparation for me"*.

Moderate self-efficacy, with a mean value of 2.77 is also noted with the statement, *"If parents comment that their child is showing more interest in biology at school, I am sure it is due to my performance as his/her teacher because I get the same comments with my colleagues and principal"*. On the other hand, teacher respondents are confident enough of their effectiveness in teaching as reflected on the mean value of 2.91 on the statement, *"I am confident enough of my effectiveness as a teacher, thus low achievement of my students should not be blamed on me because I know I am good and has performed well according to my principal"*.

Different trends can be noted from the following statements on biology teaching outcomes expectancy: *"I can find more effective teaching approach to improve my students' grades in Biology by attending in-service training"; "I can improve the performance of my students in Biology if I exert extra effort in teaching the subject by observing other teachers and learning techniques from them"; "I can help my students overcome their inadequacy in Biology background through exerting extra time and effort in teaching them as what I have observed being practiced by my senior colleagues"; and, " I can make my students achieve high grades in Biology since I am good at motivation. They can see how enthusiastic and passionate I am when I explain and do lectures during our classes"*. They have higher mean values of 3.61, 3.43, 3.43 and 3.26 with standard deviation values of 0.05, 0.51, 0.50 and 0.45, respectively.

In general, respondents' biology teaching outcomes expectancy has a composite mean of 2.99 with a standard deviation value of 0.75 which falls on the category moderate self-efficacy belief. Same factors were revealed that would probably affect the moderate self-efficacy belief of teachers, these are mastery experiences and verbal persuasion. It could be noted that the teacher respondents are unsure that they can make positive influence on their students because the subject is a new preparation.

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Table 2. Respondents' Biology Teaching Outcomes Expectancy (BTOE)

Statements	Mean	Standard Deviation	Verbal Interpretation
Mastery Experiences			
I can find more effective teaching approach to improve my students' grades in Biology by attending in-service training.	3.61	0.05	High
If my students are underachieving in Biology, it is most likely due to my shortcomings in teaching them because I, myself, still learning since this is a new preparation for me.	2.35	0.70	Moderate
Even if I exert more effort in teaching Biology, I cannot improve their achievement because I have limited knowledge with different teaching pedagogies.	3.13	0.50	Moderate
I can make my students succeed or fail in Biology since I apply different approaches I learned from my professors in college.	3.05	0.60	Moderate
Vicarious Experiences			
I can improve the performance of my students in Biology if I exert extra effort in teaching the subject by observing other teachers and learning techniques from them.	3.43	0.51	Moderate
I can help my students overcome their inadequacy in Biology background through exerting extra time and effort in teaching them as what I have observed being practiced by my senior colleagues.	3.43	0.50	Moderate
Verbal Persuasion			
I am confident enough of my effectiveness as a teacher, thus low achievement of my students should not be blamed on me because I know I am good and has performed well according to my principal.	2.91	0.70	Moderate
If parents comment that their child is showing more interest in biology at school, I am sure it is due to my performance as his/her teacher because I get the same comments with my colleagues and principal.	2.77	0.61	Moderate
Somatic and Emotional States			
I can make low-achieving students progress in biology because I know how to motivate them to do good through my confident demeanor during classes.	3.17	0.39	Moderate
I can make my students achieve high grades in Biology since I am good at motivation. They can see how enthusiastic and passionate I am when I explain and do lectures during our classes.	3.26	0.45	Moderate
Composite Mean	**2.99**	**0.75**	**Moderate**

Technological Knowledge

In terms of technological knowledge, it is shown on Table 3 that the level of technological knowledge of biology teachers' evaluation vary among group of raters. Similar trend can be seen on the students' and teachers' self-evaluation with 3.04 and 3.28 mean values, respectively, equivalent to moderate proficiency. Conversely, with the rating from class observation, the value of mean, 2.38 which is equivalent to slight proficiency is significantly lower with that of the other two groups of evaluators.

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This is probably because there are two specific statements regarding technological knowledge that were rated as "low proficiency". Teachers were rated low in the statements, *"I make use of laboratory equipment with ease"* with mean value of 1.40 and, *"I apply certain techniques when using laboratory instruments"* with mean value of 1.48.

Table 3. Biology Teachers' Technological Knowledge

Statements	*Students' Evaluation*	*Teachers' Self-Evaluation*	*Class Observation Evaluation*
1. I use projector and laptop in the class.	3.38 Moderate	3.70 High	3.24 Moderate
2. I know how to solve simple technical glitches.	3.09 Moderate	3.35 Moderate	2.76 Moderate
3. I use downloaded animations and videos in support to our lesson in biology.	3.14 Moderate	3.61 High	2.88 Moderate
4. I reach out to my students regarding our lesson thru online messaging.	3.08 Moderate	3.22 Moderate	2.64 Moderate
5. I create online group account for our class intended for sharing files and other learning materials relevant to our topics in biology.	2.94 Moderate	3.09 Moderate	2.48 Slight
6. I include multimedia platforms like Facebook, YouTube, in some of our activities.	2.80 Moderate	3.00 Moderate	2.60 Moderate
7. I mention during classes jargons which are somewhat "techie" in nature.	2.78 Moderate	3.05 Moderate	2.50 Moderate
8. I give activities that involve use of technology.	3.12 Moderate	3.30 Moderate	1.79 Slight
9. I make use of laboratory equipment with ease.	3.06 Moderate	3.27 Moderate	1.40 Low
10. I apply certain techniques when using laboratory instruments.	2.98 Moderate	3.18 Moderate	1.48 Low
Composite Mean	**3.04 Moderate Proficiency**	**3.28 Moderate Proficiency**	**2.38 Slight Proficiency**

The use of multimedia platforms like Facebook and YouTube, as well as the use of jargons which are somewhat "techie" has low mean values of 2.60 and 2.50, respectively. On the other hand, different trend is exhibited when it comes to the use of projector and laptop in class. This statement has the

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highest mean value in all groups of evaluators. These quantitative results were confirmed by the researcher during class observations. More than half of the population of the teacher respondents were observed.

Pedagogical Knowledge

Table 4 presents the respondents' pedagogical knowledge or the knowledge of the SHS biology teachers to deliver, thru different strategies and approaches, the lessons in biology to their students.

As shown in the table, students evaluated their teacher as moderately proficient with a mean value of 2.99. Same trend can be seen with biology teachers' self-evaluation as well as the result of class observations with mean values of 3.45 and 2.65, respectively. It is worthy to note that evaluation of biology teachers' pedagogical knowledge is the same in all group of evaluators which means they all agree that there is still aspects on the use of appropriate biology teaching methodologies that should be addressed to achieve highest proficiency.

It is important also to mention, however, that the statements, *"I use variety of strategies in presenting topics in biology", "I try another strategy when my students are having difficulty understanding the lesson"* and, *"I provide activities that can help students cope with the subject"* were rated as slightly proficient by class observers. And, it is quite obvious that the ratings from class observations are somewhat different with that the students' and teachers' self-evaluation as indicated in its mean value although it is also verbally interpreted as moderate proficiency.

Content Knowledge

Table 5 shows the SHS biology teachers' content knowledge. It can be gleaned from the table that SHS biology teachers of Batangas City perceived their content knowledge as moderately proficient with a mean value of 3.33. However, the evaluation from students and classroom observation say otherwise. Mean values of 2.48 and 2.14 are lower which indicate slight proficiency.

It can be seen from the table that the statement, *"I use non-traditional authentic assessment techniques like concept mapping, debates and practical exam"* has the lowest mean value of 1.40 with a verbal interpretation of slight proficiency. Other statements with low mean values include: *"I give simple yet innovative laboratory activities"*, *"I provide updates related to our topic"*, *"I can discuss lessons in my own language and do not rely much on the book"*, *"I explain difficult topics as if it is easy, thus, my students understand them better", and "I relate biology topics to other subject areas"*. These aspects of

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biology teachers' content knowledge were all rated as slightly proficient by both students and class observers, which is obviously, different from teachers' self-evaluation. This suggests that even the students recognize that their biology teachers are not well-versed in general biology course based on the indicators mentioned in the table, and their assessment is strengthened by class observers' evaluation who know more about the nature of the subject.

Table 4. Biology Teachers' Pedagogical Knowledge

Statements	*Students' Evaluation*	*Teachers' Self-Evaluation*	*Class Observations Evaluation*
1. I use variety of strategies in presenting topics in biology.	2.85 Moderate	3.43 Moderate	2.36 Slight
2. I know how to assess students' performance in the classroom.	3.12 Moderate	3.39 Moderate	2.50 Moderate
3. I show fairness in dealing with students.	3.11 Moderate	3.70 High	3.24 Moderate
4. I try another strategy when my students are having difficulty understanding the lesson.	2.89 Moderate	3.39 Moderate	2.49 Slight
5. I correct students' misconceptions about certain topic.	3.16 Moderate	3.48 Moderate	2.67 Moderate
6. I adapt my teaching style based on the level of understanding of my students.	2.98 Moderate	3.57 High	2.69 Moderate
7. I adjust teaching method when some of my students are having difficulty in understanding the lesson.	2.89 Moderate	3.39 Moderate	2.64 Moderate
8. I know how to manage and organize our classroom.	3.05 Moderate	3.39 Moderate	2.74 Moderate
9. I provide activities that can help students cope with the subject.	2.81 Moderate	3.43 Moderate	2.36 Slight
10. I know the pace of lessons appropriate to the needs and difficulties of my students.	2.98 Moderate	3.35 Moderate	2.86 Moderate
Composite Mean	**2.99 Moderate Proficiency**	**3.45 Moderate Proficiency**	**2.65 Moderate Proficiency**

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Table 5. Biology Teachers' Content Knowledge

Statements	*Students' Evaluation*	*Teachers' Self-Evaluation*	*Class Observations' Evaluation*
1. I discuss biology lesson clearly.	2.60 Moderate	3.35 Moderate	2.57 Moderate
2. I encourage my students to ask questions even practical ones.	2.65 Moderate	3.61 High	2.24 Slight
3. I relate biology topics to other subject areas.	2.49 Slight	3.43 Moderate	2.05 Slight
4. I set lesson objectives within the experiences and capabilities of my students.	2.57 Moderate	3.43 Moderate	2.31 Slight
5. I show confidence in delivering the lesson.	2.72 Moderate	3.43 Moderate	2.71 Moderate
6. I can discuss lessons in my own language and do not rely much on the book.	2.39 Slight	3.13 Moderate	2.26 Slight
7. I provide updates related to our topic.	2.41 Slight	3.26 Moderate	2.05 Slight
8. I give simple yet innovative laboratory activity.	2.39 Slight	3.09 Moderate	1.69 Slight
9. I use non-traditional authentic assessment techniques like concept mapping, debates and practical exam.	2.12 Slight	3.26 Moderate	1.40 Low
10. I explain difficult topics as if it is easy, thus, my students understand them better.	2.49 Slight	3.26 Moderate	2.14 Slight
Composite Mean	**2.48 Slight Proficiency**	**3.33 Moderate Proficiency**	**2.14 Slight Proficiency**

Self-Efficacy Belief and TPACK

Pearson correlation was computed to determine the relationship of the SHS biology teachers' self-efficacy belief and their technological, pedagogical and content knowledge. As shown in the Table 6, the computed Pearson's *r* of 0.401 with p-value of 0.058 implies that there is no significant relationship between these two variables.

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Table 6. Biology Teachers' Self-Efficacy Belief and their TPACK

	Pearson's *r*	***p*-value**	**Interpretation**
Self-efficacy Belief VS TPACK	0.401	0.058	No Significant Relationship

DISCUSSION

Biology Teachers' Self-Efficacy Belief in terms of Personal Biology Teaching Efficacy

In terms of PBTE, the results revealed that they have moderate self-efficacy belief which suggests that most of the SHS biology teachers believed they can deliver lessons to their students well but there is still room for doubt. This feeling of non-absolute answer is expected from teachers who are newly hired, transferred into a new workplace or even to those who are exposed to new subject preparations, most especially to those who did not attend trainings on this particular subject area, more so if the teacher's educational background is non-science or graduate of other disciplines, which is actually the case of the SHS biology teachers of Batangas City. Focus group discussion revealed that some of them are not well versed in doing usual biology experiments especially some are graduates of other courses like Computer Engineering and Food Technology which do not require similar laboratory activities in general biology course. In addition, most of them are first timers in handling the subject and were not able to attend trainings on biology teaching.

In this context, the results confirm Lekhu's (2013) idea that mastery experience is one of the identified factors that greatly influences teachers' efficacy belief. Francis (2016) on the other hand, mentioned that strong mastery experiences support teachers' attitudes and desired professional goals result into high levels of self-efficacy belief. Hence, SHS biology teachers of Batangas City are not so confident that they can teach the subject based on their background.

Ravikumar (2013) however, focused on how influential people in the teachers' lives like that of their principal and colleagues strengthen their

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beliefs through persuasion and giving positive comments about their teaching. It can be assumed then that teacher-respondents rarely received these encouraging feedbacks.

It could be noted from the findings that mastery experiences and verbal persuasions are two factors that cover the statements with the lowest mean values, therefore, SHS biology teachers of Batangas City need some boost in these aspects influencing their self-efficacy belief, attendance to training workshops, specifically with activities that require development of laboratory skills and conducting experiments. Abaan et al. (2012) also suggested collaborative planning sessions, team meetings, peer observations and mentoring relationships should be included because these provide opportunities to enhance self-efficacy belied through verbal persuasions.

Biology Teachers' Self-Efficacy Belief in terms of Biology Teaching Outcomes Expectancy

Based on their self-assessment, teachers concluded that they have moderate self-efficacy belief. Mastery experiences and verbal persuasions are also the two factors that need to be addressed to be able to improve on this aspect. This is probably because the subject is a new preparation under a new curriculum.

The findings clearly show then that the teacher preparation program is very important. Since teacher efficacy plays an important role in promoting students' learning achievement and their self-efficacy development in the classroom. Mastery experience was found to be the main predictor for academic achievements (Choy and Loo 2013). Hence, teacher educators must provide better teacher preparation programs for efficacy development (Chang 2015; Incikabi 2013).

In addition, verbal persuasion is also a factor because they believed that encouraging words and praises from colleagues and immediate supervisor like their principal uplift their self-efficacy belief (Ravikumar 2013). Thus, it can be deduced that they did not receive those compliments. Validation coming from the parents with their children performing well, showing interest in biology and getting high grades are affirmations that they are doing the right thing in teaching the subject. Absence of these lessen self-efficacy belief which in effect will also affect student outcomes. These mentioned areas are needed to be considered in planning and drafting of any kind of development programs for SHS biology teachers of Batangas City.

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Technological Knowledge

It can be noted from the results that weaknesses of the SHS biology teachers of Batangas City on this aspect are mostly on the knowledge on how to come up with activities that involve technology, manipulation laboratory equipment and laboratory techniques.

It was mentioned during the focus group discussion that most of the respondents are having a hard time coming up with laboratory activities. One teacher even commented, which everybody agreed, that if ever there are training plans, a sort of compilation of laboratory activities which is simple and easy to do that can be provided and photocopied would be helpful. This statement is a clear indication that they do not have enough knowledge on these matters, hence, cannot come up with their own good laboratory activities. These findings are similar with the findings on the study of Tibyehabwa et al. (2017) wherein in-service teachers' lab-based training was suggested to address this problem. Furthermore, experience could be a factor because most of them are less than five years in teaching. The proposition of Tondeur et al. (2012) that technological knowledge is the most critical area for "novice" teachers because they have not participated in many learning experiences that were enriched by technology must be true then.

In addition, numerous studies concluded that technology, by their very nature are tools that encourage and support independent learning (Lowther et al. 2012). Thus, teachers' technological knowledge is very important to educate millennial learners. The technological knowledge consists of having an understanding of the standard technologies as books and chalk and more advanced technologies such as the internet and soft wares. Teachers need this kind of knowledge so that they know how lessons have been planned or designed to integrate technology into their classroom activities (Agyie and Voogt 2012). It could then be assumed that technological knowledge of the respondents need enhancement based on the findings.

Pedagogical Knowledge

It is apparent from the results that the teachers are using the same teaching strategies as they deliver the lesson, however, this should not be the case in biology teaching. Tanner (2018) emphasized the importance of using various strategies in teaching because if a teacher chooses a singular approach – always lecturing or always concept-mapping, regardless of the nature of approach – it could result in the alienation and exclusion from learning of a subpopulation of students. The biology teacher should then know how to utilize different teaching strategies.

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In the case of the respondents, it was mentioned that some of them are non-science education graduates, hence, it is probably the reason why this particular aspect of pedagogical knowledge was only rated as moderately proficient. Van Driel et al. (2014) mentioned that a higher level of subject matter knowledge, which cannot be expected from teachers who are graduates of other disciplines, is typically associated with more confidence and more interactive and adventurous ways of teaching. Moreover, general biology is under the new K to 12 curriculum, most of the teachers are young and new in the teaching profession and everything is on its initial stages, these could probable the reasons.

Content Knowledge

Results implied that SHS biology teachers of Batangas City are not inclined much on giving updates on the subject and providing relevant activities as shown in the ranking of their mean values. It is also very evident that among the three categories of TPACK, content knowledge has the lowest rating which should not be the case. Biology is science and teachers should know the latest information from different sources like journal articles. They are lifelong learners who continue to keep current with the latest news, discoveries and research findings (Silver undated)

Teachers with high content knowledge can discuss the lesson spontaneously and use their own words and explain difficult topics as if they are easy. This clearly reflects the mastery of the teacher with the subject matter. Teachers who depend much in the book or references during discussions show lack of comprehensive knowledge. Relying solely on the book restricts the ideas that teachers can share to their students (Ball et al. 2008).

Self-Efficacy Belief and TPACK

Senior high school biology teachers of Batangas City have moderate proficiency in both aspects of self-efficacy belief: personal biology teaching efficacy (PBTE) and biology teachers' outcomes expectancy (BTOE). Mastery experiences and verbal persuasions were found to be the factors that should be looked into and addressed.

On the other hand, teachers, in general, have moderate proficiency in TPACK, however, it is important to mention that there are indicators on each category which varies based on the group of evaluators that need to be noted: knowledge on the manipulation of laboratory equipment and integration of technology in laboratory activities; the use of various teaching strategies; and, use of non-traditional assessment techniques like concept mapping, debates, and practical exam, constant subject matter updating and contextualization

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are aspects that ought to be strengthened. Furthermore, since SHS is new, all biology teachers can be treated as "novice" regardless of their technological, pedagogical and content knowledge proficiency, hence, there is no relationship with their self-efficacy belief.

When it comes to the correlation of self-efficacy belief and TPACK, respondents perceived that there is no significant relationship between these two variables. This means that the respondents do not think that the teachers' conviction to teach the subject as well as the belief that they can produce positive student outcomes have something to do with their technological, pedagogical and content knowledge. Which means that their moderate proficiency in terms of TPACK has nothing to do with their self-efficacy belief, and their moderate self-efficacy does not result into their moderate proficiency in TPACK.

Results challenge the theoretical assumptions about the positive relationship of self-efficacy belief and TPACK of Joseph (2010) and Rohaan et al. (2012). The findings are quite different from most studies which demonstrated the connection between the two variables. It could then be assumed that since SHS is in its initial stages, all teachers can be considered as "novice" regardless of their technological know-how, the level of their subject matter knowledge and expertise on the use of different teaching strategies. All of them are on the new academic setting and teaching environment with new challenges and expectations so no matter what TPACK proficiency they have, it has nothing to do with their self-efficacy belief. The feeling of anxiety and being uncertain and doubtful of their capabilities are expected. However, Abbitt (2011) explained the changing nature of the relationship between TPACK and self-efficacy belief, thus, when everything is settled and in place, the relationship between these two variables might change.

The relationship properties of TPACK-self-efficacy belief can be referenced in the framework of training for pre-service and in-service science teachers (Eny Hartadiyati and Sutikno 2015). As emphasized by Joseph (2010), science educators should have a clearer understanding of their combined impacts one could expect an effective teacher to have well-structured pedagogical content knowledge and consequently, a higher sense of teaching efficacy.

Luft and Hewson (2014) pointed out that professional development can occur before a teacher ever begins teaching in the classroom and may continue until the end of the teaching career. It is then highly recommended for the SHS teachers of Batangas City to attend such trainings, especially, everything in the SHS is in its transition and adjustment period. However, a training design framework can be drafted to serve as ground principle of

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future training design plans since this present study provided baseline data. It will ensure that the trainings to be implemented are tailored to the needs of SHS biology teachers of Batangas City. It is also suggested to conduct similar study on other science disciplines like physics and chemistry in order to assess science teaching in totality.

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Effects of forest management practices on microbial biomass, litter decomposition, microbial abundance, and the soil's physical and chemical properties of replacement plantations after pine wilt disease

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ABSTRACT

The goal of this study was to determine the effects of two combinations of forest management practices employed on replacement pine plantations after pine wilt disease. The objective was to measure the soil's physical and chemical properties, microbial biomass, litter decomposition and microbial abundance as affected by thinning and pruning, and the understory weeding and applications of insecticide and nitrogen fertilizer. This study was conducted in Ryuosan, Higashihiroshima City, Japan. Results showed that the physical characteristics (color, texture, moisture and water-holding capacity) of the three study sites did not differ significantly from each other. On the other hand, the chemical properties (pH, C and N) showed significant differences among sites. The relative light intensity difference (LID) greatly fluctuated and differed. Microbial biomass and microbial abundance were shown to have seasonal variations and lower at the managed sites than at the unmanaged site whereas litter decomposition did not vary significantly. The high correlations among biological and physico-chemical properties of soil at the study sites implied high interdependence among soil's characteristics.

Keywords: forest management practices, microbial biomass, replacement plantations, microbial abundance, pine wilt disease.

INTRODUCTION

There were massive forestry losses, especially in Japan, due to pine wilt disease caused by pinewood nematode infestations (Mamiya 1983; Fukuda et al. 1998). With the occurrence of this disease, foresters and even private land owners have become more concerned about how to protect forest ecosystems from infestations. One of the solutions for protecting forests from infestations is the use of different forest management practices (Johnston and Crossley 2002). In similar process in Japan, the areas affected by pine wilt disease were

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totally deforested and replaced by plantations. These replacement plantations are then subjected to different management practices to prevent contamination and infestations and to increase commercial value of forest stands during their development (Rötzer et al. 2010).

Concerns have increased regarding the possibility of detrimental ecological effects of forest management practices (Johnston and Crossley 2002; Bird et al. 2004; Lundmark et al. 2017) and have been placed under scrutiny with respect to their impacts on the environment and on site productivity and biodiversity (Burger and Zedaker 1993; Gupta and Malik 1996; Günter et al. 2011). Many studies have been conducted to show the effects of forest management practices, like clear-cutting (Donegan et al. 2001; Gondard et al. 2003; Lundmark et al. 2017), forest harvesting (Marshall 2000; MacDonald and Thompson 2003; Bird et al. 2004), understory weeding (MacDonald and Thompson 2003), fertilizer applications (Lee and Jose 2003; Williamson and Neilsen 2003; Zhang et al. 2017), and prescribed burning (Pietikäinen and Fritze 1995; DeLuca and Zouhar 2000), on the soil's physical and chemical properties and biological components. Each of these forest ecosystems studied had their own history of disturbances, but there is no record yet of any study conducted on forest management practices after pine wilt disease.

Soil microorganisms have been proven to be sensitive indicators of environmental disturbances (Torsvik and Øvreås 2002). They are responsible for most soil processes but the knowledge of the relationship between them and aboveground processes is still incomplete (Colombo et al. 2016). Reports have been very varied about microbial responses to different disturbances. The disturbance history of the forest ecosystem may contribute to the varying responses of microorganisms towards different management practices (Johnston and Crossley 2002).

Two combinations of management practices were considered in this study, both employed in young pine plantations. The first combination was biomass thinning and pruning, and the second combination was understory weeding and applications of nitrogen fertilizer and insecticide. Biomass thinning and pruning which involve selective removal of small diameter trees and the cutting of the appropriate amount of branches, are practices traced with very long history (Forest management solutions 2004; Lundmark et al. 2017). Thinning and pruning are considered important management techniques for plantations, and are undoubtedly indispensable for the sustainable development of forest (Seiwa and Kikuzawa 1994; Ooishi et al. 1998; Krauchi et al. 2000; Montagu et al. 2003; Rötzer et al. 2010). Thinning and pruning creates canopy openness or gaps, which are critical in the community dynamics of many types of forest (Gray and Spies 1996). For example, tree harvesting and site preparation practices have resulted in a significant loss of nutrients and organic matter, thereby decreasing site

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productivity (Pritchett and Fisher 1987; Bormann and Likens 1994). Another study conducted by Donegan et al. (2001) showed that clear-cutting decreased the populations of nematodes, microarthropods, bacteria and fungi in both litter and soil. In addition, clear cutting could have similar genetic effects as pest outbreaks, wildfires or storms (Alfaro et al. 2014).

Fertilization and understory weeding of forest plantations have also become an increasingly important part of intensive management in recent years (Allen et al. 1990; Fox 2000). The few existing studies have reported conflicting results of the effects of fertilization, some reporting positive effects (Gallardo and Schlesinger 1994; Williamson and Neilsen 2003), and others reporting negative effects (Haynes and Gower 1995; Lee and Jose 2003). These conflicting results could be attributed to differences in allocation patterns among tree species, soil condition, stand's age and disturbance history. Fertilization increased the arthropod abundance in the harvested site which was comparable to an unharvested site (Bird et al. 2004).

The goal of this study was to determine the effects of two combinations of forest management practices employed on replacement pine plantations after pine wilt disease. The objectives were to measure the soil's physical and chemical properties, microbial biomass, litter decomposition and microbial abundance as affected by thinning and pruning, and the understory weeding and applications of insecticide and nitrogen fertilizer.

METHODS

Study Site

This study was conducted in Ryuosan, Higashihiroshima City, Japan. Three study sites were chosen for this study. The first one (Figure 1) was a 6-year old stand (P6) maintained by under-storey weeding, application of nitrogen fertilizer and spraying of insecticide once a year, without pruning or thinning. The second site (Figure 2) was a 10-year old stand (P10) maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year. These two sites are pine plantations which are replacement stands after total deforestation and eradication because of pine wilt disease. The third site (Figure 3) was an unmanaged pine forest (UM) about 30 years old, which is not affected by the disease and without any previous history of pine wilt disease. In these study sites, three 10m x 10m plots were established. Sampling was conducted every other month for a period of 13 months.

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Figure 1. A 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without thinning or pruning. During the first 4 years in this plantation, the under-storey grasses were removed but this was then stopped.

Figure 2. A 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year.

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Figure 3. An unmanaged pine forest without any previous history of pine wilt disease.

Soil Sampling

For all the soil analyses conducted, top soil samples were taken from 12 different points, to a depth of 5 cm, randomly in each of the study sites using a sterile hand corer. Samples were then homogenized and big particles, such as litter, rocks and macrofauna, were hand-removed aseptically. Samples were then subjected to sieving (of desired mesh size depending on the analysis done). Sampling was done once every other month.

Light Intensity Difference (LID)

The relative light intensity in each study area was measured at noonday using an LI-210SA photometric sensor (LI-COR, USA). The light intensity, both outside and inside the forest, was measured, and the difference between the two was recorded as LID (light intensity difference).

Physical and Chemical Properties of the Soil

The moisture content was measured by oven-drying the samples at 105°C for 24 hours. The color of the soil was determined through ocular inspection (Munsell 1976; Oyama and Takehara 1997). The water-holding capacity was measured using the Hilgard method (Childers et al. 1996). The soil texture was determined using the modified 'jam jar' method (Anonymous 2000; Gardeners Supply Company 2002). The pH at each site was determined using an electrode pH meter (Tateishi et al. 1989). The total carbon and total nitrogen were measured using a C-N analyzer (Tateishi et al. 1989). Except for

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soil color and texture, all other physical and chemical analyses of soil samples were conducted bimonthly.

Litter Decomposition

Freshly abscised pine needles were collected in July 2003 from the study area. The litter samples were air-dried until constant weight was achieved. Five grams of litter was placed in nylon (1 mm mesh size) litter bags. These litter bags were exposed to UV light for 10 minutes in a laminar flow as a form of sterilization. The litter bags were placed randomly in each plot, where each bag was tied firmly to a stick and covered with litter/soil. Three replicates of litter bags from each plot were collected bimonthly to determine the mass loss. Mass losses were determined after oven-drying the samples to a constant weight at 80°C.

Microbial Analyses

Microbial biomass carbon. The microbial biomass carbon was measured using the chloroform-fumigation and direct extraction method (Vance et al. 1987). This procedure was then followed by dichromate digestion and titration.

Microbial abundance. The dilution plate count technique was used in this study (Tateishi et al. 1989) to enumerate the functional groups of microorganisms using selective culture media. For Gram positive bacteria, an Albumin agar medium was used. The same Albumin agar medium was used for Gram negative bacteria but added with 10 $ml.L^{-1}$ 5% crystal violet. Actinomycetes and fungi were enumerated using Dextrose-nitrate agar medium and Rose Bengal agar medium, respectively. For cellulase-producers and amylase-producers, the Most Probable Number (MPN) technique was used (Acea and Carballas 1996).

Three sub-samples were used from each plot and Petri plates were then incubated, at 28°C for 2-5 days for bacteria and actinomycetes, at 25°C for 7-10 days for fungi, and at 28°C for 4-9 weeks for cellulolytics and amylolytics microbes. All the results were obtained in triplicates.

Data Analyses

All the data obtained were subjected to analysis of variance (ANOVA) to show the significant differences among means, and Tukey's test was employed to separate means. Correlation analysis was used to test the relationships between the litter decomposition, light intensity difference, microbial biomass and abundance, and the soil's physical and chemical characteristics.

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RESULTS

Light Intensity Difference (LID)

The relative light intensity difference (LID) of P6, P10 and UM greatly fluctuated and differed from each other. The average LID at P6 was 3.75 klux, at P10 3.01 klux and at UM 4.36 klux (Table 1). The LID at UM was then around 19.5% and 30.9% greater than that at P6 and at P10, respectively.

Table 1. Mean light intensity difference (LID) and the physical and chemical properties of the soil.

Study sites	LID (Klux)	Color	Texture	Sand (%)	Silt (%)	Clay (%)	Moisture (%)	WHC (%)	pH	C (%)	N (%)
P6	3.75	10YR5/6	loam	52.2	31.1	16.7	26.2	66.77	5.97	2.14	0.207
P10	3.01	10YR4/6	loam	53.0	29.5	17.5	23.4	66.03	4.36	2.35	0.247
UM	4.36	10YR4/4	loam	51.3	29.5	19.2	27.5	68.3	4.9	4.85	0.302

Physical and Chemical Properties of the Soil

The soil color at P6 was yellowish brown (10YR5/6), at P10 brown (10YR4/6) and at UM brown (10YR4/4). The soil texture was loam for all three study sites (Table 1).

The soil moisture content at each site was high from April to July and low from December to February. The mean moisture content at P6 was 26.2%, at P10 23.4%, and at UM 27.5% (Table 1). The moisture content at UM was around 4.7% and 14.9% higher than that of P6 and P10, respectively.

The water holding capacity (WHC) in all study sites was almost constant throughout the whole year during which the WHC at the three sites did not differ significantly. The mean WHC at P6 was 67.77%, at P10 66.03% and at UM 68.3% (Table 1)

The pH was slightly acidic at all sites. The mean pH at P6 was slightly high, 5.97, probably because of fertilization. The mean pH at P10 was 4.36, while it was 4.9 at UM (Table 1). Tukey's test showed that the pH in P6 was significantly higher than that of P10 and UM.

The total carbon and nitrogen were higher in UM than in P6 and P10 (Table 1). At UM, the averages of soil carbon and nitrogen contents were 4.85% and 0.302%, respectively. At P6, the average soil carbon and nitrogen contents were 2.14% and 0.207%, respectively. For P10, the average soil carbon and nitrogen contents were 2.35% and 0.247%, respectively.

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Litter Decomposition

The litter decomposition rate was faster at P10 but not significantly different from P6 and UM. At the end of 13 months, 40.3% of litter samples remained in P6, 36.7% in P10 and 45.8% in UM. The decomposition process underwent a fast initial phase, followed by a slow intermediate phase, and then a fast terminal phase (Figure 4). ANOVA showed no significant difference in decomposition rate between sites (Table 3). Litter decomposition also showed no correlation to any of the LID and the soil's physico-chemical properties (Table 2).

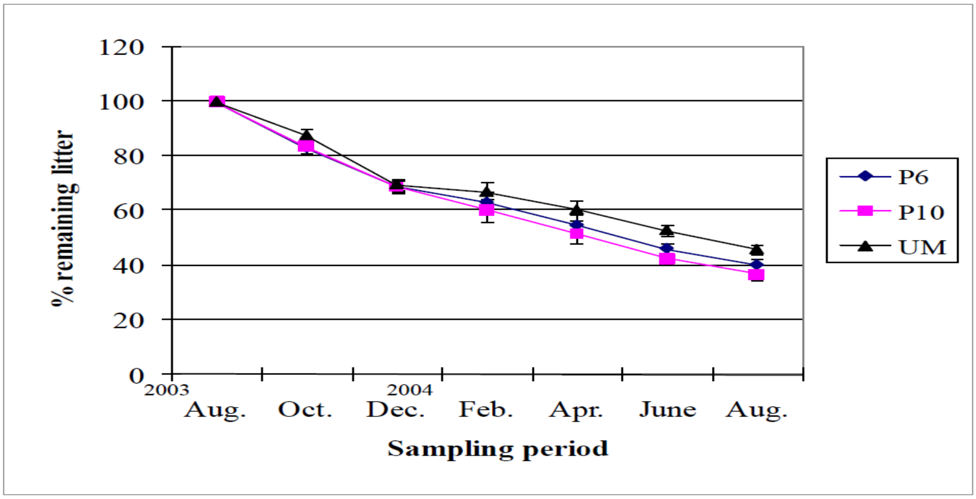

Figure 4. Average (±se) litter decomposition at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

Microbial Biomass Carbon

The ANOVA (Table 3) showed that the microbial biomass carbon differed significantly between the study sites ($p<0.05$). UM showed the highest microbial biomass carbon, followed by P10, and then P6. Tukey's test proved that P6 and P10 were not significantly different from each other, but they were significantly different from UM. High values were recorded in October 2003, and from April to June 2004. A little fluctuation occurred from December 2003 to February 2004, but the lowest value was recorded in August 2003 at P10 (Figure 5). Correlation analysis proved a significant relationship between biomass carbon and moisture content (r^2=0.584, $p<0.01$), LID (r^2=-0.628, $p<0.01$), total carbon (r^2=0.568, $p<0.01$), and total nitrogen (r^2=0.760, $p<0.01$).

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Table 2. Correlation analysis among the parameters tested.

Parameters tested	Moisture %	WHC %	pH	Light (LID)	C %	N %	Litter decomposition
Litter decomposition	0.242ns	0.080ns	-0.056ns	-0.304ns	0.194ns	0.116ns	1
Biomass carbon	0.584**	0.013ns	-0.198ns	-0.628**	0.566**	0.760**	0.506*
Gram + bacteria	0.330ns	0.014ns	-0.387ns	-0.446*	0.678**	0.633**	0.264ns
Gram – bacteria	0.211ns	0.204ns	-0.280ns	-0.205ns	0.852**	0.861**	0.096ns
Fungi	0.503**	0.138ns	-0.268ns	-0.066ns	0.817**	0.861**	0.109ns
Actinomycetes	0.699**	0.028ns	-0.367ns	-0.509**	0.808**	0.807**	0.167ns
Cellulolytics	0.342ns	0.186ns	-0.217ns	-0.033ns	0.680**	0.704**	0.410*
Amylolytics	0.448*	0.226ns	-0.245ns	-0.019ns	0.612**	0.656**	0.464*

** significant at p<01; * significant at p<05; ns not significant

Table 3. Summary of Analysis of variance (ANOVA) for all determined parameters.

Parameters tested	Sum of Squares	df	Mean square	F	Sig.
Moisture	59.16	2	29.58	1.97	.169 ns
WHC	3.69	2	1.85	0.61	.554 ns
pH	4.30	2	2.17	2.61	.101 ns
C	5.16	2	2.58	11.68	.001**
N	5.63	2	2.82	11.86	.001**
Biomass C	203829.80	2	101914.90	4.68	.023*
Gram+bacteria	10220.60	2	5110.30	17.47	.000**
Gram-bacteria	1212.64	2	606.32	13.22	.000**
Fungi	1583.71	2	781.85	8.66	.002**
Actinomycetes	5403.81	2	2701.90	25.77	.000**
Cellulolytics	268.93	2	134.46	5.74	.012*
Amylolytics	487.27	2	243.63	4.88	.020*
Litter decomposition	115.65	2	57.83	0.13	.877 ns
LID	18.04	2	9.02	25.95	.000**

** significant at p<01; * significant at p<05; ns not significant

Microbial Abundance

For all the six groups of microorganisms studied, UM showed the highest abundance, followed by P10, and then P6. The highest abundance was observed in spring and summer and the lowest was observed in winter. Great fluctuations were also observed and the lowest abundance was recorded in February 2004 (Figure 6, 7, 8, 9, 10, 11). ANOVA showed significant differences between study sites (Table 3) (p<.01). Tukey's test proved that the abundance of Gram positive and Gram negative bacteria and actinomycetes at P6 was not significantly different from P10, but P6 and P10 were significantly different from UM. The fungal, cellulase-producers' and the amylase producers' abundance at P6 did not differ significantly from P10, and P10 did not differ significantly from UM, but P6 did differ significantly from UM.

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Table 2 (correlation analysis) shows the close correlation between microbial abundance and the physicochemical properties of the soil. The Gram positive bacteria were highly correlated with total carbon (r^2=0.678, p<0.01), and total nitrogen (r^2=0.633, p<0.01). The Gram negative bacteria showed close correlation with total carbon (r^2=0.852, p<0.01), and total nitrogen (r^2=0.861, p<0.01). Fungi was highly correlated with moisture content (r^2=0.503, p<0.01), total carbon (r^2=0.817, p<0.01), and total nitrogen (r^2=0.861, p<0.01). Actinomycetes proved to be highly correlated with moisture content (r^2=0.699, p<0.01), LID (r^2=0.509, p<0.01), total carbon (r^2=0.808, p<0.01), and total nitrogen (r^2=0.807, p<0.01). Cellulase-producers were closely correlated with total carbon (r^2=0.680, p<0.01), and total nitrogen (r^2=0.704, p<0.01). Amylase-producers showed close correlation with total carbon (r^2=0.612, p<0.01), and total nitrogen (r^2=0.656, p<0.01).

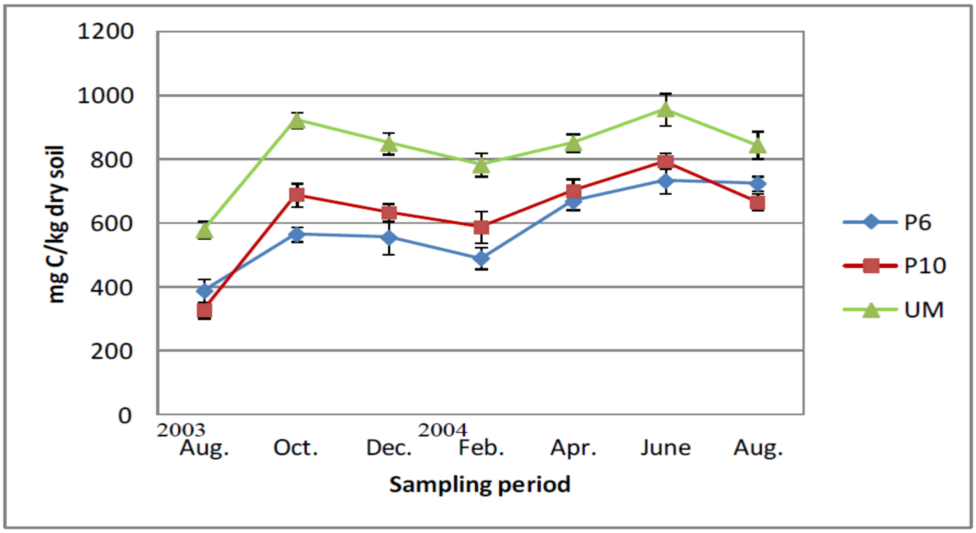

Figure 5. Average (±se) microbial biomass carbon at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

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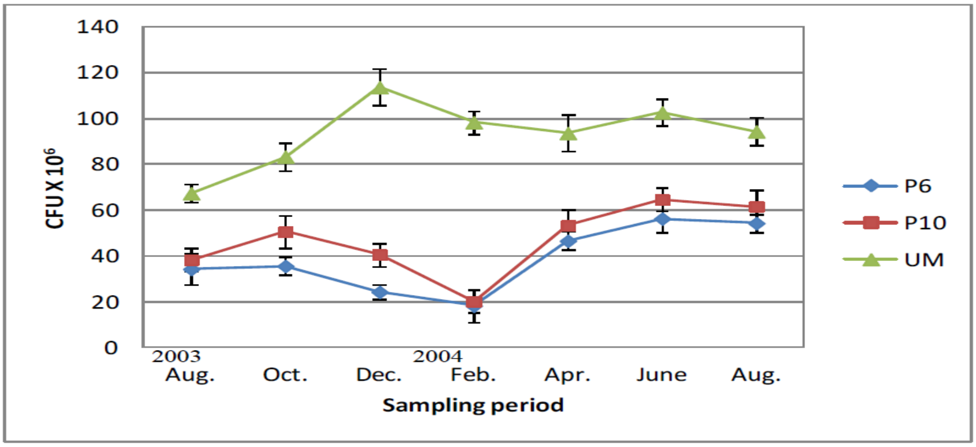

Figure 6. Average (±se) Gram positive bacterial abundance at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

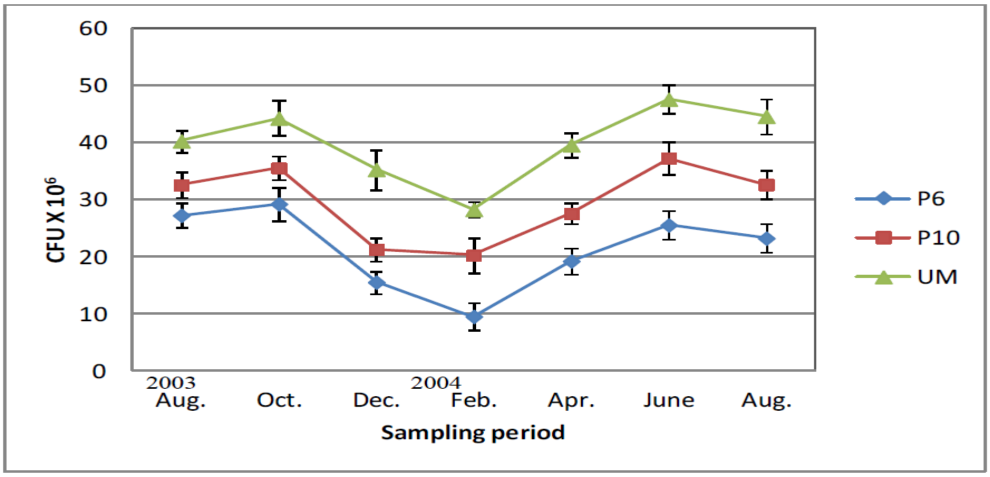

Figure 7. Average (±se) of the Gram negative bacterial abundance at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

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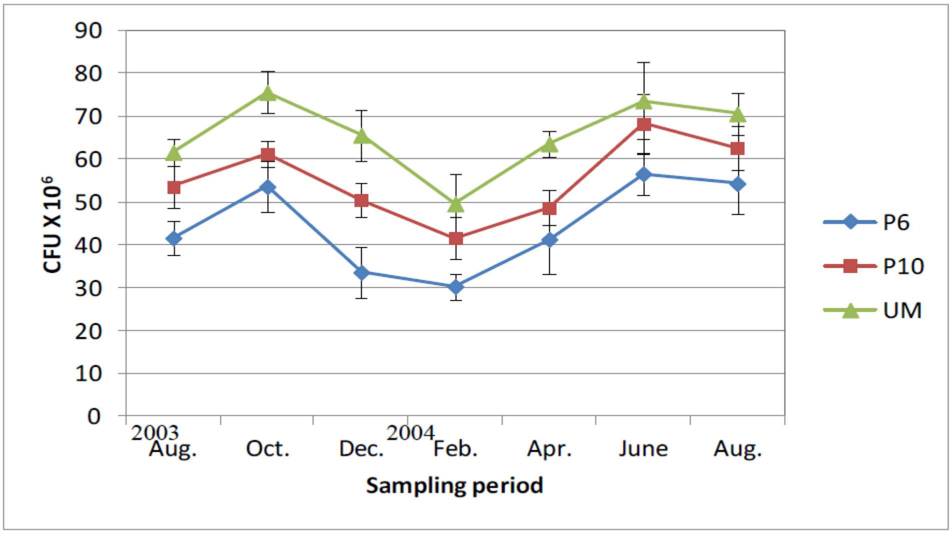

Figure 8. Average (±se) fungal abundance at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

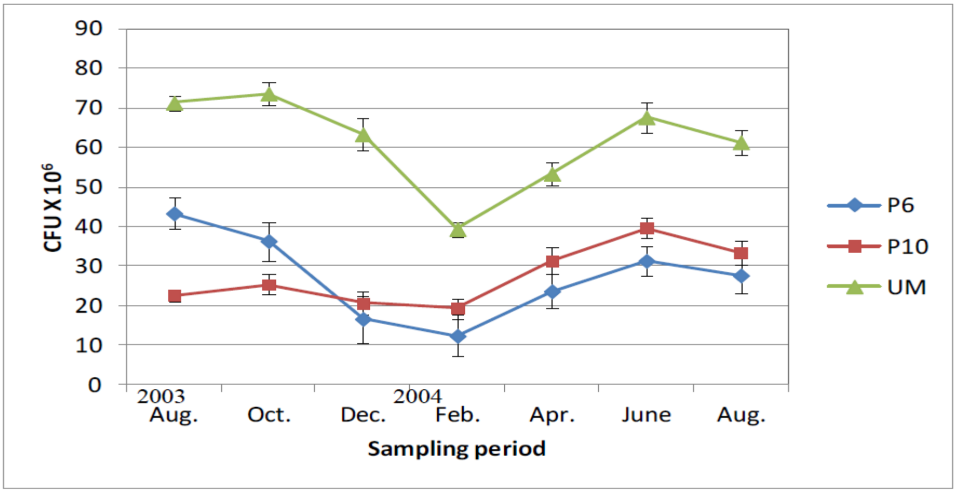

Figure 9. Average (±se) abundance of the actinomycetes at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

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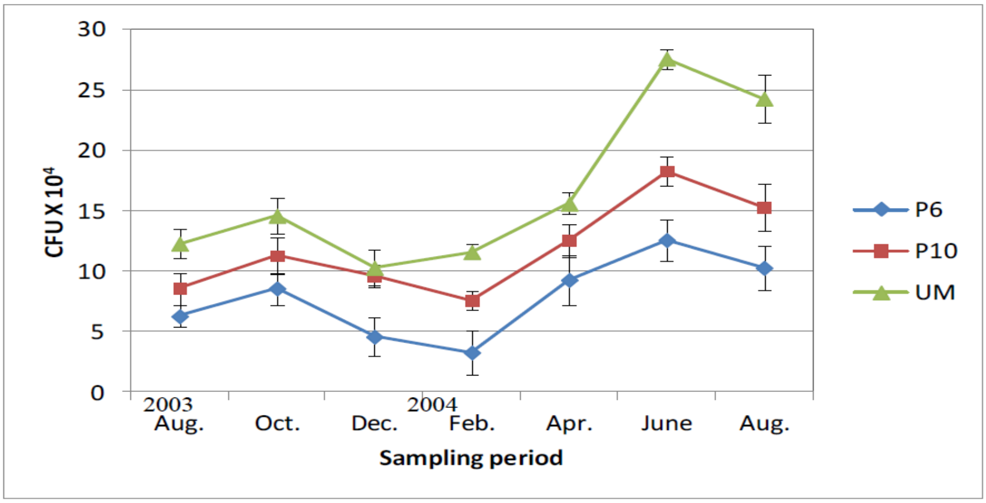

Figure 10. The average (±se) abundance of the cellulase-producers at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

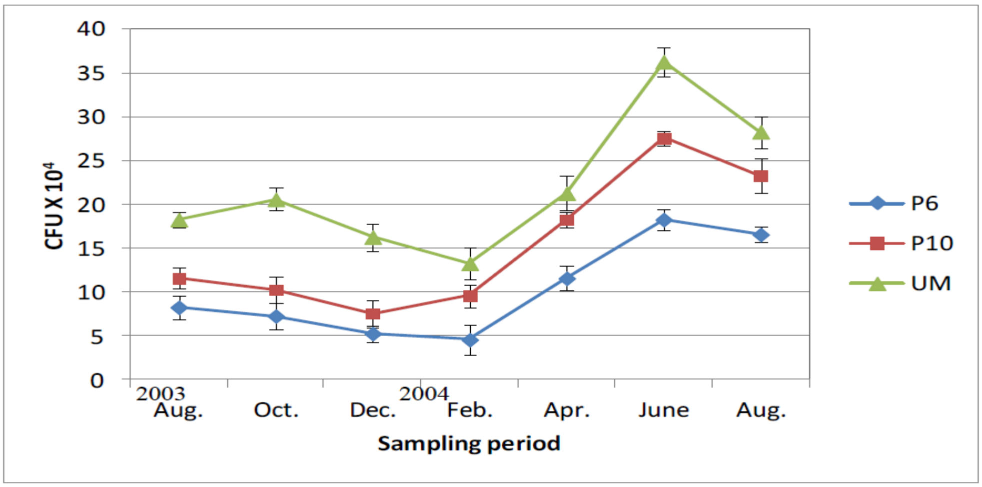

Figure 11. The average (±se) abundance of the amylase-producers at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

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DISCUSSION

The physical characteristics (color, texture, moisture and water-holding capacity) of the three study sites did not differ significantly from each other. On the other hand, the chemical properties (pH, C and N) showed significant differences between sites. The high correlations among biological and physico-chemical properties of soil implied high interdependence among soil's characteristics. The unmanaged site (UM) showed C and N content two times higher than the two managed sites (P6 and P10). These results were in contrast to what Boerner and Sutherland (1997) had observed where thinned forest had greater mineral nutrients than the unmanaged plot. The pH in P6 and P10 were higher than UM by around 20% and 9% respectively. The slightly high pH in P6 was probably because of nitrogen fertilization (Guan 2016). The nitrification process (bacteria convert ammonium to nitrate) releases hydrogen ions (H^+), which react with hydroxide ions (OH^-) released by plants during the process of taking up of nitrate. The overall effect on soil pH is close to neutral (Guan 2016). The low C and N content can be attributed to the young age of the stand where most vegetation was still undergoing vegetative growth, needing higher nutrient allocation. P6 was a fast growing stand and since no thinning or pruning had yet been employed, competition for nutrients among vegetation was high and fertilization activity was not sufficient for the vegetative demands. In the case of P10, the thinning and pruning processes should have minimized the competition for available nutrients (Vesterdal et al. 1995), but because no fertilization was being done and the vegetation was still growing fast, nutrient pooling was not possible. In addition, the litter input decreased because thinned trees and cut branches were not left to decay but were gathered to prevent infestations. Thinning and pruning promote natural regeneration and expression of some inhibited understory vegetation (Zhu et al. 2003), thereby increasing nutrient consumption.

The rate of litter decomposition did not differ significantly between sites. There was also no indication of close correlation of litter decomposition to any of the physical, chemical and biochemical parameters measured in this study. The litter decomposition underwent different phases. This could be due to microbial communities which colonized leaf litter, underwent major temporal changes, both seasonally and over the course of litter decomposition (Voříškova and Baldrian 2013). Because of high light penetration to the forest floor after thinning and pruning, a faster rate of decomposition was expected, but this was proven to be not true. Although the thinned and pruned site showed the lowest mass remaining after 13 months of litter exposure, it was not significantly different from the other two sites. On the other hand, nitrogen fertilization was expected to increase the rate of decomposition by increasing microbial abundance and other decomposers like arthropods (Bird et al. 2004; Zhang et al. 2017), but this was also proven not to be the case in this study. If N limits microbial activity like decomposition (Kaye and Hart

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1997; Hobbie and Vitousek 2000), then population and activity would increase after N addition, but this hypothesis was also not supported by the results of this study. Many studies have shown that N additions decreased or did not influence decomposition (Fog 1988; Aber 1998; Carreiro et al. 2000; Ågren et al. 2001).

Microbial biomass and microbial abundance were shown to be lower at the managed sites than at the unmanaged site. The stand type always has a strong influence on microbial biomass and abundance (Compton et al. 2004). Microbial biomass and the processes carried out by soil microorganisms depend upon complex interactions with plants (Singh et al. 1989; Bohlen et al. 2001; Zheng et al. 2017). Soil microorganisms are generally C limited (Wardle 1992) and, as a result, microbial biomass and abundance depend upon soil organic matter. In this study, the C and N content at the managed sites were a lot lower than that of the unmanaged site. This explains the low microbial biomass and abundance at managed sites. For P6, although nitrogen fertilization was being employed, the opposing effects of insecticide should also be considered. Because of the threat of pine wilt infestations, as the surrounding areas were still affected by the disease, this young stand was maintained by insecticide application to prevent infestation. Insecticide, on the other hand, is not just harmful to insects but its penetration to the soil kills microorganisms and other microflora and fauna (Aktar et al. 2009). Also, understory weeding was conducted, which made soil moisture evaporation increase and litter input decrease. With this, nutrient pools were also decreased. Although quite surprising, many studies have reported that nitrogen addition suppressed microbial biomass even in forest ecosystems where productivity is primarily N limited (Prescott et al. 1992; Smolander et al. 1994; Fahey et al. 1998; Scott et al. 1998; Colombo et al. 2016). For P10, because of thinning and pruning, gaps were created whereby light radiation towards the forest floor increased (Demarais et al. 2017). These gaps contributed to high air and soil temperatures in the forest microenvironment (Arunachalam and Arunachalam 2000). This kind of microclimate initially facilitated rapid decomposition on the forest floor (Arunachalam et al. 1996), but, because the litter input was decreased by thinning and pruning, decomposition of the organic layer happened instead. On the other hand, since P10 is in a hilly area, soil fertility may also be affected by heavy rainfall penetrating through the gaps, which may have caused erosion of top soil and leaching of organic matter.

There were seasonal variations in microbial biomass and the six groups of microorganisms studied. In all cases, high values were observed in autumn and spring. On the other hand, low values were observed in winter. These results are supported by the studies of Wüthrich et al. (2002) and Singh et al. (1989), who observed that microbial biomass is usually affected by season, with high amounts occurring in spring. On the other hand, this was in

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contrast to the findings of Arunachalam and Arunachalam (2000), that microbial biomass peaks in winter.

It was proven by Tukey's test that the abundance of Gram positive and negative bacteria and actinomycetes at P6 was not significantly different from P10, but P6 and P10 were significantly different from UM. On the other hand, the fungal, cellulose-producers' and amylase producers' abundance at P6 did not differ significantly from P10, and P10 did not differ significantly from UM, but P6 differed significantly from UM. These relationships are a little complicated to explain just looking at the effects of the management practices on the microbial populations. This could probably be attributed to the conflicting results on the effects of N additions on microbial populations and their activities, and to the harmful effects of insecticides applied to prevent nematode contamination.

It was shown in this study that the sites maintained by thinning/pruning and nitrogen/insecticide applications had lower C and N content, and low microbial biomass and abundance than the unmanaged site. Similar results were observed by Zheng et al. (2017) where they observed that the effects of fertilization on microbial communities correlated with variations in pH, moisture and N availability. Despite these facts, it is difficult to conclude that all these results were due to the silvicultural practices employed at each site. The disturbance history has a strong relationship with the soil's current physical, chemical and microbial conditions. In one of the studies the authors conducted (Mabuhay and Nakagoshi 2012), it was observed that microbial biomass carbon was greater, and the rate litter decomposition was faster, in the site currently affected by pine wilt disease than in an unmanaged site without pine wilt disease. On the other hand, the abundance of Gram positive and negative bacteria, fungi, actinomycetes, and cellulase- and amylase-producers were low at the site affected by pine wilt disease. These general effects of pine wilt disease on the soil's properties may also determine the condition and responses of microorganisms to forest management practices following pine wilt disease. These negative impacts may have been enhanced by total deforestation and pest eradication prior to reforestation. This subject should be studied more in detail for better understanding and conclusion.

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Saving Almaciga (*Agathis philippinensis*): means of cultural preservation and species rehabilitation in Palawan, Philippines

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ABSTRACT

Agathis philippinensis Warb. (Almaciga) is a coniferous tree that forms a dominant component of upland primary forest in Palawan, Philippines. Almagica is valued for its high-quality timber and for its resin, which is used in the manufacture of varnish and linoleum. The species is categorized as "vulnerable" by the IUCN due to illegal logging, destructive methods of resin tapping, and land-use change. In Palawan, collection of Almagica resin provides up to 80% of income for indigenous communities and other inhabitants living in close proximity to the forests. A recent study into the effect of resin harvesting suggested that the resource is being collected unsustainably, leaving trees highly susceptible to outbreaks of pests and diseases, reproductive failure and death of trees, increasing the risk of local extinctions. Thus, the economic livelihood and future of indigenous peoples who depend on Almaciga resin is uncertain. This study aimed to provide protocols on propagation, reforestation, and sustainable management of *A. philippinensis*. This knowledge can be used to rehabilitate depleted populations of Almaciga. Between July and December 2016, three nurseries were established across two indigenous communities in the Cleopatra's Needle Mountain range, Palawan, where more than 10,000 seeds were collected and sown in seedbeds. These seeds generated more than 6,000 seedlings that were propagated, nursed and monitored throughout the study. Information on the collection of cones, seeds and seedlings, propagation and nursery management, identification and mitigation of pests and diseases, and environmental requirements for growth and survival were documented. The propagated seedlings were used to reforest declining populations of this species, thereby ensuring the future livelihood of the indigenous communities is preserved through conservation of Almagica populations.

Keywords: Threatened species, resin collection, conifer, nursery propagation, reforestation, aboriginal, sustainable forest management, rainforest tree.

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INTRODUCTION

Agathis philippinensis Warb. (Almaciga) is a species of conifer native to the Philippines and Indonesia. It is a large tree reaching up to 60 meters height, 300 centimeters in diameter and occurs in upland forests at altitudes between 250 to 2,200 meters. It occurs in the Babuyan Islands, from Northern Luzon to Palawan and Mindanao (Ella and Domingo 2011). Almaciga yields high quality resin, also known as Manila copal that is used as raw material for varnish, lacquer, paper paint driers, linoleum, and ink, among others (Brown 1921; Saminao and Ella 2014). Manila copal is considered an important dollar earner among the country's non-timber forest products. In the Philippines, Palawan is the largest producer of Almaciga resin (Razal 2013). Collection of resin is an important source of income for indigenous peoples (IPs) and other rural communities, along with collection of cashew and seaweed (Goloubinoff et al. Undated). Due to the current high market demand of resin however, the number of collectors has increased, including non-IPs, and many practice unsustainable tapping methods leading to decline in resin yield and quality and, at worst, the death of trees due to pests and disease (Vermeer et al. 2017) and other factors that remain to be determined. Almaciga timber is also of high quality and excellent for paneling; it commands a high price in the world market. Logging of Almaciga is currently banned by the Philippine government (Mittelman et al. 1997; Ella and Domingo 2011) but illegal logging still occurs. Sustained pressure from logging and resin collection has contributed to declining populations of *A. philippinensis* in the Philippines. If these malpractices continue, Almaciga is prone to local extinction, affecting the livelihood of communities depending on it. Although little is known of the ecology of this particular species, studies have shown that closely related *Agathis* species, throughout their natural distribution, contribute greatly to the maintenance and balance of soils and nutrient cycling, plant-water relations, forest succession and many other related ecological relationships (Steward and Beveridge 2010; Bieleski 1959). Finally, with climate change as a global concern, recurrence of natural calamities like typhoons and forest fires will have impacts on forest ecosystems, particularly vulnerable and threatened species like Almaciga (Razal 2013), and thus it is important we understand more about these ecologically and economically important plants.

The Cleopatra's Needle Mountain Range (CNMR) in Puerto Princesa, Palawan, is inhabited by three indigenous communities being their ancestral territory. The largest remaining population of Batak tribe inhabits the inner forests of CNMR. The Tagbanua and Cuyunon tribes inhabit the marginal civilized areas in close proximities of the CNMR, together with several other migrant populations from Visayan groups. Almaciga resin collection serves as the main livelihood for these communities. For the Batak tribe, the Almaciga tree has remarkable value as a big portion of their income revolves around this tree, providing roughly 80% of the tribe's total income (Jose unpublished data). The tree provides several benefits, including providing traditional

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medicine, fire starter, firewood, torches, and paste and caulking substance, coloring agents for artifacts, smudge against mosquitoes and ritual incense and accessories, among others. For the Batak tribe, ownership of individual trees is passed down from generation to generation. However, overharvesting is evident due to an influx of resin collectors from areas where the trees have vanished; this threatens the remaining population of the species in the CNMR. The trees eventually become weak due to over-tapping of resin and will collapse in the near future if no action is taken to restore the health of trees. This means that the traditional livelihood and thus the future for the tribes and locals depending on Almagica is very uncertain.

Promisingly, the Centre for Sustainability received grants from the Flagship Species Fund of Fauna and Flora International and the Philippine Tropical Forest Conservation Foundation. This flagship species project aimed to rehabilitate the population of Almaciga in CNMR and conduct research on the biology and ecology of the species. Furthermore, the project aimed to raise awareness within the communities on the species' conservation status, understand the links between the overharvesting of resin and the collapse of the trees and reduction in fertility of seeds, and develop sustainable management plans for the species. The goal of the project was to generate 10,000 seedlings in local nurseries, to be planted in the surroundings of the Cleopatra's Needle Critical Habitat. Efforts and strategies were developed to equip the communities with the capacity of understanding biology and ecology, sustainable use and rehabilitation of Almaciga trees, to save it from local extinction in the future. As part of this wider project goal, this study focused on providing protocols on propagation, nursery, reforestation and management of *A. philippinensis* in the forest of CNMR. Specifically, the study aimed to:

1. Test various methods of cone, seed and seedling collection;
2. Germinate seeds for use in reforestation of declining populations;
3. Monitor growth and survival of seedlings in the nursery;
4. Identify, manage and mitigate pests and diseases of seedlings in the nursery;
5. Identify pressures and approaches to address unsustainable resin harvesting.

METHODS

There were five major activities initiated in each nursery. Experimental treatments of seed propagation were performed for comparison purposes, considering that these activities were the first of its kind for any Almaciga nurseries in the country. The study was conducted between July and October, 2015.

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Establishment of Nurseries

We aimed to establish nurseries in areas where Almaciga are naturally occurs since it is assumed that these areas are most suitable for seed propagation and growth of seedlings. However, after the reconnaissance survey done in the area this turned out to be impractical considering slopes, distance to the community and the difficulties in the monitoring, research timeframe, workload and budget. Therefore, three nurseries were established in more accessible areas, each in a distinct location differing in altitude and topography for purposes of comparison (Figure 1). The first nursery was established in the lowland area of Bgy. Binduyan, with an elevation of ≈1 meter above sea level (masl). The second nursery was constructed at the edge of the forest at a higher elevation (Pulang Bato, ≈100 masl, Bgy. Tanabag) while the third nursery was established at an elevation of ≈200 masl (Lipso, Bgy. Tanabag) where Almaciga trees naturally occur. A nursery in high montane elevation (1000+ masl), although desired, was not established. However, the three nurseries are sufficient to provide baseline data on Almaciga propagation, and nursery related requirements and studies.

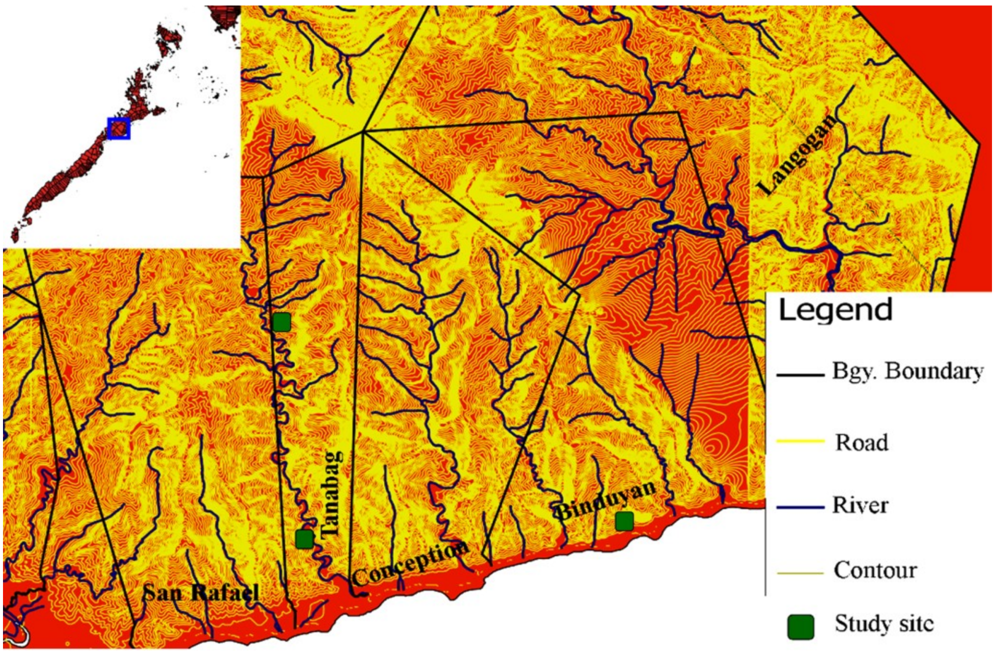

Figure 1. Map of Palawan (inset), CNMR and the nursery sites (green quadrangles). Nursery 1 – Binduyan; nursery 2 – lower Tanabag; and nursery 3 – upper Tanabag, Puerto Princesa City.

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Cone and Seedling Collections

Preliminary consultations with local nursery experts and operators were performed to acquire information on nursery protocols prior to the actual surveys. Most of these experts and Almaciga nursery operators were propagating Almaciga seedlings collected from the wild; none had attempted collection of cones and seeds. In order to provide insights and options for cone/seed collection, field surveys were initiated in areas where Almaciga trees are located in the CNMR. Mother trees were identified for the presence of cones and seeds. These trees are found on top of high ridges with deep slopes in the forest, making the collection of fallen matured cones, seeds and/or seedlings for propagation and nursery very difficult. Fallen rotting cones, cones attacked by ants and termites and other signs of animal-eaten seeds, were observed. Moreover, wild seedlings are scarce in all of the surveyed areas. Based on these *in-situ* situations, three methods of cone collection were trialed. First, trapping of cones and/or seeds from pre-identified mother trees was undertaken by deploying a series of net traps, covering a total of 225 square meters (m^2) surrounding a mother tree, which were monitored for two months. Net traps were deployed and monitored in one mother tree, in the montane forest area of Bgy. Tanabag. Secondly, wildlings that were < 12 cm in height and below 5 mm diameter, that were encountered in the surveyed sites were collected and transplanted into seedling pots in the nurseries. Lastly, groups of local Batak tree climbers were employed during expeditions for cone collection.

Treatments of Cones and Seeds, Experimental Seedbeds and Seed Pot Transplanting

Considering the limited knowledge on propagation and nursery of the species and related germination data in wild populations, three methods of handling cones and seeds were trialed. In spite of our knowledge gap on the biology of the species, we tried to evaluate its seed viability. Seeds having distinguishable cotyledons inside the seed coat were separated from seeds without obvious cotyledons. Seventy-six seeds with cotyledons and 124 without obvious cotyledons were sown in a seedbed as preliminary trial. The rest of the sorted seeds with obvious cotyledons collected were immediately sown in experimental seedbeds. Medium-ripe cones were treated with readily-available fungicides and insecticides (Carbofuran – Furadan: FMC Corp.; Malathion: BIOSTADT) and sprayed with water (0.003 milliliter per liter concentration) every two days to evade dryness and potential attack from insects and pests that can damage cones whilst maturing. Some other cones were hung in a nearby firewood hob, allowing smoke to deter insects and/or pests that could potentially damage them as an alternative protection method during cone maturation. These cones were sorted for seeds after they became fully mature, which took between 7 and 14 days, and seeds were then planted in experimental seedbeds.

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Three types of seed bedding treatments were initiated. Soil from the seedbeds of 1 m width and 2 m long were dug about 10 cm deep and mixed top soils available in the nurseries. Linear furrows of about 3 cm deep, at 20 cm distance from each other, were prepared and seeds were distributed in the furrows with about 5 cm distances from each other. In all, approximately 200–250 seeds were planted per treatment, and approximately 4500 seeds were used in total for the experiments. Seeds were covered with soil of about 1 cm thick. The soil was analyzed for nitrogen, phosphorus and potassium content, alkalinity, moisture, temperature and humidity using a Rapitest soil test kit (Spoerri, Inc.), a 3 way soil meter (Shenzen Yago Technology Co., Ltd.) and a GM1360 humidity and temperature meter (Shenzen Leyi Industrial Co., Ltd.).

The effect of watering quantity (factor A) and pesticide/insecticide application (factor B) were tested for the germination of seeds. Two replicates were performed for each factor in the experimental seed beds. To assess water quantity effects, four seedbeds were covered with a tarpaulin (to protect soil from possible overwatering due to rainfall) and supplied with water twice a week, to a total of 30 liters per seedbed. Four seedbeds were covered with fine nets, which allowed equal distribution of rainwater. These seedbeds were not supplied with additional watering. The control treatment had no roofing system and received no additional watering. Within each watering treatment, one seedbed was treated with Furadan at the time the seeds were sown, and sprayed with Malathion every three days to control insects and pests. The other two seedbeds within each treatment were treated by either one of the two types of pesticide/insecticide used respectively. One seedbed without any chemical treatment was also prepared. Similar pesticide/insecticide treatments procedures were performed in the net roofed experimental seedbeds. Control seedbeds received no pesticide/insecticide treatment for both factor A and factor B (Table 1). The layout of the experiments is presented in Table 2.

Table 1. Details of the factors tested in the experiment.

Factor A - Watering effect	Factor B - Insecticide/pesticide effect
A1- seedbed covered with tarps and supplied with 30 Liter of water per week	B1 – seedbed treated with both Furadan and Malathion
A2 – seedbed covered with fine net that blocks direct rainwater	B2 – seedbed treated with Furadan
A3 – seedbed without roof cover (Control)	B3 – seedbed treated with Malathion
	B4 - No insecticide/pesticide treatment (Control)

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Table 2. Layout of the Almaciga seed propagation experiments. A refers to the effect of watering quantity, and B refers to the pesticide/insecticide application.

A1B1	A1B2	A1B3	A1B4
A2B1	A2B2	A2B3	A2B4
A3B1	A3B2	A3B3	A3B4

Five days old sprouting seedlings in the seedbeds were transplanted to individual seed pots, using topsoil acquired from local nurseries and the seedlings were counted. Seedlings in the nursery were monitored on a weekly basis parallel to monitoring of cone/seed traps and data gathering.

Identification and Mitigation of Pests, Diseases and Monitoring of Seedlings

Information on the presence of pests and diseases was documented. The number of seedlings germinated in the experimental seedbeds was recorded. Mortality was monitored when the seedlings were transplanted in seed pots. Heights and leaf sizes were measured prior to reforestation time. Initial data of mortality, growth and survival rates recorded from October to November 2015, were the only available data and are treated in the analysis.

Group Meetings and Key Informant Discussions

Group meetings and key informant discussions with members of stakeholder communities, particularly with the Batak and Tagbanua tribes, were performed to identify pressures and approaches to address unsustainable resin harvesting and to develop a management plan.

Data Analysis

Data gathered from the experiments was organized for analysis. A Canonical Correspondence analysis (CCA) was carried out to test correspondence of seed germination, seedling growth and survival, with the measured soil nutrient contents, soil and external environment temperature and humidity and moisture contents of soil and seed pot substrates. A single factor Analysis of Variance (ANOVA) was performed for the total propagation performances of seedlings in the three nurseries of different elevations. Multivariate ANOVA was used to analyze the effects of watering and pesticide/insecticide treatments on the germination of the seeds during the seed bedding processes. A Tukey's test was done for all ANOVA results that were found significant.

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RESULTS

Six cone/seed collection expeditions were initiated yielding nearly 12,000 viable seeds from cones collected from six mother trees. These seeds were sown and propagated in experimental seedbeds in the nurseries. A total of 6,397 seedlings were germinated from the experiment. Eighty-three wildlings were also collected during expeditions and were grown successfully in seed pots in the Lipso and Binduyan nurseries. No cones and/or seeds were collected from net traps.

Rots caused by molds and fungus were common problems with cone and seed storage. These might be the consequences of incalcitrant seeds which might require more technology, laboratory and expertise in cone/seed handling and storage. Nevertheless, stored cones and seeds treated with fungicides and insecticide retained their viability within two weeks of keeping them in the field prior to seed bedding processes.

Generally, the propagation performance of the seedlings in seed pots from the three nurseries showed significant difference ($F_{(2,57)} = 3.135$; $p = 0.05$) in favor of lower Tanabag (Pulang Bato) over the Binduyan nursery site (Tukeys $Q = 3.49$;$p = 0.04$). The lower and upper (Lipso) Tanabag nursery sites showed no significant difference (Tukeys $Q = 2.29$; $p = 0.25$) as well as the upper Tanabag and Binduyan (Tukeys $Q = 1.20$; $p = 0.68$).

The result of experiments on the effects of watering (factor A) and pesticide/insecticide (factor B) on the germination of seedlings is shown in Table 3. Analysis showed high significant difference in watering treatments to the germination of the seedlings.

Table 3. Multivariate ANOVA of the germination data revealed from the seedbed experiment. Factor A = watering; Factor B = Pesticides/insecticide.

Parameters	Sum of Squares	df	Mean Square	*F*	*p*
Factor A	10250.00	2.00	5123.00	15.80	0.01
Factor B	3403.00	3.00	1134.00	3.50	0.05
Interaction (A X B)	1556.00	6.00	259.40	0.80	0.59
Within Groups	3890.00	12.00	324.20		
Total	19100.00	23.00			

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Tukey's post hoc comparisons on the effects of controlled watering to the germination of seedlings is significantly different to that of the uncontrolled watering treatments in the experiments ($F_{2,21}$ = 12.16; p = 0.01). Pesticide/insecticide on the other hand, appears to have no significant effects in the seedlings germination ($F_{3,20}$ = 1.4; p = 0.26) except that treatment C (Malathion) has more reliable data compared to the other three treatments and the control group has lesser data reliability compared to other two treatments (Figure 2). A separate ANOVA for Factor B however, indicated no significant difference ($F_{3,20}$ = 1.4; p = 0.25).

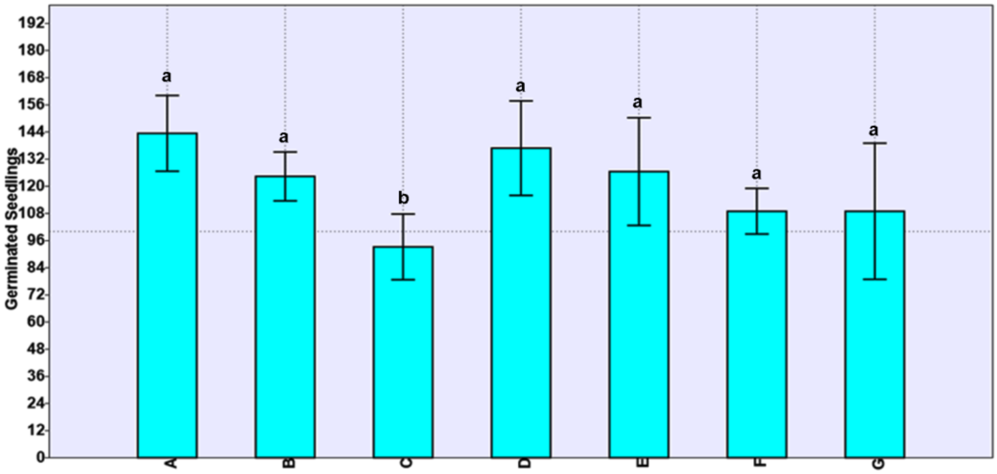

Figure 2. Effects of watering (A-C) and pesticide/insecticide treatments (D-G) to the germination of seedlings in the experimental seedbeds. A = Controlled Water; B = partly controlled water; C = Uncontrolled water; D = Treated with two types of pesticide/insecticide; E and F = Treated with one type of pesticide/insecticide; and; G = no pesticide/insecticide treatment.

Seeds without obvious cotyledons did not germinated in the preliminary seed germination test. Results of experimental seedbeds are shown in Figure 3. Propagation natality for Treatment 3, the control treatment, greatly corresponded towards trajectories of soil moisture and temperature and external temperature. Treatment 1 corresponded only to nitrogen and treatment 2 corresponds only to pH. Humidity, light and potassium did not correspond to any of the experimental treatments. This implies that seeds cannot tolerate too much or too little water and this greatly affects its success at propagation.

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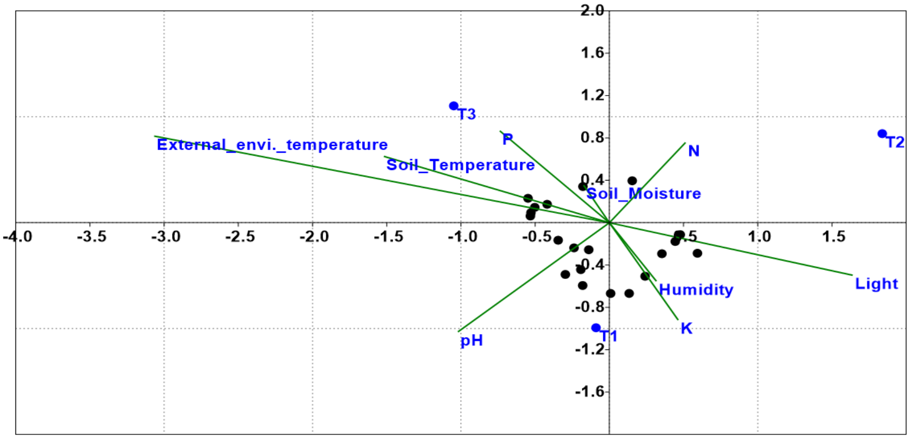

Figure 3. Canonical Correspondence Analysis showing environmental factors that correspond to the number of propagated seeds in the experimental seedbeds. T = treatments. Environmental parameters: N, P, K = soil nutrients; pH; soil moisture, humidity; light; and temperature.

Adequate nitrogen and slightly acidic substrate appears to be more favorable for germination in the case of Treatment 1 and 2 respectively. The pesticides used in these treatments might contributed to the amount of acidity in the case of treatment 2, while decaying organisms due to pesticide use might have added to the nitrogen content of the soil substrates of the Treatment 1, although this assumption requires further investigation. Additionally, rodents, ants and hoppers are the common problems consuming and/or damaging seeds and seedlings in the nurseries. Specific taxonomic identification of these pests is a necessity. Samples were collected and sent to the entomology expert in one of the local universities in Palawan for further investigations. Results of the taxonomic identification were not yet finalized during the course of this study, and therefore are not included in this paper. Finally, too much rain water caused seeds to rot, and after heavy rains, the soil substrates clump when dried, preventing sprouting seedlings from coming out. Cultivation to soften the soil substrates or replacing the entire soil substrates in the seed pots is required in such cases.

The mortality rate of the seedlings in the three nurseries was averaging 2.72% per month over three months. Mortality is negatively correlated with the age of the seedling (r = -0.5; p = 0.12) ranging from about 6% in the first month to nearly zero mortality in the fourth month (Figure 4). This implies that as seedlings mature they become more tolerant to causes of mortality.

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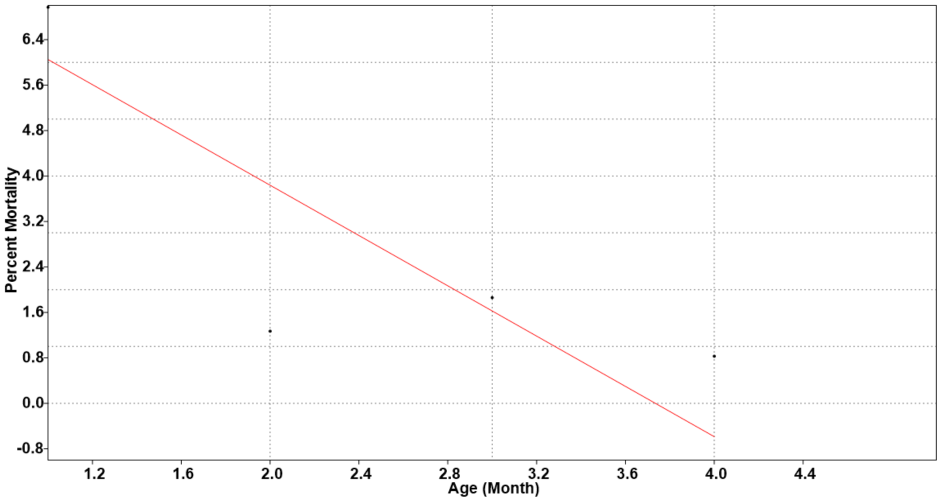

Figure 4. Linear correlation of the percentage mortality with the age of the seedlings.

DISCUSSION

The results imply that propagation of Almaciga is manageable and straightforward. Seedlings are not sensitive to elevation gradients and can be propagated from ≈1 to ≈200 meter above sea level. These biophysical limits were likewise documented for *A. microphylla*, which is tolerant to light salt sprays, well adapted to grow in windy and cyclone-prone locations, and likely to tolerate short dry periods (Orwa et al. 2009). The only factor that significantly affected seedling natality/mortality among sites was due to overwatering and/or water shortages in the seedbed and pot substrates, and fungi and pests as manifested in the canonical correspondence analysis result. Similar observations have been documented from other *Agathis* species. Pacific Kauri (*A. macrophyla*) seedlings had been proven susceptible to termite and beetle attacks, moths and root fungi that are usually associated to waterlogged substrate or sites (Orwa et al. 2009). Optimum mixtures of soil components in the seed pots are still subject for further experiments; however, our present result revealed that mixtures of 50% sand, 20% clay and 30% topsoil are appropriate for the growth and survival of the seedlings. Soil or soil mixed with sand has likewise been used and recommended as germination medium for *Agathis loranthifolia* in the greenhouse (Nurhasyni and Sudarat 2002). Despite wild-collected seedlings successfully surviving in the nurseries, we do not recommend collecting wild seedlings for it reduces the natural populations of the species and further increases workload, as these are being replanted back to the forest. Moreover, this practice could post additional threats as natural population recruits can be depleted while expanding inappropriate locations that is favored for plantation of the species. Based on

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the results of seeding experiments and nursery monitoring, the critical age of seedlings which needs ultimate care is from seed bedding up to four months of age – the stage where only primary root and cotyledon leaves support the growth of the seedlings. Seedlings growth becomes quicker once the true leaves and secondary roots start to come out, normally four months and beyond from time of transplanting in seed pots.

Kauri Dieback Management in New Zealand provided a brief guide for growing Kauri. This guidebook provided the basics of Kauri biology and ecology including care, seed germination and growing plantations. In the New Zealand setup, Kauri seedlings and young trees are susceptible to droughts and waterlog. Moreover, growth of Kauri occurs best in soil pH range of 4.8 and 6 but growth can be affected in soil pH below 4.5. The lack of knowledge and skills in monitoring and initiating appropriate measures to mitigate the threatening effects of catastrophic events occurring in the nurseries is likewise a problem in this study. This is also because the activity is the first of its kind for any Almaciga nursery in Palawan and the entire Philippines. The knowledge and experience gained in the whole process of the project together with the nursery manual subsequently developed can be used by the indigenous communities in the creation of community-based Almaciga nursery projects and by other interested parties on Almaciga propagation.

Options to conserve Almaciga include replacement of dead or badly damaged trees in the forest by either planting seedlings or allowing growth of naturally-generated young trees prior to extracting resins (Lacuna-Richman 2004; 2006). As such, this project/research intended to provide seed-propagated seedlings to reforest the degrading population of the species in the forest of Cleopatra's Needle. Development of a closed canopy and high diversity forest farming system that replaces slash and burn forming practices, preserves biodiversity, re-establishes ecosystem and ecological functions and provides subsistence to farmers, had been established in Leyte during the 1990s (Margaraf and Milan 1996; Göltenboth et al. 1999; Schulte 2002; Göltenboth and Hutter 2004). The seedlings propagated and planted in the CNMR added significantly to the number of surviving individuals in the population of the species, thus restoring the normal ecosystem and ecological functions in this ecologically important area, while also ensuring the future livelihood of the Batak and other inhabitants in the area that depend on Almaciga products. Although such projects will need external support from government and other concerned agencies for continuity. Moreover, similar projects should be adopted by the National Greening Program of the DENR for additional technical and financial support. Currently, the National Greening program of the DENR is rehabilitating degraded forest areas in the Philippines with non-native tree species which can potentially becoming additional threats in the future, such as possibilities of species invasion. Furthermore, monitoring and care of the planted seedlings in the reforestation areas is necessary to ensure the survival of the plants.

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Based on discussions with the tribal people and key informants, the major challenges in the conservation of Almagica are unsustainable resin collection methods. This is due to the high demand of the resin product that causes an influx of resin collectors from areas where Almagica is locally scarce. Resin and other non-timber products are only allowed to be harvested by the tribal collectors and/or members of forest tenured agreements including community based forest management (CBFM), social industrial forestry management agreements (SIFMA) and other forest management agreement programs set by the government (Department of Environment and Natural Resources and Palawan Council for Sustainable Development). However, the documentation process in securing necessary permits for these forest management tenure instruments seems to be financed by middlemen buyers of the products. In such way, the local collectors or members of these community-based organizations (mostly tribal people) are obligated to pay for debt, and at worst, the debt of gratitude (as the tribal people treat it as such), by supplying more products to the middlemen financers. Moreover, middlemen financers are allowed to send non-IP workers to the sites to collect and meet the volume quota of resin they need. Apparently, pricing of these products are likewise being controlled by the middlemen financers. This allows the middlemen financers to profit greatly from Almaciga resin, while local collectors only receive a nominal income. A community share (PhP 1.00 per kilogram of resin sold to middlemen buyers) which was charged from the collectors and is intended for conservation and permitting processes. However, these shares are normally not liquidated and turned-over to the community to implement its purpose, but rather remain in the custody of the middlemen financers for unknown purpose (Nicknik Saavedra pers. comm. 2017.)

Finally, the indigenous peoples in the project area realized the imbalance in profit benefits that runs between them and middlemen financers. However, they do not have enough knowledge and finances to process and manage the Almaciga business on their own. This research, together with the other components of the project, helped the tribal people realized the importance of conservation which have prepared them to defend Almaciga and the CNMR – their ancestral domain – to the challenges of habitat and environmental destruction. The CNMR is finally declared as a protected area in 2016. Future plans for Almaciga management include the creation of tribal organization that sustainably controls and manages the resin collection business (Nicknick Saavedra pers. comm. 2017).

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Mammals of Cleopatra's Needle Critical Habitat: Outcomes of a rapid assessment

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ABSTRACT

A rapid biodiversity assessment of mammalian fauna was conducted in the Cleopatra's Needle mountain range in the biodiversity hotspot province of Palawan, Philippines to determine species diversity of, anthropogenic activities affecting and conservation status of the species in the area. Standard methods of research sampling for capturing or observing volant and non-volant mammalian species included: baited cage trapping, drift-fenced pitfall trapping, camera trapping, mist-net trapping and opportunistic hand-capturing or observing. A total of 12 mammalian species were documented. *Maxomys panglima* and *Crucidura palawanensis* were the most commonly captured species with the use of baited cage traps and pitfall traps, respectively. The rare *Chiropodomys calamianensis* was also captured with a baited cage trap. *Aonyx cinereus* is listed as Vulnerable in the International Union for Conservation of Nature Red List of Threatened Species; *Prionailurus bengalensis heaneyi* and *Sus ahoenobarbus*, are listed as Vulnerable under the Department of Environmental and Natural Resources' 2017 National List of Threatened Philippine Fauna and their Categories. The results revealed this area is an important habitat for threatened and poorly studied species of mammals, most of which are imperiled by individual threats and habitat loss.

Keywords: biodiversity, mammals, Red List, survey

INTRODUCTION

Tropical forests support at least two-thirds of the world's biodiversity (Raven 1988). Unfortunately, global biodiversity rates are steadily declining, with anthropogenic land use change being of primary concern to the sustenance of terrestrial ecosystems (Balmford et al. 2003; Jenkins et al. 2003; Butchart et al. 2010). Alroy (2017) estimates global biodiversity loss will cause the 6th mass extinction event if tropical deforestation persists at the same

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degree. Conserving tropical forest biodiversity thus lies in the proper management of vital protected area networks that house threatened species and global ecosystem functions (Laurance et al. 2012).

The Philippines is home to at least 5% of the world's endemic mammals (Ceballos et al. 2005). The unique Philippine island of Palawan boasts mammal diversity that is reflected in its distinctive biogeographical history: Palawan is part of the Sunda shelf and may have been connected to Borneo via a land bridge; all other Philippine islands are oceanic and were probably never connected to mainland Asia (Heaney 1986; Esselstyn et al. 2004). Thirteen of Palawan's 58 native mammal species are endemic to the island region; while 8 of Palawan's 11 native rodent species are endemic to the province (Esselstyn et al. 2004). Although Palawan's mammals have been documented for over a century, there is a general lack of information regarding their ecology, distribution, and phylogenetic relationships (Esselstyn et al. 2004).

In 2016, the Cleopatra's Needle mountain range (CNMR) in Puerto Princesa City, Palawan, Philippines was officially protected as a 'critical habitat' – a local conservation initiative which aims to safeguard valuable, endemic and/or endangered species in areas adjacent to national protected areas in the Philippines. This 41,350-hectare critical habitat, named Cleopatra's Needle Critical Habitat (CNCH), is home to 85% of Palawan's endemic and endangered plant and wildlife species (Hoevenaars and van Beijnen 2015). The declared critical habitat aims to protect the flora and fauna within its domain from logging, mining, and land-grabbing activities that continue to occur throughout Palawan. However, even with the protected status of the forest, approximately 20 hectares of newly cleared swidden agriculture have been recorded in the core zone of the critical habitat between February and April of 2017 alone (Karina Reyes-Antonio Pers. Comm.). Hence, continuous research and cooperation with the Batak and Tagbanua tribes in the area is necessary to innovate effective management strategies within the CNCH that will respect the traditional practices and welfare of indigenous communities without compromising conservation.

An assessment conducted by Ambal et al. (2012) shows this area is among the key biodiversity areas in the Philippines in need of conservation action. Yet, mammalian surveys surrounding the CNMR are minimal. Esselstyn et al. (2004) was able to record three non-volant mammal species and two bat species between 1300 and 1600 masl on Cleopatra's Needle over 740 trap nights; while Marler et al. (2015) was able to record eight medium-bodied mammals using camera traps throughout the CNMR over a five-month period. We initiated this survey as a prerequisite for declaring the CNMR a critical habitat. The survey further aimed to determine the mammalian species diversity, the anthropogenic threats, and the conservation status of species in the CNCH.

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METHODS

Study Area

This study was conducted in two political districts (locally called 'barangays') within the CNCH in Puerto Princesa, Palawan, the Philippines: Langogan and Concepcion. These two districts have direct contact with the Sulu Sea on their eastern coasts and touch Cleopatra's Needle Mountain on their land locked borders (Figure 1). The CNCH is adjacent to the Puerto Princesa Subterranean River National Park (PPSRNP), which extends to the west coast of the island. Together, these two protected forest areas comprise a corridor connecting the northern and southern portions of Palawan. Habitat types in the CNCH include riverine, montane, beach or littoral, lowland evergreen, secondary, and swamp forests (Marler 2016).

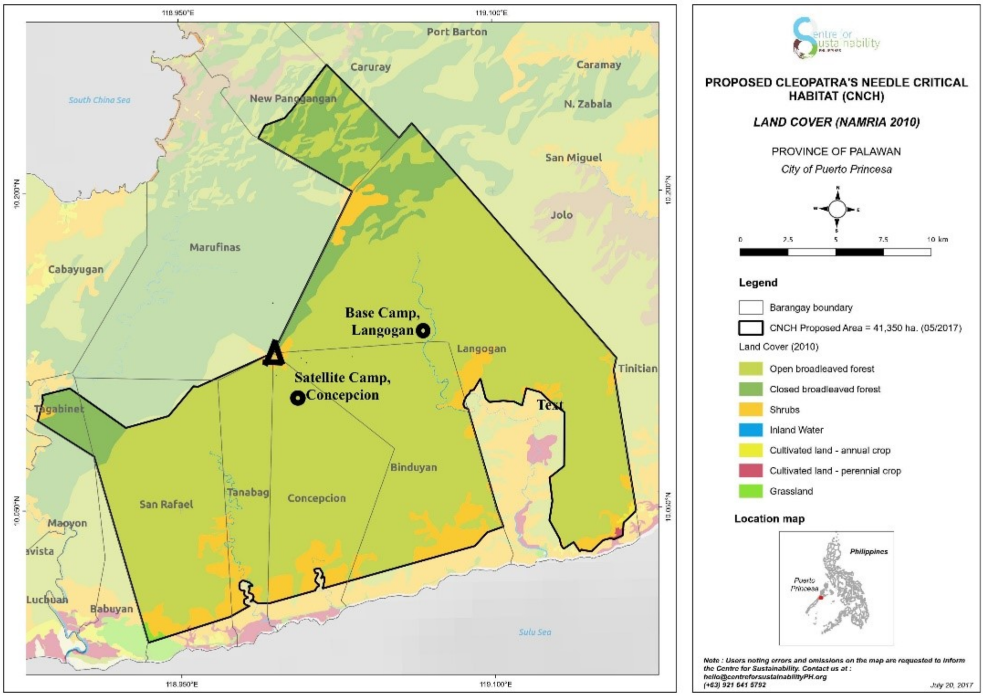

Figure 1. Map of Cleopatra's Needle Critical Habitat (the two dark circles indicate the base camps and the triangle represents Cleopatra's Needle Mountain). ©Centre for Sustainability PH, Inc.

The study areas were approximately 50 km north of Puerto Princesa City proper. The sampling areas were focused in the forest surrounding Cleopatra's Needle (CN) mountain (10° 07' 26" N, 118° 59' 43" E; 1,593 masl), the centerpiece of the critical habitat, two years prior to its declaration. A base

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camp was established in Langogan approximately 15.5 km from the main highway and 300 m from the Langogan River (Figure 1). Secondary succession vegetation was evident in the surroundings of the area, which is mainly caused by seasonal slash-and-burn farming systems by local inhabitants. From the base camp, we created three sub-sites: L_{S1}, L_{S2}, and L_{S3} (Figure 2). Sub-sites L_{S1} (180 masl) and L_{S2} (324 masl) were in riparian habitat, where large dipterocarp trees dominate, and smaller understory trees create dense vegetation on the forest floor. Sub-site L_{S3} (1010 masl) was located on a mountain ridge; this sub-site had similar ground foliage to L_{S1} and L_{S2} with large Almaciga *Agathis philippinensis* trees interspersed throughout the terrain. In Concepcion, our surveys were centralized around a satellite camp located approximately 12.6 km from the main highway at 828 masl (Figure 1). From this camp, three sub-sites were created: C_{S1}, C_{S2}, and C_{S3} (Figure 2).

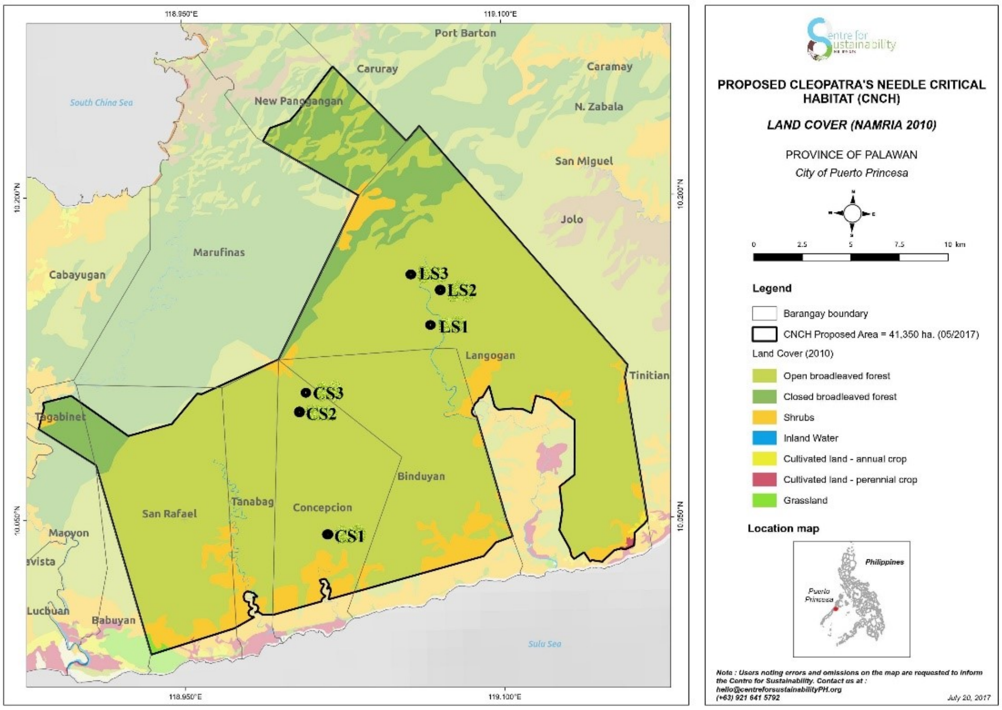

Figure 2. Map indicating the location of subsites that were surveyed for mammals. ©Centre for Sustainability PH, Inc.

Sub-site C_{S1} (151 masl) was in riparian habitat and sub-sites C_{S2} (796 masl) and C_{S3} (805 masl) were in lowland evergreen habitat. Large dipterocarp trees with smaller understory trees again dominated these three habitats. However, *A. philippinensis* was found throughout sub-sites C_{S2} and C_{S3}. For one night each, a camera trap and one cage trap were placed near the peak of Cleopatra's

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Needle Mountain (1584 masl) in montane habitat dominated by *Nepenthes mira* pitcher plants, ferns and moss-covered trees.

Rapid Assessment Survey

This rapid assessment occurred at the end of the rainy season in the first two weeks of December 2014. Approximately five days were spent surveying each barangay. Considering the rapid nature of the research, five methods were employed for short periods of time to document and, when possible, capture all possible species present in the area. Unfortunately, due to varying numbers of team members over the brief time frame of the expedition, it was not possible to replicate all methods for each of the six sub-sites; in some cases, only one method was employed at certain sub-sites throughout the expedition. The five methods are discussed below:

Cage Trapping. Forty-five single-door cage traps with dimensions of approximately 30 cm x 15 cm x 15 cm were used to catch ground-dwelling small non-volant mammals. Four different baits were used to attract the animals: coconut (*Cocos nucifera*), banana (*Musa cf. acuminata*), peanut butter (store bought), (Esselstyn et al. 2004) and oats (store bought). Baits were used at relatively equal proportions for each of the trapping sub-sites. Various bait types were employed to ensure mammal captures as information was not found to determine which bait would prove most effective. For one cage trap, one bait was selected, placed into a hand-made mesh bag and tied inside the cage. Line transects were then created in each of the different study areas on the forest floor; for each transect, cages were placed in approximately 20 m intervals in a line transect (Pearson and Ruggiero 2003). The number of cages per transect varied from 10 to 26 cages, depending on the terrain and availability of cages when reaching each of the sub-sites. Geographic Positioning System (GPS) coordinates were taken from a central location in each sub-site. These traps were checked daily. Any animals caught were collected and prepared as voucher specimens following the instructions and limitations specified in the Wildlife Gratuitous Permit 2014-15, issued by the Palawan Council for Sustainable Development (PCSD). The cages were then reset for the next day.

In Langogan, one transect was established each at sub-sites L_{S1} and L_{S2} for three nights each for a total of 120 trap/nights. In Concepcion, one transect was established each at sub-sites C_{S1}, C_{S2}, and C_{S3} for four, two and two nights, respectively, for a total of 92 trap/nights. One cage trap was placed at the peak of CN for one night.

Drift-fenced Pitfall Trapping. Twenty-five-liter water buckets, polyethylene plastic sheets and iron stakes and/or wooden sticks were used to construct the drift-fenced pitfall traps (DPTs). The DPTs were created approximately 50 m from cage trap transects, with one DPT being

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independent of a cage trap location. We followed Voss et al. (2001), using a linear design for the pitfall traps by digging 10 holes at about 70 cm deep and large enough to fit a 25-L water bucket. Ten buckets were spaced an average of 3 m from one another and polyethylene plastic sheets were affixed between buckets with iron stakes or wooden sticks to create a barrier between each bucket (Voss et al. 2001). DPTs were inspected daily and any animal caught was collected.

For sub-sites L_{S1}, L_{S2}, and L_{S3}, DPTs were left to function for three, three, and two nights, respectively. In Concepcion, only one DPT transect was established in sub-site C_{S2} for two nights.

Camera Trapping. For our Langogan site, a Truth Cam 35 was used. In Concepcion, we used a Bushnell 8MP Trophy Cam. We followed Gerber et al. (2010) and placed camera traps opportunistically alongside the trail. When possible, camera trap placement was near a sign of animal presence; examples of such include: animal feces and feeding sites (O'Brien et al. 2003; Meek et al. 2012). Once a camera trap location was found, a camera was strapped to a nearby tree approximately 30 cm from the ground and 1 m to 2 m in front of a clearing (Ancrenaz et al. 2012; Meek et al. 2012). The cameras use a passive infrared motion sensor to detect heat within the detection cone of the infrared sensor, triggering the camera. A five-second delay was used in between trigger events. Once camera traps were retrieved, their SD cards were safely stored. The photographs were analyzed from a computer and labeled according to the species recorded.

In Langogan, a camera trap was set for two nights at an animal feeding site, 200 m from L_{S3}. In Concepcion, two cameras were established at C_{S3}; one camera was directed towards carnivore scat and the other camera was set up 100 m from this site for two nights each. One camera was set up on CN Mountain peak for one night. Camera traps were used to survey for a total of seven trap/nights.

Mist-net Traps. A 12 m by 3 m mist-net was used. Both sides of the net were hung on trees at about 8 m in height in fly ways. The nets were checked every hour starting from 17:00 until 6:00 the next morning. If an animal was caught in the net, the nets were lowered to untangle the volant mammals, such as bats, and other animals caught were promptly released.

In Langogan, a mist-net was set up in sub-site L_{S1} for two nights. In Concepcion, a mist-net was set up and monitored in sub-site C_{S3} for one night only, due to time constraints that hindered the researchers from staying in one site for more than one night.

Hand Capturing, Observing and/or Opportunistic Sampling. When possible, hand-captures of small volant and non-volant mammals were

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employed. During daylight hours, crevices on big rocks were inspected for roosting bats and tree holes were inspected for arboreal mammals. Large-bodied mammals that were seen, heard, smelled or encountered by opportunity were also recorded. Animals identified by their tracks, such as droppings, were also recorded.

Preservation of Specimens

Only the allocated number of specimens under the Wildlife Gratuitous Permit 2014-15 were preserved. Others were promptly released from where they were caught after taking the necessary information. The collected specimens were humanely euthanized by either injecting ethanol directly into the heart or snapping the neck. Each specimen was photo-documented and preserved in 10% formalin for 24 hours and then transferred to 70% ethanol for long-term preservation. The specimens are currently being used for educational purposes at Western Philippines University, Puerto Princesa Campus and will be later transferred for museum purposes.

Anthropogenic Activities

Human activities and disturbances in the area were noted from actual observations, key informant and informal interviews, and Google Earth© satellite images.

Data Analysis

Species identification was guided using synopses and the aid of individuals with previous small Philippine mammal field experience. Cumulative number of species recorded every sampling day were plotted to determine the satisfactory of sampling over the sampling period. Generated data were analyzed for diversity and abundance using standard diversity indices (Shannon H and E) and statistics.

RESULTS

Twelve mammalian species under seven orders were confirmed to occur in the CNCH during this study, wherein eight are endemic to Palawan and four are native species (Table 1). Eight species captured in this study were photo-documented (Figure 3).

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Table 1. Species recorded during the Rapid Assessment of Cleopatra's Needle Critical Habitat with their corresponding IUCN category, method of capture/observation, number of individuals, and location. Categories: LC=Least Concern, NT=Near Threatened, VU=Vulnerable, EN=Endangered, DD=Data Deficient, NA=Not Yet Assessed. +Category provided by Department of Environmental and Natural Resources (DENR 2017).

Order	Scientific name	Common Name/Local name	Category	Method	Number of Individuals	Location
Carnivora	*Paradoxurus hermaphroditus* Pallas, 1777	Asian Palm Civet "musang"	LC	Camera Trap	1	Langogan
	Aonyx cinereus Illiger, 1815	Asian Small-clawed Otter "dungon"	VU	Opportunistic sampling	NA	Concepcion
	Mydaus marchei Huet, 1887	Palawan Stink Badger "pantot"	LC	Opportunistic sampling	NA	Langogan, Concepcion
	Prionailurus bengalensis heaneyi Groves, 1997	Palawan Leopard Cat "singgarong"	NA, VU+	Opportunistic sampling	NA	C_{S2}
Cetartiodactyla	*Sus ahoenobarbus* Huet, 1888	Palawan Bearded Pig "baboy-ramo"	NT, VU+	Observed from hunters	2	Langogan
Chiroptera	*Cynopterus brachyotis* Müller, 1838	Lesser Short-nosed Fruit Bat "bayakan"	LC	Hand Capture	3	Langogan
Eulipotyphyla	*Tupaia palawanensis* Thomas, 1894	Palawan Shrew	LC	DPT	16	L_{S1}, L_{S2}, C_{S2}
Primates	*Macaca fascicularis* ssp. *philippensis* I. Geoffroy, 1843	Philippine Long-tailed Macaque "unggoy"	NT	Observed	~7	Langogan
Rodentia	*Chiropodomys calamianensis* Taylor, 1934	Palawan Pencil-tailed Tree Mouse	DD	Cage Trap, coconut bait	1	L_{S2}
	Hylopetes nigripes Thomas, 1893	Palawan Flying Squirrel "buyatat"	NT	Hand Capture	3	Langogan
	Maxomys panglima Robinson, 1921	Palawan Spiny Rat	LC	Cage Trap, coconut & banana baits	20	L_{S1}, L_{S2}, C_{S1}, C_{S2}, C_{S3}
Scandentia	*Crocidura palawanensis* Taylor, 1934	Palawan Treeshrew	LC	Cage Trap, banana bait	1	L_{S2}

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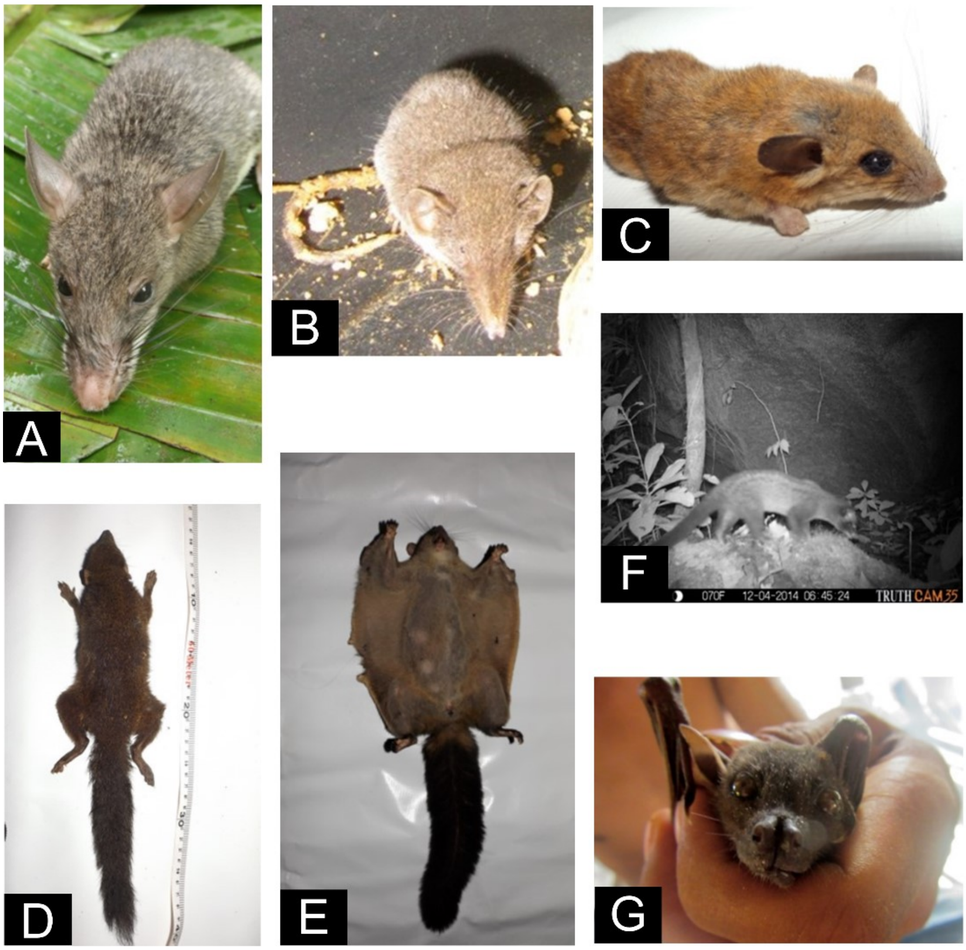

Figure 3. Photo-documentation of eight species captured during the survey: A) *Maxomys panglim*a B) *Crocidura palawanensis* C) *Chiropodomys calamianensis* D) *Tupaia palawanensis* E) *Hylopetes nigripes* F) *Paradoxurus hermaphroditus* G) *Cynopterus brachyotis*.

Aonyx cinereus spraints were found alongside a river at 240 masl. *Mydaus marchei*'s distinct odor was smelled in lowland evergreen forest and riparian habitats. The droppings found near sub-site C_{S2} are likely from *P. b. heaneyi* due to their resemblance to feline droppings and the visible presence of hair, which could belong to rodent prey. A group of *M. fascicularis* ssp. *philippensis* was observed in the tree canopies in riparian habitat. *Hylopetes nigripes* were captured from their tree nest approximately 15 m from the ground (Figure 3E). *Cynopterus brachyotis* were captured from their roost in a rocky outcrop (Figure 3G). Hunters were observed retrieving two, medium and large-sized, *S. ahoenobarbus* individuals from a hunting site at a higher elevation from our surveying sites.

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Satisfaction of sampling (Figure 4), diversity indices (Figure 5), and cumulative density (Figure 6) were analyzed using species richness and number of individuals caught and observed per species. As shown in the cumulative number of species recorded per day of sampling (Figure 4), sampling efforts in both sites proved inadequate to determine the totality of species in the study areas. Similarly, the diversity and evenness are apparently not so high, with a Shannon H value and evenness value of 1.789 and 0.6648, respectively, for Langogan and 1.496 and 0.6378, respectively, for Concepcion (Figure 5). Assuming our traps can attract animals from 10-meter distances in the surroundings, the estimated density results show the area had approximately 102 individuals per hectare. This would be dominated by 43% *M. panglima* and 36% *C. palawanensis*.

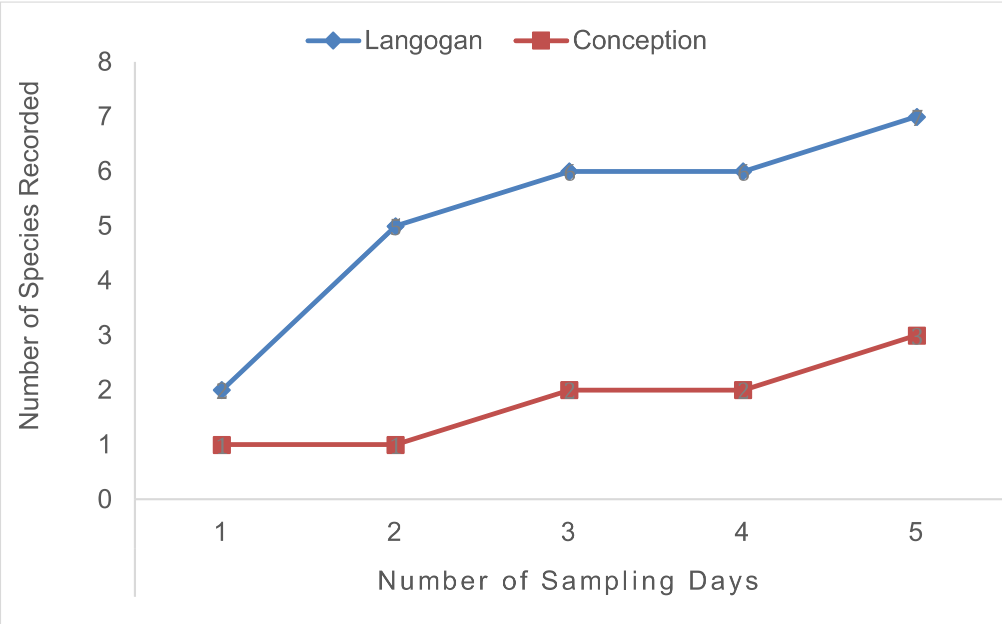

Figure 4. Cumulative number of species recorded per day from December 3 to 12 in both localities.

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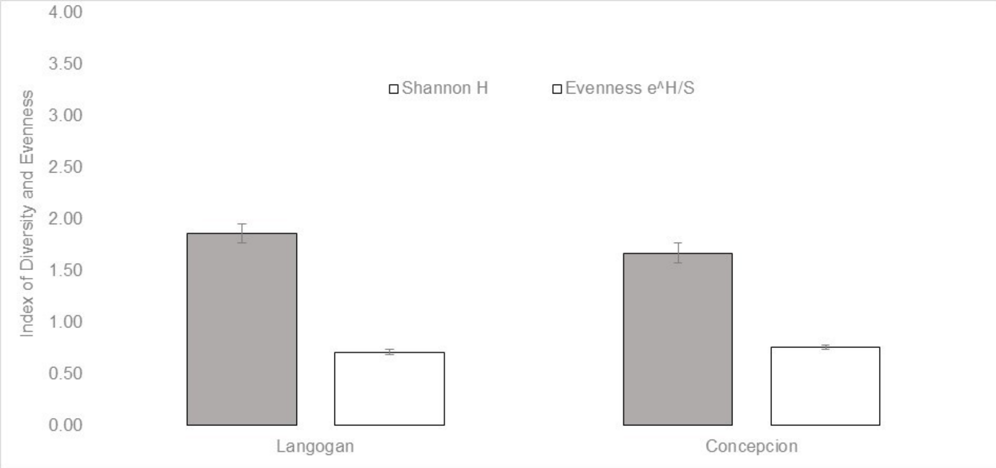

Figure 5. Diversity index revealed from the number of captured individuals per species in two sampling localities in CNCH.

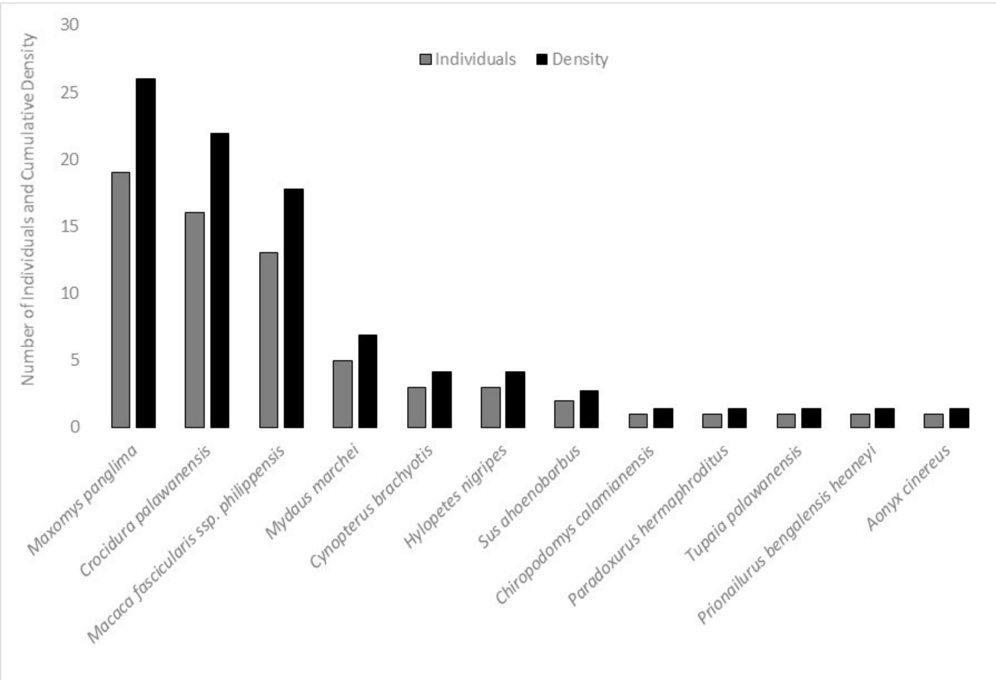

Figure 6. Cumulative density of the 12 species for each sampling locale.

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DISCUSSION

There is a need to consider potential biases in capturing method among species as this could skew the species accounts in the study. Insufficient capturing methods previously left the *C. palawanensis* population understudied (Esselstyn et al. 2004); however, recent surveys using pitfall traps have proven the species to be widespread across its known range (Cassola 2016), which is reflected in our findings.

Currently, there is a need to understand species' occurrence, behavior, and population sizes; all of which can be studied using non-invasive methods that survey multiple species over larger areas (Gompper et al. 2006). Some examples include camera trapping, satellite remote sensing, and drone technology (Ancrenaz et al. 2012; Koh and Wich 2012; Pettorelli et al. 2014). Data that can be generated from these methods are relevant in understanding the ecology of study animals such as site fidelity, home range size, social behaviors, habitat use and habitat availability.

A recent LandSat image featuring areas within the sampling sites showed a substantial portion of newly cleared areas for upland farming activities (Figure 7). This was not observed during the 2014 field research. These scenarios are of great interest for biodiversity research that aims to determine effects of land use changes on species composition, abundance and other related ecological studies.

Figure 7. LandSat image of cleared forest along the Langogan River. Image created by Google Earth©.

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Esselstyn et al. (2004) captured five mammal species between 1300 and 1600 masl over two weeks of surveys on Cleopatra's Needle: *Rhinolophus arcuatus*, *Pipistrellus javanicus*, *Tupaia palawanensis*, *Maxomys panglima*, and *Rattus tiomanicus*. Although habitats above 1300 masl were only surveyed one night, two of these five species were also recorded. Marler et al. (2015) recorded eight medium-bodied mammals in five months of camera trapping throughout the CNCH: *Hystrix pumila*, *Paradoxurus hermaphroditus*, *Prionailurus bengalensis heaneyi*, *Viverra tangalunga*, *Mydaus marchei*, *Herpestes brachyurus*, *Manis culionensis*, and *Sus ahoenobarbus*. Four of these species were opportunistically sampled and observed, one with the use of a camera trap as well. Further mammalian studies focused within the CNCH were not found in the literature. However, Widmann et al. (2008) recorded 35 species across five study areas in northern Palawan and Dumaran Island, with a significantly greater trapping effort than conducted here; the 12 species recorded in this study were all represented.

Approximately 20% of the 58 native mammal species were sampled in our survey. While the species diversity and evenness were not so high in this study, the species richness within each sampling locale proved unique in composition. *Tupaia palawanensis* was recorded only in Concepcion, while evidence of occurrence of *A. cinereus* and *P. bengalensis heaneyi* were likewise only observed in Concepcion. In contrast, *C. calamianensis*, *H. nigripes, C. brachyotis* and *P. hermaphroditus* were captured only in the Langogan sampling sites.

The Langogan sampling sites have a slightly higher density than in Concepcion, although statistically not significant (Figure 6). Assuming a cumulative area of sampling of 50 m^2 per DPTs and 25 m^2 per cage trap cover, there could be an average of 2.6 individuals and 3.2 individuals per 12.5 m^2 area in Concepcion and Langogan, respectively. From such data, there could be 2,080 individual animals per hectare in Concepcion and 2,560 individuals per hectare in Langogan. The ratio per species can then be determined by the individual percentages based on the total number of captured individuals per species. In this case, *M. panglima* and *C. palawanensis* dominated the species with 43% and 36% respectively. This was followed by *C. brachyotis* and *H. nigripes* (7%) and lastly *C. calamianensis*, *P. hermaphroditus* and *T. palawanensis* (2%).

Aonyx cinereus is among the least studied Asian otter species in the wild (Hussain et al. 2011). The species is widely distributed through Southeast Asia but is only found in Palawan within the Philippines (Wright et al. 2015). *Aonyx cinereus* holds a Vulnerable listing by the IUCN due to a past population decline exacerbated by habitat loss, exploitation, and depletion of prey species (Hussain et al. 2011; Wright et al. 2015). Since otters are effective symbols of environmental quality and are highly susceptible to degradation (Nawab and Gautam 2008), *A. cinereus* presence is seen as a healthy

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ecosystem indicator within freshwater and wetland systems in Palawan. Unfortunately, high hunting pressure and habitat loss by human modification threaten the species in Palawan (Castro and Dolorosa 2006).

Chiropodomys calamianensis is greatly understudied, with only a few specimens observed since its first scientific capture in 1918 (Kennerley 2016; Musser 1979). The species is an arboreal mouse endemic to the Palawan Faunal Region (Kennerley 2016). *Chiropodomys calamianensis*' ecological role, population status and conservation needs, if any, cannot be determined without further studies of the species.

Crocidura palawanensis, M. panglima, and *T. palawanensis* are endemic to the Palawan Faunal Region (Cassola 2016; Balete et al. 2016; Kennerley 2017). These three species seem capable to adapt to secondary habitats (Balete et al. 2016; Cassola 2016; Esselstyn et al. 2004; Heaney et al. 1998). *Maxomys panglima* seems widely distributed across primary, lowland, and low montane forests as well (Balete 2016). These three species hold a Least Concern listing, yet further studies on the species' populations will help us assess their unique ecological roles.

Cynopterus brachyotis occurrence ranges throughout South and Southeast Asia (Csorba et al. 2008). The species is common and geographically widespread in the Philippines, carrying a Least Concern listing by the IUCN (Csorba et al. 2008; Esselstyn et al. 2004). However, further taxonomic revision could reveal subspecies or separate species within the populations currently characterized as *C. brachyotis* (Heaney et al. 1998; Csorba et al. 2008). Csorba et al. (2008) fail to include the Philippines in the 'Range Description' for *C. brachyotis*. This survey and past surveys (Heaney et al. 1998; Esselstyn et al. 2004) support a revision of this assessment to incorporate Palawan and other islands in the Philippines in the range description.

Hylopetes nigripes is endemic to the Palawan Faunal Region (Ong et al. 2008). The species appears to be widespread (Heaney et al. 1998; Esselstyn et al. 2004), however, its suspected extent of occurrence is not much greater than 20,000 km^2 (Ong et al. 2008). The Batak and Tagbanua tribes hunt this species for meat. *Hylopetes nigripes* nests in cavities in large trees, thus deforestation may pose a serious risk to the species' survival (Ong et al. 2008). Further surveys are necessary to understand the species and more accurately determine a Red List category (Esselstyn et al. 2004; Ong et al. 2008).

The Philippine subspecies *M. fascicularis* ssp. *philippensis* is known to occur in agricultural areas, primary lowland forests, secondary forests, and montane forests (Heaney et al. 1998; Esselstyn et al. 2004). *Macaca fascicularis ssp. philippensis* plays important roles in seed dispersal within the forest by consuming fruit and carrying seeds on their coat (Paluga 2006).

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Within Palawan, the species seems to have relatively stable populations, yet pressures exist from hunting for meat and habitat loss (Esselstyn et al. 2004; Ong and Richardson 2008).

Mydaus marchei is endemic to Palawan, Busuanga, and Calauit islands in the Philippines (Heaney et al. 1998). There seems to be no extreme fluctuations in the population size, although the species inhabits an area under 20,000 km^2 (Widmann 2015). *Mydaus marchei* can be found near human modified areas as well as primary and secondary lowland forests (Widmann 2015). The species appears to be common with threats from occasional hunting and road kills (Widmann 2015).

Paradoxurus hermaphroditus is found throughout the Philippines, where it has been recorded in agricultural and forested areas (Heaney et al. 1998; Esselstyn et al. 2004). *Paradoxurus hermaphroditus* disperses small to large seeds via consuming fruits whole and travelling through the forest as the seed passes through the gut (Nakashima et al. 2010). The species is also known to consume rats, insects, and molluscs (Duckworth et al. 2016). It is usually the most common carnivore found where it is known to occur (Duckworth et al. 2016). However, *P. hermaphroditus* is increasingly kept as a pet and captured to produce civet coffee within Indonesia and the Philippines; the effect of these actions on the wild populations is not yet assessed but likely causing a decline (Duckworth et al. 2016).

The two Philippine subspecies of *P. bengalensis* are important predators of invasive rodents (Lorica and Heaney 2013; Fernandez 2014) and can be found in agricultural fields and forests (Heaney et al. 1998; Esselstyn et al. 2004; Lorica and Heaney 2013). The two subspecies are the only wild felids found in the Philippines and are currently listed as Vulnerable by the DENR (Ross et al. 2015; DENR 2017). The Palawan subspecies has yet to be assessed by the IUCN. Island populations of Leopard Cats in the Philippines are seriously threatened (Lorica 2008; Ross et al. 2015), so population dynamics and area of occurrence for *P.b. heaneyi* must be evaluated to determine further conservation actions.

Sus ahoenobarbus is endemic to the Palawan Faunal Region where it is the largest remaining member of Palawan's megafauna (Meijaard and Widmann 2017). *Sus ahoenobarbus* could play a key role in plant dynamics in the understory, as seen with other native pigs (Ickes et al. 2001), and with forest upkeep by eating carrion. Even though the species is protected under the National Wildlife Act of the Philippines, *S. ahoenobarbus* is still commonly hunted for subsistence and the bushmeat trade (Widmann 2017). *Sus ahoenobarbus* was recently moved from a threatened category to Near Threatened by the IUCN; while the species has a small extent of occurrence and remains heavily hunted, it seems able to adapt to various habitats

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(Widmann 2017). The species still holds a Vulnerable listing under the DENR (DENR 2017).

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Manuscript Preparation

1. The manuscript should be no more than 6,000 words; typewritten using Georgia, font 11; double-spaced, justified on A4 (8.3"x11.7") paper, with 2.54 cm margins on all sides. All pages should be numbered consecutively at the center of the bottom of the page. Line numbers should be continuous (do not restart at each page).
2. Page 1 should contain the following: title of the article, running title, author(s), affiliation(s), name and complete contact details (mailing address, telephone number, fax number, and e-mail address) of the person to whom correspondence should be sent. A superscript in Arabic numbers should be placed after the author's name as reference to their affiliations. The title of the paper should be centered, **bold** and written in a sentence form. Capitalize only the first word of the title and proper nouns if there are. Scientific name(s) when included in the title should be italicized and not enclosed in parenthesis. **Provide name and contact details of at least two potential external Reviewers**. The Editors reserve the right with regard to the selection of external reviewers.
3. Page 2 should contain a short abstract of not more than 250 words. The abstract should contain facts and conclusions, rather than citation of the areas and subjects that have been treated or discussed. It may start with the hypothesis or a statement of the problem to be solved, followed by a

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description of the method or technique utilized to solve the problem. It should end with a summary of the results and their implications. The abstract is to be followed by a maximum of six **Keywords**.

4. The paper should be organized with the following main headings: **ABSTRACT, INTRODUCTION, METHODS, RESULTS, DISCUSSION, ACKNOWLEDGEMENTS, REFERENCES**. First subheadings should be in **bold** with each main word capitalized (example: **Study Site**). For second sub-headings, the first letter of the first word should be capitalized. Paper written in other formats will not be accepted or sent for review, instead it will be returned to the author for revision.

Figures and Tables

1. Figures and tables should be numbered (Arabic numerals) chronologically. Captions for figures and tables should be double spaced and have justified margins; First line not indented.
2. References to the tables and figures in the text should be cited as: Table 1; Figure 1; Tables 1 and 2; Figures 1 and 2. Photos, maps and drawings should be treated as Figures.
3. The Table or Figure if possible should appear in the same page where it is firstly mentioned in the text.
4. Figures must be in black and white if possible with a background free from major grid lines (of y-axis); the x and y axes are labeled and legend is provided.
5. Illustration should be original line drawings of good quality and should not exceed A4 size paper. Inscriptions should be readable even if the drawing is reduced by 75%. Drawings should be scanned and saved in TIF or PDF format before embedding on the manuscript. Separate file of the photos/illustrations maybe requested upon the acceptance of the manuscript.
6. Photographs – if possible, all photos used in the paper must have been taken by the author(s). Photos taken by other researchers/individuals/organizations must be duly acknowledged in the paper. The use of photos downloaded from the web/internet is strictly forbidden unless a written permission from the copyright holder (of that photo) is presented.

Scientific, English and Local Names

1. All organisms must be identified by their English, scientific names and local names if possible.
2. Scientific names must be cited for all organisms at first mention. Subsequently, only the initial of the genus should be written except when starting a sentence with a scientific name. All scientific names should be italicized. Example: *Tectus niloticus*; *Anadara* sp. *Musa* spp. Do not italicize the higher levels of taxonomic classification (example: family Echinometridae).
3. Local names should be in double quotes (example: locally called “saging” not ‘saging’; “palay” not ‘palay’).
4. Research articles dealing on species list should provide the authorities for each species (example: *Conus magus* Linnaeus, 1758; *Enosteoides philippinensis* Dolorosa & Werding, 2014).

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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

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).

1. 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).

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2. 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).

3. Alphabetize authors with the same year of publications. Use semi colons to separate each publication (example: Balisco and Babaran 2014; Gonzales 2014; Smith 2014).

4. Write journal’s name in full (examples: The Palawan Scientist, not Palawan Sci; Reviews in Fisheries Science, not Rev. Fish. Sci.).

5. The list of citation at the end of the paper should include only the works mentioned in the text and should be arranged alphabetically.

6. 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

7. 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.

8. 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.

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

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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. https://www.cites.org/. Accessed on 5 January 2014.

10. 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.

11. 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.

12. 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.

13. 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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