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Cover Photos

The Blue-naped Parrot *Tanygnathus lucionensis* and Hill Myna *Gracula religiosa* (inset) are commonly found in Palawan, Philippines. However, their populations are threatened with poaching and continuous habitat degradation. In Talakaigan, Aborlan, Palawan, the nesting trees of these two bird species in the interior part of the forest were characterized and georeferenced by Mr. A. Bernardo Jr.

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EDITORIAL

Researches and publications are the very foundation of the country's development. This is manifested by the world's super powers being on top on the list of the countries with the most numbers of scientific publications. For example, for the year 2004-2008, 21% of the world's scientific publications were written by researchers from USA, followed by China (10%) and Japan (8%). Spain on the 10th place contributed 3%, while India shared 2% (http://www.statista.com/). The Philippines population is comparable to Japan and twice that of Spain, but our contribution to global scientific publications is far less than these countries let alone our ability to write and speak the English language.

In the Western Philippines University, 30% of the faculty members and staff conduct researches and present their research results in conferences and symposia. However, only around 11.1% have consistently published papers in scientific journals, in spite of varied research incentives offered by the University. Nevertheless, there are positive inspiring signs of improvement for the last five years of Research and Development (R&D) of the University: the number of university researchers has doubled and the number of paper publications increased 7x in the last five years, and their publications tends to shift from national to international level. Although promoting and enhancing R&D in a small university like WPU is difficult and complex, it's not a reason to yield our efforts to do so.

The Palawan Scientist is one of the University's strategies to increase the outputs of its researchers. It is noteworthy that The Palawan Scientist delivers only peer reviewed articles for the best interests of the readers and the next generation. The Palawan Scientist is globally accessible through its web site (www.palawanscientist.org) and the Philippine E-Journals (http://ejournals.ph/journals.php?letter=t). Its indexing is also in progress with the Aquatic Science and Fisheries Abstract (ASFA) and Aquatic Commons. This 8th volume of The Palawan Scientist is supposed to be the year with the most (11 articles) numbers of submitted and reviewed papers, but only five articles have made through the journal's editorial board and external reviewers.

To the authors of the five articles and for those who made this volume possible, thank you for your patience and persistence.

Congratulations!

Benjamin J. Gonzales, PhD
Vice President
Research Development and Extension, WPU

Composition, size and relative density of diatoms in the stomach of 4 to 75 day-old juvenile abalone *Haliotis diversicolor* (Reeve)

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ABSTRACT

The diatom biofilm that naturally grow on polyvinyl chloride plates serve as food of postlarva and juvenile abalone *Haliotis diversicolor* Reeve, called "tokobushi" in Japanese. Composition, size and relative density of diatoms in the stomach of 4, 7, 10, 13, 17, 21, 27, 35, 50 and 75 day-old tokobushi were evaluated to characterize their diatom intake. Stomach in glycol methacrylate resin was sectioned and examined under the light microscope, then analyzed using an image processing software. The diatoms present in the stomach of tokobushi were *Thalassiosira*, *Melosira*, *Triceratium*, *Odontella*, *Asterionella*, *Licmophora*, *Thalassionema*, *Cocconeis*, *Navicula* and *Nitzschia*. Only four varieties of diatoms were observed in 4 to 10 day-old tokobushi which coincided with initial feeding. The number and size of diatoms increased in 13 to 75 day-old juvenile, which were exhibited in its exponential growth pattern. The stomach of 4 to 13 day-old tokobushi contained small-sized diatoms (<67 µm) while both small and large-sized diatoms (>123 µm) were observed in 17 to 75 day-old juveniles. Higher relative densities (8.7 – 15.8 diatom/1000 µm^2) of diatoms were documented in 4 to 10 day-old tokobushi while 17 to 75 day-old exhibited lower relative densities (1.2 – 4.2 diatom/1000 µm^2). Generally, as young tokobushi increases in size, the diatom intake increases in composition and size but density decreases with increasing size of diatom ingested.

Keywords: abalone, biofilm, diatom, glycol methacrylate, plates, stomach

INTRODUCTION

The abalone *Haliotis diversicolor* Reeve (= *H. diversicolor diversicolor*, *H. diversicolor supertexta*, *H. diversicolor aquatilis* (Geiger 1999), or 'tokobushi' in Japanese, are mass-produced in the hatchery for mariculture and fishery resource enhancement purposes (Alcantara and Noro 2006). Mass culture of tokobushi is done in outdoor concrete tanks provided with corrugated polyvinyl chloride (PVC) plates (hereafter referred to as plates) for settlement that have been naturally grown with biofilm of diatoms. Diatoms and associated microorganisms serve as food of tokobushi for the first three months in culture (Chen 1989).

The postlarval to juvenile stage is critical for survival and growth of abalone, which may be partly related to the kind and quantity of diatoms ingested as food (Day et al. 2004). Several adhesive diatoms would grow on plates but grazing rates may depend on factors such as preference and age of abalone. *Haliotis midae* postlarva prefers prostrate diatoms like *Cocconeis sublittoralis* but also ingests overstorey species like *Nitzschia palea* (Matthews and Cook 1995). On the other hand, one week-old *Haliotis rufescens* postlarva had minimal grazing on a prostrate diatom *Navicula incerta* but increased rapidly when the larvae reached 2 to 3 week-old (Martinez-Ponce and Searcy-Bernal 1998). On a commercial scale nursery culture of *Haliotis rubra*, postlarvae grow fast on the macroalga *Ulvella lens* which was further enhanced by the addition of the diatom *Navicula* sp. (Daume et al. 2004).

The gut content of young juvenile abalone in terms of diatom composition, size and relative density is a manifestation of its feeding and nutritional characteristics which has application in the aquaculture. The present paper reports on the diatom content of the stomach of 4 to 75 day-old tokobushi reared in outdoor tanks.

MATERIALS AND METHODS

One cohort of young tokobushi used in this study was hatched and cultured in the hatchery-nursery facility of the Kagoshima Mariculture Society in Tarumizu City, Kagoshima, Japan from 21 September 2004 to 21 February 2005. During hatching, the age of tokobushi is considered zero (Bryan and Qian 1998). The larvae were stocked in outdoor rectangular concrete tanks (~4 t) filled with plates naturally grown with diatom biofilm. Samples were collected randomly from 4, 7, 10, 13, 17, 21, 27, 35, 50 and 75 day-old tokobushi. Five pieces of cut-plates (~25 cm^2) with settled tokobushi were collected from each of the five tanks used for the cohort. Biofilm of diatoms that grew naturally on upright plates after 2 – 4 weeks in outdoor tanks with flow-through seawater served as the natural food of postlarval and juvenile tokobushi for about three months of culture. The different diatom populations that randomly and naturally attached on plates were the source of diatoms ingested by tokobushi in culture. The composition and density of diatoms growing on the plates were not monitored during the culture period. Continuous aeration and about eight hours flow through of seawater were provided in culture tanks.

In this study, 10 individuals of juvenile tokobushi at different ages were photographed under the stereo microscope (Nikon SMZ-U, Japan) to measure the shell length (SL) and monitor the growth rate. Microphotographs were analyzed using ImageJ: Image processing and Analysis in Java (Baggethun 2006). Another five samples were fixed in 5-10% seawater formalin for 24 – 48 hours, then transferred to 70% Ethyl Alcohol for storage. Postlarva and juvenile tokobushi were decalcified in 5% Acetic Acid, dehydrated in Ethyl Alcohol series of 70%, 80%, 90%, 95% and 100%, then infiltrated and embedded in Glycol Methacrylate (Technovit 7100®, Heraeus Kulzer GmbH, Germany) resin. Serial microtome (Yamato RV-240, Japan) sections (2 – 3 µm) were stained with Hematoxylin-Eosin or Toluidine Blue, and then photographed under a light microscope (Nikon Eclipse E600, Japan). Ingested diatoms were identified, measured and counted per 1000 μm^2 area of the longitudinally sectioned stomach to determine the composition at genera level, size in length or diameter and relative density. Using the ImageJ software, a known area (average: 1000 μm^2) in the stomach was used as the sample area of ingested diatoms (Figure 1). Identification of diatom genera were validated from diatom samples obtained from plates where tokobushi attached and grazed following the descriptions of Round et al. (1990). Relative density was calculated from the number of diatoms found in every 1000 μm^2 area in the photomicrograph of the stomach. Ingested diatoms found in the stomach of tokobushi (Figure 1) were the principal materials analyzed for the composition, size and relative density of diatom intake in this study.

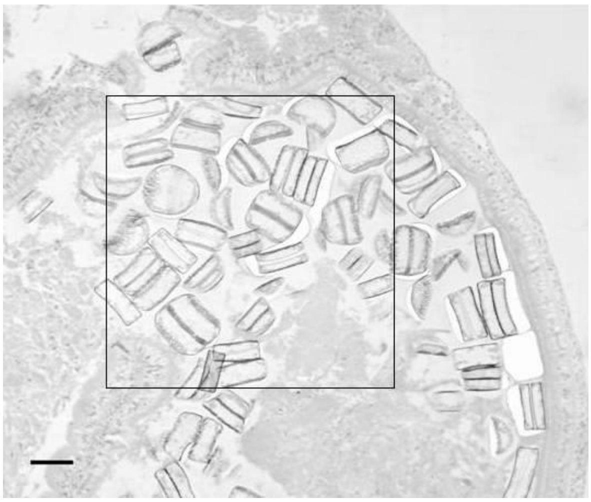

Figure 1. Photomicrograph of the stomach of a 75 day-old *H. diversicolor* showing the sample area (in a square) where ingested diatoms (shown here: *Thalassiosira*) were measured and counted using the ImageJ software; scale bar = 100 µm.

To establish the significant differences among the sizes and densities of diatom content of the digestive gut at corresponding ages, ANOVA followed by multiple comparisons was carried out using the software SIGMASTAT (Systat Software Inc., California, USA).

RESULTS

A total of 10 genera belonging to three classes, nine orders and nine families, namely *Thalassiosira*, *Melosira*, *Triceratium*, *Odontella*, *Asterionella*, *Licmophora*, *Thalassionema*, *Cocconeis*, *Navicula* and *Nitzschia* were observed in the stomach of tokobushi. These diatoms were also observed in the intestine, digestive gland and digestive caecum. *Thalassiosira* and *Melosira* were centric diatoms while the rest were pennate diatoms.

All the above diatoms were also found in the biofilm examined on PVC plates during the culture of tokobushi. However, not all diatoms were observed in the stomach of tokobushi at all ages examined (Table 1). The most commonly ingested diatoms were *Cocconeis*, *Nitzschia*, *Melosira*, *Navicula*, *Thalassionema* and *Licmophora* found in 100% to 70% of the ages examined. On the other hand, the other diatoms were only observed in 50% to 10% of the tokobushi. Moreover, 4 to 13 day-old postlarvae had ingested 4 to 6 genera of diatoms while 17 to 50 day-old tokobushi had 7 to10 diatoms in their stomach. In addition, the 4 to 75 day-old tokobushi exhibited an exponential growth pattern (Figure 2).

Table 1. Distribution of diatoms observed in the digestive gut of *H. diversicolor* at different ages in mass culture (x=present).

Diatoms	**Day-old**									
	4	**7**	**10**	**13**	**17**	**21**	**27**	**35**	**50**	**75**
Thalassiosira						x	x	x	x	x
Melosira		x	x	x	x		x	x	x	x
Triceratium					x			x		
Odontella						x		x		
Asterionella					x		x	x	x	
Licmophora	x	x		x	x	x	x	x	x	
Thalassionema		x	x	x	x	x	x	x	x	
Cocconeis	x	x	x	x	x	x	x	x	x	x
Navicula	x		x	x	x	x	x	x	x	x
Nitzschia	x	x	x	x	x		x	x	x	x

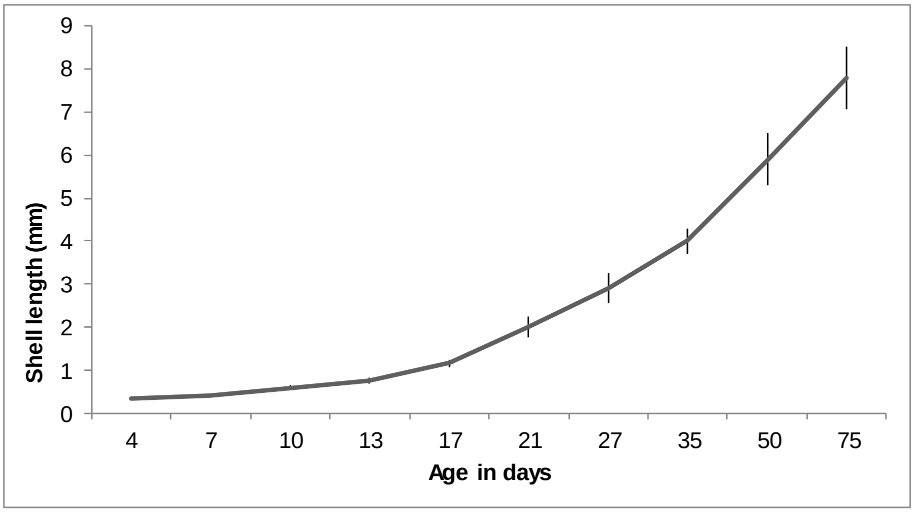

Figure 2. Growth pattern of the shell length of 4 to 75 day-old *H. diversicolor* in culture (error bar = SD).

Ingested *Cocconeis*, *Nitzschia*, *Navicula*, *Melosira*, *Odontella* and *Triceratium* were relatively small-sized diatoms ranging from 11 to 49 µm in length or diameter. On the other hand, *Thalassionema*, *Licmophora*, *Thalassiosira* and *Asterionella* were relatively large-sized ranging from 20 to 123 µm. The sizes of ingested diatoms ranged from 10 to 67 µm for 4 to 13 day-old tokobushi while 12 to 123 µm for 17 to 75 day-old (Table 2). Pooled data revealed that the size of ingested diatoms for 4 day-old was significantly smaller than those in 17, 27, 35 and 50 day-old postlarvae ($P<0.001$).

Relative densities of ingested diatoms were noted to be highest in 4, 7 and 10 day-old tokobushi which ranged from 8.7 to 15.8 diatom/1000 μm^2 of the stomach area. Such relative density was significantly different ($P<0.001$) from those in 17, 21, 27, 35, 50 and 75 day-old which ranged from 1.2-4.2 diatom/1000 μm^2 of the stomach area. Moreover, the relative density of 13 day-old (5.4 diatom/1000 μm^2) was not significantly different from those in 4 (8.7 diatom/1000 μm^2), 7 (9.7 diatom/1000 μm^2), 10 (15.8 diatom/1000 μm^2), 17 (4.2 diatom/1000 μm^2), 21 (4.5 diatom/1000 μm^2), 27 (2.5 diatom/1000 μm^2) and 50 (2.6 diatom/1000 μm^2) day-old tokobushi (Figure 3).

Table 2. Size (µm) distribution of diatoms observed in the digestive gut of *H. diversicolor* at different ages.

Diatoms	**Day-old**									
	4	**7**	**10**	**13**	**17**	**21**	**27**	**35**	**50**	**75**
Thalassiosira						58	91	116	81	123
Melosira		11	32	17	19	25	30	47	21	34
Triceratium					49			46		
Odontella								44		
Asterionella					114	99	86	112	104	
Licmophora	32	47		67	80		102	109	89	
Thalassionema		26	20	23	34	20	29	77	81	
Cocconeis	12	11	10	17	15	12	16	12	12	14
Navicula	22		17	24	19	20	25	20	23	19
Nitzschia	16	27	28	37	32	31	45	36	42	33

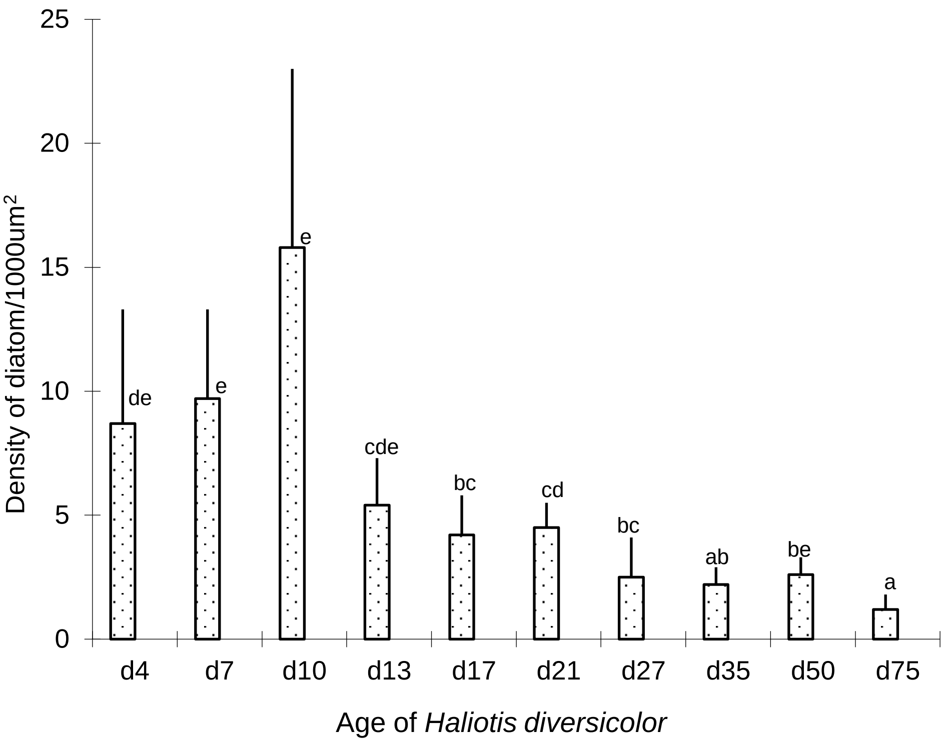

Figure 3. Relative density of ingested diatom in the digestive gut of *H. diversicolor* at different ages (d = day; error bar = SD; common letters denote no significant difference, a<b<c<d<e; n = 10).

DISCUSSION

Previous methods used to describe ingested diatom of abalone were by video recording (Martinez-Ponce and Searcy-Bernal 1998) and analysis of fecal material (Kawamura et al. 1998a). Norman-Boudreau et al. 1986 firstly described the types and kinds of diatoms actively selected by newly-settled abalone and cited by many succeeding authors. Diatoms serve as the main food of postlarval and juvenile tokobushi in the wild and in culture and are important for their growth and survival (Carbajal-Miranda et al. 2005). Diatoms are nutrient-rich because in addition to their cellular contents they have extracellular polymer substances or mucilages, such as the tubes of *Navicula*, pads of *Asterionella*, fibrils of centric diatoms and adhering film of pennate diatoms that act as collecting apparatus for nutrients and microbes (Hoagland et al. 1993). Tokobushi appears to start feeding after settlement since diatoms were present in the stomach of 4 day-old, which was also suggested by Chen (1989). Similar observation was reported in 2 to 4 day-old *H. rufescens* (Martinez-Ponce and Searcy-Bernal 1998) and 2 to 6 day-old *H. midae* (Matthews and Cook 1995).

Most of the diatoms ingested by tokobushi were similar to those given to other abalone during culture. *Cocconeis* dominates the initial prostrate diatom biofilm (Matthews and Cook 1995) and has high digestion efficiencies (Takami 2005). *Nitzschia* and *Navicula* are abundant in a diatom assemblage (Daume and Ryan 2004, Kawamura et al. 2004) and produce high survival (Najmudeen and Victor 2004) and growth (Uki and Kikuchi 1979) to young abalone. *Navicula* and *Melosira* become abundant at the later phase of culture in tanks (Stott et al. 2004), which may partly explain the composition and size of diatom ingested by tokobushi. Other diatoms ingested by tokobushi such as *Thalassionema*, *Triceratium*, *Odontella*, *Licmophora*, *Asterionella* and *Thalassiosira* were not frequently mentioned in abalone literatures probably because they were not cultured or unidentified thus unreported. In general, the growth of postlarval and juvenile abalone is influenced by density, nutritional value and digestibility of diatoms (Kawamura et al. 1998b, Searcy-Bernal et al. 2001, Gorrostieta-Hurtado and Searcy-Bernal 2004, Gordon et al. 2006). These observations were also corroborated by Capinpin (2007).

Data from this study suggest that smaller tokobushi postlarvae could take in small diatoms only but larger ones could have small and large diatoms at the same time. Small diatoms like *Cocconeis* and *Navicula* were consistently ingested by 4 to 75 day-old tokobushi while large or long ones like *Asterionella* and *Thalassiosira* were taken in only by 17 to 75 day-old. The ability of larger tokobushi to ingest larger diatoms appears to be related to increasing size of the digestive system. Roberts (1999) also showed that larger abalone had better developed radula that can be used to detach different types of diatoms. Smaller ones have less developed radula (i.e., highly curved teeth, low clearance angle) that merely function as scoops to collect smaller diatoms. This was corroborated by Onitsuka et al. (2004). The ability of larger tokobushi to ingest larger diatoms appears to be related to the increasing size of the digestive system which coincides with their increasing growth. This may also explain the significantly smaller size and fewer kinds of diatoms ingested at 4 day-old tokobushi compared to 17, 27, 35 and 50 day-old. Similarly, *H. discus hannai* larva of about 500 µm SL could ingest *Cocconeis* with 19 µm cell length and 13 µm cell width (Kawamura and Takami 1995) but *H. iris* postlarva less than 1300 µm SL could not take in *Achnanthes* with 76 µm cell length, 27 µm cell width and long stalks (Kawamura et al. 1998a). The young postlarvae (4 to 10 day-old) may not be able to digest diatoms at this stage may be due to poor digestibility as seen through the fecal material (Kawamura et al. 1998b) and because they lack digestive enzymes to do so (Takami et al. 1998). Hence, they were observed whole and intact in the gut. On the other hand, larger postlarvae are able to break open the frustules and digest the diatoms; that is why maybe few intact diatoms (particularly *Cocconeis*) were observed in the gut.

Other diatom grazers are passive feeders and do not feed based on morphology or size of microalgae but shift from one food to another due to changes in relative amounts or attachments of different diatoms (Aberle et al. 2005). Tokobushi at juvenile stage are capable of grazing on some young macroalgae but they remain feeding on diatoms present or epiphytic to macroalgae. This condition was also observed in other young and even adult abalone (Takami et al. 2003, Simental et al. 2004). The inconsistencies in the number, size and relative density of ingested diatoms by 75 day-old when compared with other younger tokobushi may be related to its tendency to graze on young macroalgae as observed on the plates in tanks, which may result to lesser diatom intake. In *H. discus discus*, the shift in feeding habit from microalgae to macroalgae occurs at about 20 mm SL (Kiyomoto and Yamasaki 1999).

The exponential growth pattern exhibited by 4 to 75 day-old tokobushi is probably due to increasing diatom intake in terms of its composition and size. Similar pattern of growth has been reported in *Haliotis varia* (Najmudeen and Victor 2004). At this stage, metabolic rates are high (Shilling et al. 1996) and there is a greater need for nutrients to support increasing growth rate (Searcy-Bernal et al. 2001, Daume and Ryan 2004). Moreover, most of their energy budget is utilized for somatic growth thus the exponential increase in size (Peck et al. 1987).

The high relative density of diatom ingested by 4 to 10 day-old tokobushi was probably due to small radula, mouth and stomach at this stage (Onitsuka et al. 2004, Roberts et al. 1999). Again, this may be due to the fact that the diatoms were not efficiently digested by young postlarvae at this stage, hence can be seen whole and intact. At this stage, they may even utilize the bacteria, mucus, and diatom extracellular substances as their initial source of energy (see Kawamura et al. 1998b). As mentioned previously, a young postlarval tokobushi has slow growth and just begun feeding on small diatoms able to fit its mouth. It follows therefore that intake maybe maximal relative to size to get enough nutrients for survival and much-needed growth. Ingestion and digestion of diatoms by tokobushi can be affected by diatom size, morphology, attachment and frustule strength (Kawamura et al. 1998a). In this study, the ingested diatom in the stomach of young tokobushi increases in terms of composition and size but inversely proportional to relative density as tokobushi grows bigger. This study shows that histological examination can be one of the methods to characterize the diatom composition, size and relative density of ingested diatoms.

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Georeferencing and characterization of nesting trees of commonly traded wild birds (Blue-naped Parrot *Tanygnathus lucionensis* and Hill Myna *Gracula religiosa*) in Talakaigan Watershed Aborlan, Palawan, Philippines

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ABSTRACT

Forest degradation endangers the survival of nesting trees in the wild. Despite this threat, the nesting trees that support the commonly traded birds in Talakaigan Forest are not well documented. Hence, the study was conducted to provide information on the characteristics, species composition and geographical distribution of nesting trees. The study was participated by the indigenous Tagbanua bird poachers in the area. Preliminary information was obtained using key informant interview and focused group discussion. Appropriate parabiology training was conducted to equip the participants with practical skills needed in the data gathering. The study unfolded that there were only two species of birds commonly poached in the watershed, the *Tanygnathus lucionensis* and *Gracula religiosa*. These birds nest only on four species of trees which typically towers above the canopy layer. Remarkably, more than 60% of the recorded nesting trees belong to a single species of tree, the *Koompassia excelsa*. The GIS analysis unveiled that most nesting trees were located in the interior part of the forest. The skill needed to venture into the jungle highlights the role of Tagbanua Tribesmen in the poaching activity in the area. The study recommends an education campaign, enforcement of relevant environmental laws as well as conservation activities focusing on both the nest trees and the poached bird species to be implemented in the area.

Keywords: georeferencing, avifauna, poaching, nesting tree

INTRODUCTION

The Philippines is one of the countries in Asia that is gifted with plenty of natural resources. The country supports more than 552,177 described species, of which more than half are considered endemic (Ong et al. 2002). Among its component islands, Palawan is one with remaining pristine terrestrial and marine ecosystem. Because of the vast tracts of verdant vegetation with remarkable remaining old growth forest, the province was commonly called the “last biodiversity frontier”. Talakaigan Watershed is one of the remaining unspoiled forests in the municipality of Aborlan. It is part of the Victoria –Anipahan Mountain Range, a vast tract of forest that is considered as one of the 11 important bird areas in Palawan with Important Bird Area (IBA) Code PH053 (Mallari et al. 2001). This place is endowed with luxuriant forest that supports diversity of wildlife species. Its massive trees and thick understory vegetation harbor unique assemblage of resident and endemic birds such as the Palawan Peacock-pheasant, Palawan Hornbill, Blue-headed Raquet-tail, Palawan Tit and others. The bountiful foods in the forest also attract some forest dwelling migratory birds like the Chestnut-winged Cuckoo and the Grey-streaked Flycatcher that pass along this region, making it as one of their staging areas.

The towering trees in the emergent layer of the forest provide great nesting places for canopy and trunk cavity nesting bird species. Some of these birds like the Hill Myna, Blue-naped Parrot and Palawan Hornbill are highly priced species and are commonly collected by poachers and sold in the local, national and international pet markets (Cruz et al. 2007).

In the midst of the flourishing biodiversity, human activities in the area are also getting intense. Tagbanua, a cultural minority group that lives within and along the forest edges of the watershed are the major users of the forest resources in the area. According to Sopsop and Buot (2011), this indigenous group together with other settled migrants cultivate the fertile soil in the foot hills and also gather timber and non-timber forest products in Aborlan forest system. They also mentioned that other than rattan, wild fruits, resin, orchids, honey and plants with medicinal properties, Tagbanuas also collect and hunt for wildlife such as mynah, parrot, jungle fowl, hornbill and wild pig.

Like in other municipalities, harvesting forest resources in Aborlan is an emerging problem which threatens the sustainability of these resources. The demand for lumber, fuels the proliferation of small scale logging operations. Widmann (1998) supported this by mentioning that albeit logging was banned since 1989, illegal logging is still practiced in Palawan. Moreover, some of the economically important trees cut by the lumberjacks are known as nesting trees of birds.

Considering the vital function of these birds as primary agent of seed dispersal in the forest and the potential impacts of poaching, habitat degradation and loss of nesting trees due to logging merits an immediate conservation efforts that are not only focused on the commonly poached bird species but on the conservation of nesting trees as well. One of the important information needed in making strategies to control and stop the poaching activities in Talakaigan Watershed and at the same time conserve the nesting trees of the commonly marketed bird species is establishing a baseline data on the geographical distribution of the nesting trees. Moreover, documenting the attributes of nesting trees is also of utmost importance. These pieces of information might help us understand further the nesting preferences and nest site selection behavior of commonly poached birds. The information derived from this study is a useful tool that can be used by the Local Government Unit (LGU) and Department of Environment and Natural Resources (DENR) in intensifying their efforts in conserving the commonly traded wild birds and their nesting trees as well.

This study aimed to identify and characterize the nesting trees of commonly traded wild birds in Talakaigan Watershed Aborlan, Palawan. Specifically, the nesting trees were characterized in terms of average canopy spread, height and girth. It also aimed to determine the location of the nesting trees by getting the coordinates using Global Positioning System (GPS) and georeferencing it in a digital map of the watershed using the Geographic Information System (GIS) interface.

MATERIALS AND METHODS

The study was conducted in Talakaigan Watershed from June to August 2014. The researcher used Key Informant Interview (KII) method in identifying the actively operating poachers and traders in the area. While Focused Group Discussion (FGD) with local poachers was conducted to obtain preliminary information on poaching activity such as number of poachers, number of buyers, price of birds, breeding season, commonly poached bird species and commonly used nesting tree species. The participants were also tasked to draw the detailed map of the Talakaigan Watershed (Figure 1) which includes the relative locations of the landmarks such as river, tributary streams, ridges, foothills, trails, reforestation areas, campsites, dam, community water intake, and all known nesting trees in the area.

The drawn map with marked locations of nest trees in the watershed was used as a guide in selecting starting points, routes, location of base camps and period of time needed in the survey.

A hands-on training on data collection was also conducted to equip the local poachers who agreed to participate in the nesting trees survey with appropriate data gathering skills. To ensure the reliability of the data, each trainee took a practical examination prior to the actual data collection.

Figure 1. Group of local poachers drawing the detailed map of the Talakaigan Watershed and marking the location of the nesting trees occupied by commonly traded wild birds.

The survey prioritized the nesting trees identified by the local poachers. However, exhaustive searching of other nesting trees in the area was also done. When finding a nesting tree, attributes of the nest trees (tree height, trunk diameter, average canopy spread), digital photographs and coordinates were recorded.

The trunk diameter of each nesting tree was measured according to the guidelines set by College of Forestry, University of the Philippines (Carandang 1996). The nest tree height was calculated using the angle of elevation and the horizontal distance of the tree from the observer, while the average canopy spread was determined using the suggested method defined by Maryland Department of Natural Resources (MDNR 2013).

Descriptive statistics including frequency counts and percentages were used to describe the relative abundances of nesting tree species and species of commonly poached birds that use the specific nesting trees. The selected attributes of the different nesting trees were described using the mean and range. Meanwhile, the GPS coordinates of nesting tree species and bird species using it, were georeferenced in the digital map of Talakaigan Watershed using the GIS interface. The collection of data was limited within the slopes where most of the poaching activities were concentrated based on the information revealed by the participating poachers.

RESULTS AND DISCUSSION

Status of Poaching Activity in Talakaigan Watershed Area

The eight actively operating poachers were identified by the informants, all were residents of the community and members of the indigenous Tagbanua Tribe. This information uncovers the role of Tagbanua Tribesmen in the poaching activity in the area. These people are highly experienced hunters and forest product gatherers. Equipped with indigenous knowledge from their ancestors and skills honed by experience of living almost entirely in the forest, they can easily locate nests of Blue-naped Parrot and Hill Mynah even in the deepest part of the forest. Moreover, they are also good and agile tree climbers; they can easily climb the tall nesting trees using only few indigenous tools.

The key informants disclosed that the poaching activity in the area was rampant and trading of wild bird nestlings was openly done in the community. The presence of abandoned temporary shelters and carved footholds (Figure 2) in most of the tall *Koompassia excelsa* (Becc.) Taub trees were noticed during the initial visit to Talakaigan watershed and confirmed the poaching activity in the area.

The poaching activity in Talakaigan Forest begins from January and lasts until August every year. It coincides with the breeding season of Blue-naped Parrot (*Tanygnathus lucionensis)* and Hill Myna (*Gracula religiosa*), two important commonly poached bird species sought after by the local buyers in the area. The poachers divulged that the breeding activities of the Hill Myna starts as early as January and lasts until August, while breeding of Blue-naped Parrot begins from March and lasts until July. The breeding season of Blue-naped Parrot in Watershed as disclosed by poachers is two months longer than those mentioned by other researchers who claimed to last from April to June (Kennedy et al. 2000; Birdlife International 2012). Moreover, Kennedy et al. (2000) also reported a nest of the Hill Myna with two chicks in Culion, Palawan during the month of September. This observation indicates a much longer breeding period compared to the data provided by the local poachers. These findings indicate that the breeding period observed in Talakaigan Watershed might not be the same in other places within the geographical range of these bird species. Thus, the actual extent of the breeding period in a much wider scale is still inconclusive and remains to be investigated.

Figure 2. One of the temporary shelters used by poachers in guarding the nest trees of commonly traded birds in Talakaigan Watershed Aborlan, Palawan (Left); carved footholds on the trunk of one of the *Koompassia excelsa* trees locally known as “Manggis” (right).

Similar to the information given by the key informants, all of the FGD participants agreed that there were eight poachers actively operating in the area and all of them are members of the indigenous Tagbanua Tribe. They also disclosed that there were three known buyers and all of them are lowlanders who belong to other ethno-linguistic groups. It was also unfolded that the buyers were funding the poaching operation by giving advanced payments in terms of cash or goods to Tagbanua poachers.

The local bird poachers also divulged that they have verbal ownership claim on every nesting trees they climbed, once the nesting tree was claimed by one poacher it is respected by most of them. However, some poachers coming from other communities occasionally steal the nestlings. Because of this occasional stealing in claimed nests, most poachers guard the nests after the hatching of eggs until the nestlings were collected. This activity was confirmed by the presence of makeshift shelters near the nesting trees (Figure 2).

Both the key informants and FGD participants agreed that the price of Blue-naped Parrot and Hill Myna nestlings was pegged at 500 pesos each and it was dictated by the buyers. According to their estimates, there were about 50-90 nestlings sold every season and most of them was Hill Myna. The participants also believed that RA 9147 or the Wildlife Resources Conservation and Protection Act of the Philippines was weakly enforced or not enforced at all in the area.

Commonly Poached Bird Species in Talakaigan Watershed

The Talakaigan Watershed is a home to variety of attractive bird species that could be potential targets of poachers. However, only the Blue-naped Parrot and Hill Myna nestlings were sought after by the local buyers in the area (Figure 3).

Figure 3. Commonly traded birds in Talakaigan Watershed. Hill Myna (left) and Blue-naped Parrot (right).

The Hill Myna has wider geographical distribution and is listed as Least Concern in the IUCN Red List of Globally Threatened Wildlife (Birdlife International 2012). On the contrary, Blue-Naped Parrot is classified as a near threatened species in the IUCN Red List of Globally Threatened Wildlife (Birdlife International 2012). Under the Philippines Wildlife Conservation and Protection Act (RA 9147), both species were classified as threatened species. Moreover, both Hill Myna and Blue-naped Parrot were listed in appendix II of Convention on International Trade of Endangered Species of Wild Flora and Fauna (CITES 2013). However, the Philippines/Palawan population of Blue-naped Parrot is put into danger by the growing demand for pet trade. This bird has a much smaller geographical range because of its near endemic status (it is once considered as Philippine endemic, but populations were now found in Borneo and Indonesia – mostly due to per trade where birds were either deliberately or accidentally released in the wild and had established their population).

The consistent pressure of poaching in the area and the continuously receding forest cover might have a significant impact on the population of this near threatened and endemic species. If not given full conservation effort, Blue-naped Parrot might as well become locally extinct in the watershed similar to the Red-vented Cockatoo (*Cacatua haematuropygia*). The Red-vented Cockatoo once abound in the forest of Talakaigan, but excessive poaching resulted in a localized extinction of this species in the watershed. At present, the Red Vented Cockatoo is protected and conserved in Rasa Island, Narra, Palawan.

Composition of Nesting Trees

In the present study, a total of 38 nesting trees were recorded, georeferenced and characterized. There were four species of nesting trees represented by four families (Table 1). The species with the highest number (25) was *Koompassia. excelsa* or locally known as ":Manggis" (65.8% of the total nesting trees) followed by "Bagtik" or "Palawan Almaciga" *Agathis celebica* (Koord.) Warb. of the Araucariaceae family represented by eight individual nesting trees (21.1% of the total number of the nesting trees). "Lomarau" *Swintonia foxworthyi* Elmer of the Anacardiaceae family was represented by only four individuals (10.5% of the total nesting trees recorded). Finally, the species with the least number of individual is the *Macaranga ovatifolia* Merr. or locally known as "Indang" of the Euphorbiaceae family, it is represented by only one individual nesting tree.

Table 1. Nesting trees used by commonly poached birds in Talakaigan Watershed

Family	Scientific Name	Local Name	Frequency (*f*)	Percentage (%)
Caesalpiniaceae	*Koompassia excelsa* (Becc.) Taub.	"Manggis"	25	65.8
Araucariaceae	*Agathis celebica* (Koord.) Warb.	"Bagtik"	8	21.1
Anacardiaceae	*Swintonia foxworthyi* Elmer	"Lomarau"	4	10.5
Euphorbiaceae	*Macaranga ovatifolia* Merr.	"Indang"	1	2.6

Characteristics of Nesting Trees

The results showed that only few species of trees were utilized as nesting trees (Table 2). All nesting trees have large trunks with deep cavities, massive, tall and mostly reaching the emergent layer. Among the recorded nest trees, *K. excelsa* has the tallest average height of 59.7 m. It was followed by *A. celebica* which is also a massive emergent layer tree with an average height of 56.1 m followed by a moderately size *S. foxworthyi* Elmer which has an average height of 41.8 m, which is still considerably taller than most of the trees in the area. The shortest recorded nest tree species is the *M. ovatifolia* and is represented only by one individual with the height of 26.5 m.

Table 2. Selected characteristics of nesting trees found in Talakaigan Watershed.

Species of Nesting Tree	**Crown Spread (m)**		**Tree Height (m)**		**Diameter (dbh) (m)**	
	Mean	Range	Mean	Range	Mean	Range
Koompassia excelsa (Becc.) Taub.	28.2	12.5-45.0	59.7	33.7-79.5	2.5	1.6-3.1
Agathis celebica (Koord.) Warb.	32.3	24.0-42.5	56.1	39.0-67.2	3.1	2.6-3.8
Swintonia foxworthyi Elmer	13.5	11.0-14.5	41.8	31.4-50.3	1.5	1.2-1.7
Macaranga ovatifolia Merr.	13.5	---	26.5	----	2.1	---

The species of nesting tree with largest average trunk diameter is *A. celebica* (3.1 m). Meanwhile, *K. excelsa* the nesting tree with the tallest average height has a slightly smaller average trunk diameter of 2.5 m. Likewise, the trunk diameter of the only representative of *M. ovatifolia* (2.1 m) is bigger than the average trunk diameter of the much taller *S. foxworthyi* (1.5 m).

Similarly, the average crown spread (32.3 m) of *A. celebica* was found to be wider than the average crown spread (28.2 m) of species with the tallest average height the *K. excelsa.* Meanwhile, the average canopy spread (13.5 m) of the much taller *S. foxworthyi* is only the same with canopy spread of the single representative of *M. ovatifolia*.

The trunk diameter of nesting trees does not always translate into the tree height. Similarly, the average crown spread also does not always follow the linear pattern of tree height and trunk diameter. However, the two species of nesting trees with the largest average trunk diameter, widest average canopy spread and tallest average height (*A. celebica* and *K. excelsa*) also have the highest number of recorded individual trees with nests of commonly traded birds in Talakaigan Watershed. The presence of trunk cavities is the primary reason why trees were selected for nesting sites.

There are four woodpecker species that thrive in Talakaigan Watershed, the Common Flameback *Dinopium javanense*, Greater Flameback *Chrysocolaptes lucidus*, White-bellied Woodpecker *Dryocopus javensis* and Great Slaty Woodpecker *Mullerripicus pulverulentus*. These birds ensure a steady supply of nesting holes for trunk cavity nesting birds in the watershed. As woodpeckers glean and forage on fungus infested invertebrate rich branches, they create holes which in turn hasten the fungal attack and invertebrate penetration. This cyclical process is repeated through time, making the holes big enough to be used as a cavity nest.

The findings clearly revealed that most of the nesting trees preferred by the commonly poached birds (Blue-naped Parrot and Hill Myna) are massive, with large trunk, wide canopy and are exceptionally tall that reach the emergent layer. This highlights the need for conserving these old stands of nesting tree species which are found only in primary forest or very old secondary forest.

Nesting Tree Species Preference of Commonly Poached Birds

The findings revealed that most of nest trees of Blue-naped Parrot and Hill Myna were found in trunk cavities of *K. excelsa* compared with other species of nesting trees recorded in the study area (Table 3).

Table 3. Frequency of nesting tree species of the commonly poached species of birds (Blue-naped Parrot and Hill Myna) in Talakaigan Watershed

Species of Nest Trees	*Tanygnathus lucionensis*		*Gracula religiosa*	
	Frequency (f)	Percentage (%)	Frequency (f)	Percentage (%)
Koompassia excelsa (Becc.) Taub.	9	23.7	17	44.7
Agathis celebica (Koord.) Warb.	0	0.0	8	21.1
Swintonia foxworthyi Elmer	1	2.6	3	7.9
Macaranga ovatifolia Merr.	1	2.6	0	0.0

Out of the 38 nesting trees georeferenced and characterized, 17 *K. excelsa* nest trees (44.7% of all the recorded nest trees) had Hill Myna nests, while nine *K. excelsa* nest trees (23.7% of all the recorded nest trees) had Blue-naped Parrot nests. Moreover, the results also revealed that while there was no nest of Blue-naped Parrot recorded in *A. celebica* this tree species was the next preferred nesting tree by Hill Myna. The data showed that out of the 38 total nests trees recorded, eight *A. celebica* trees (21.1%) had nests of Hill Myna. Although relatively much fewer than *K. excelsa* and

A. celebica, four S. *foxworthyi* trees were also found to have nests of these two commonly poached bird species. One, with nest of Blue-naped Parrot, while the other three had nests of Hill Myna.

Finally, no nest of Hill Myna was found in the *M. ovatifolia* tree, the only one recorded individual belonging to this species was occupied by Blue-naped Parrot. Furthermore, it was also observed that one *K. excelsa* tree with massive canopy and multiple trunk cavities had nests of both bird species at the same time.

One factor that possibly influenced the nest hollow development in these major nesting tree species was their exposure to strong wind surges during typhoon seasons. Having canopies reaching the emergent layer, they are exposed to strong winds without the protection of the adjacent trees. As a result more branches are broken which may serve as an entry point for fungal attack. However, further studies on nesting preferences of cavity nesting birds and the relative abundance of these tree species remain to be investigated. The locals claimed that Blue-naped Parrot also nests in *A. celebica* but no Blue-naped Parrots were found nesting on this tree species during the survey.

The salient feature of the data is that not all species of huge trees in the watershed is used as nesting places by the commonly poached birds. The findings revealed that only a relatively few species of trees were used as nesting places by these two commonly poached birds. Another noteworthy finding was that majority of the nesting trees used by commonly poached birds (Hill Myna and Blue-naped Parrot) in Talakaigan Watershed belongs to *K. excelsa*. This brings to light the importance of this species as nesting tree of commonly poached birds in the area. However, the reason why this tree species is commonly used as nesting tree remains to be investigated.

The parabiologist divulged that this species is not a common target of illegal loggers. However, they also disclosed that the demand for lumber is continuously increasing and they also noticed that the availability of commonly chosen species for lumber production is also declining at a very fast rate. This scenario is alarming, because there are possibilities that the next target of the illegal logging operators will be the huge stand of nesting trees.

Georeferencing of Nesting Trees

The coordinates of individual nesting trees obtained by using a GPS transceiver together with other specific data such as species of nesting trees and species of the nesting commonly poached birds were recorded and georeferenced using a GIS interface. The output was projected in Environmentally Critical Areas Network (ECAN) zoning map.

The yellow area in the ECAN map represents the multiple use zone, yellow green area as the controlled use zone, blue area as the restricted use zone and red area as the core zone. For the nest tree species, blue represents *Koompassia*, green for *Agathis*, white for *Swintonia* and black for *Macaranga*.

The map projection of the location of nesting tree species revealed that almost all the nesting trees of commonly poached birds in Talakaigan Watershed are located at the boundary of the controlled use zone and the core zone (Figure 4). This place is relatively far from the multiple use zone. According to the information disclosed by the parabiologists who frequently roam the Talakaigan Forest, massive and tall trees are still abundant in this particular area because the distance made it difficult for illegal logging activity to operate. Moreover, the unforgiving terrain and relative distance of the area from human settlements made the upland cultivation also impractical. This conforms to the observations of Mallari et al. (2011), where they mentioned that upland cultivation was more likely practiced at lower altitudes, while steep and inaccessible areas were spared and old growth forest were most likely preserved in these places . It was also noted that only few nesting trees represented by a single species (*K. excelsa*) are located near the multiple use zone or near the human settlement area. This species together with other nest tree species (*A. celebica, S. foxworthyi* and *M. ovatifolia*) were recorded only in the interior part of the forest. The distance of these nesting trees, the difficulty of the terrain and the needed skill to venture out in the jungle for several days most likely explain why all the poachers in the area are Tagbanua Tribesmen since their way of living is known to be closely interwoven with the forest. Being knowledgeable and experienced in the forest, they can easily endure the elements when they venture in the deepest part of the forest to look for the nesting birds. Using only minimal indigenous climbing and safety equipment, they bravely climb and collect the nestlings in the cavities of the towering nesting trees. Meanwhile, the nest trees occupied by Blue-naped Parrot and Hill Myna (Figure 5 and 6) showed no major difference in terms of geographical distribution.

CONCLUSION AND RECOMMENDATIONS

The poaching and bird trading activity in Talakaigan Watershed is overtly done in the local community. It can be considered that the RA 9147 or known as the Wildlife Resources Conservation and Protection Act of the Philippines is weakly implemented or totally not enforced at all in this area.

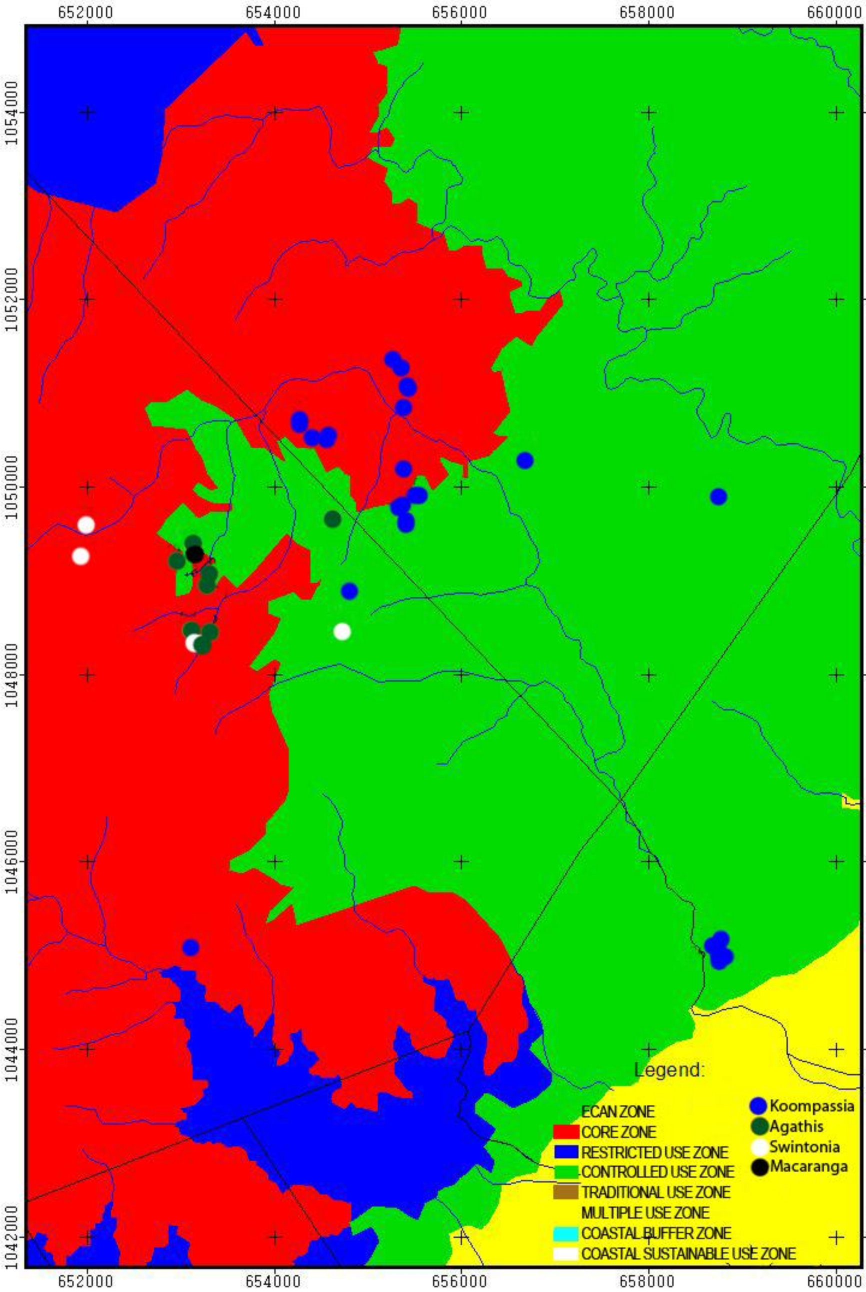

Figure 4. ECAN zoning map of Talakaigan watershed showing the location of all the recorded nesting trees occupied by commonly poached bird species (Blue-naped Parrot and Hill Myna).

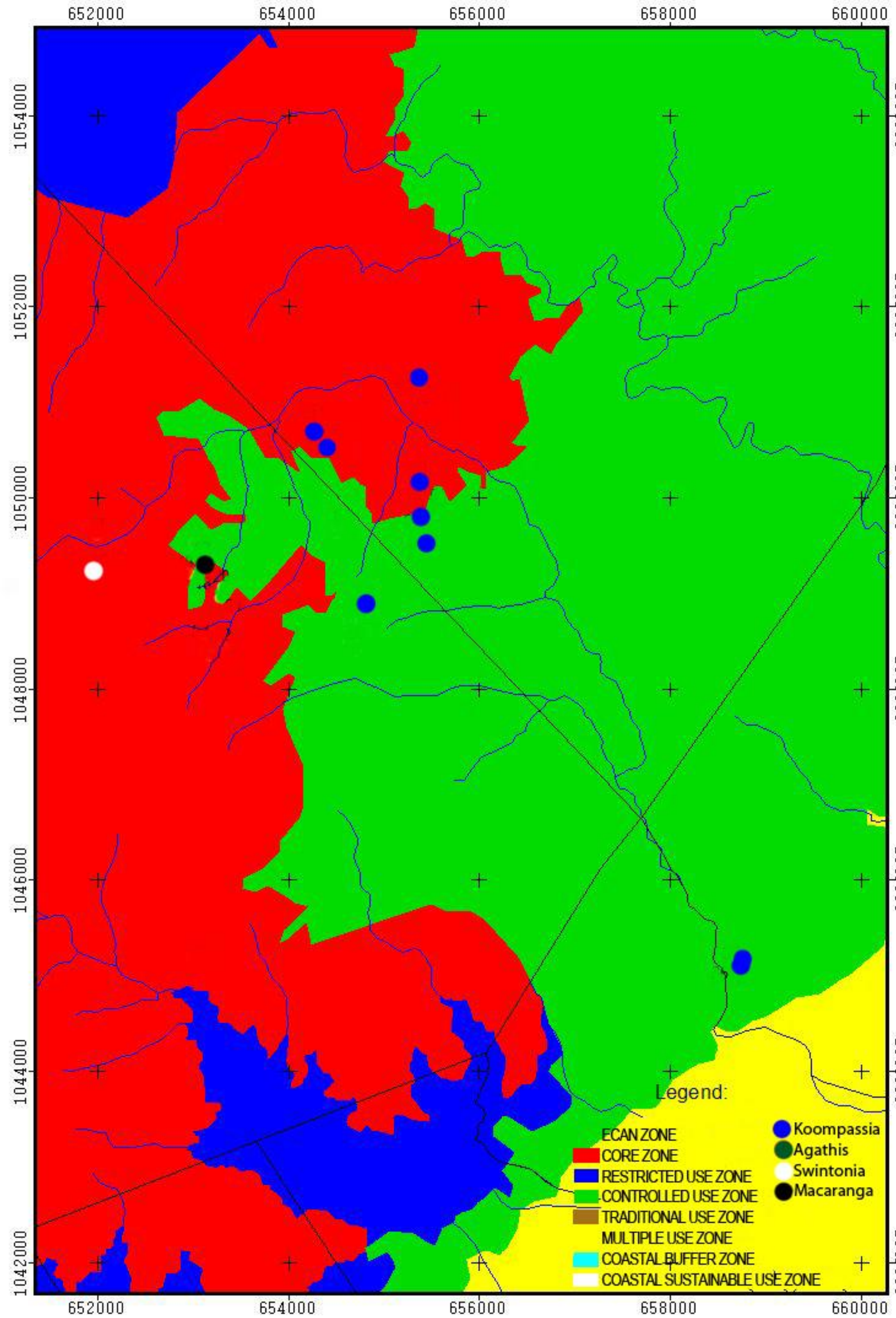

Figure 5. ECAN map showing the location of nest trees occupied by Blue-naped Parrot (*Tanygnathus lucionensis).*

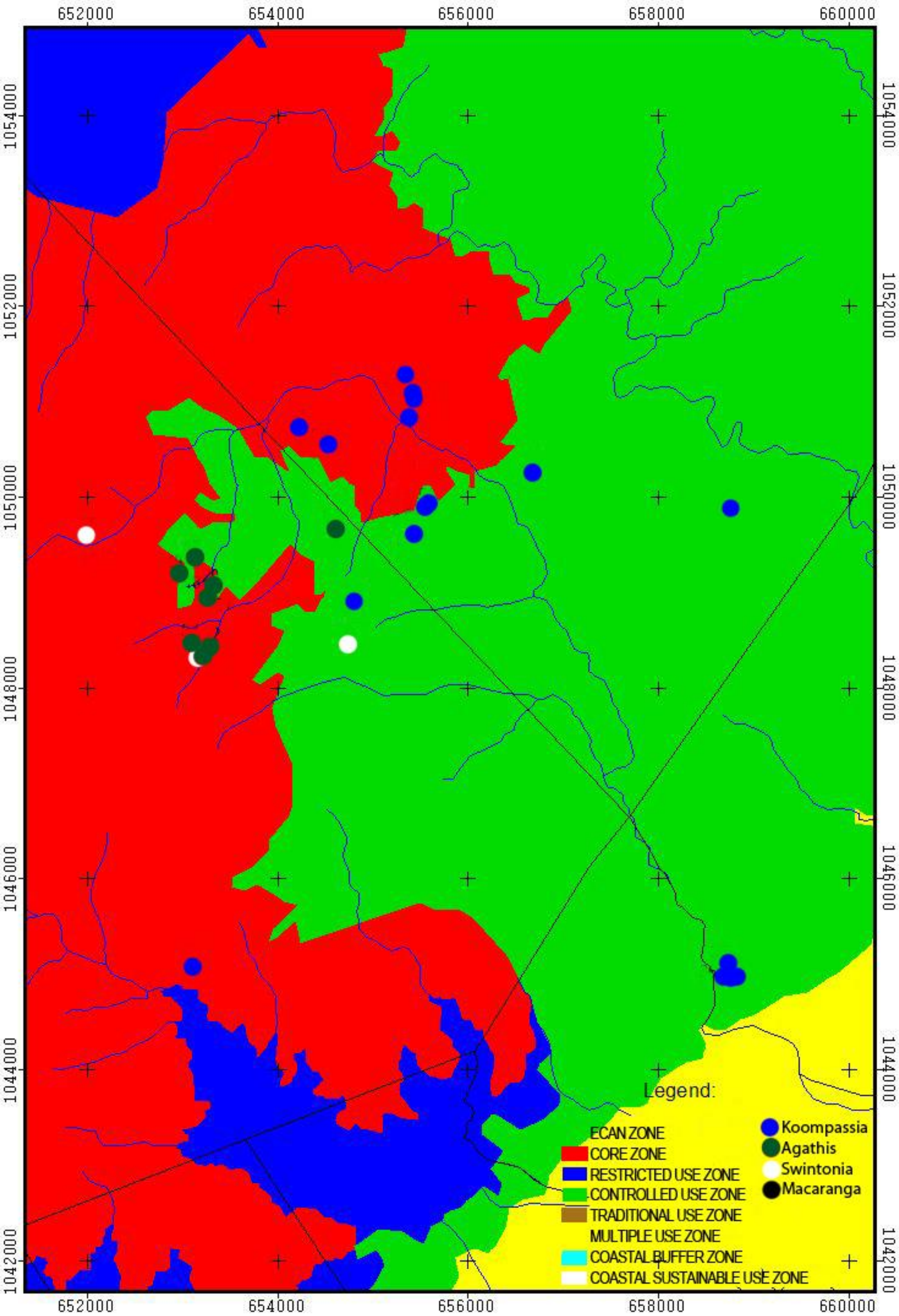

Figure 6. ECAN map showing the location of nest trees occupied by Hill Myna (*Gracula religiosa).*

The local indigenous Tagbanua Tribesmen who are experienced forest going hunters and forest product gatherers play an important role in the poaching of wild birds such as the Blue-naped Parrot and Hill Myna in the watershed area.

The two commonly poached bird species in Talakaigan Watershed appear to be selective in choosing the species of nesting trees. All the recorded nesting trees occupied by the commonly poached birds in the area were represented by only a few number of species and more than half of them belong to *K. excelsa* This brings to light the important function performed by these selected nest tree species in the reproduction and maintaining the healthy population of commonly poached birds in Talakaigan Watershed.

Aside from tree species selection, the chosen nesting trees generally have massive trunk, large canopy and usually are very tall reaching the emergent layer. This suggests that the nest trees commonly used by these birds are very old and found only in the old growth forest. Losing these trees in the forest might as well affect the nesting of commonly traded birds in the watershed. These findings highlight the need for a more strict and effective protection and conservation measures for the nesting tree species.

Furthermore, most of the nesting trees of commonly poached birds are concentrated near the boundary of the controlled use zone and the core zone, only few remains in the area near the multiple use zones where anthropogenic activity is at the greatest scale. This suggests that the anthopogenic activity in the multiple use zone most likely reduced the number of nest trees in the area. Finally, the majority of the nest trees recorded are concentrated in the innermost part of the forest where human activity is minimal. Future increase in the population of settlers in the periphery of the watershed might as well increase the anthropogenic activity that may lead to the demise of the nesting tree population.

It is highly recommended that the community of people living near the Talakaigan Watershed be involved in any conservation endeavours in the future. The more the people participate in natural resource conservation efforts, the deeper they understand how ecosystem works. Understanding such a concept might as well influence their actions leading towards sustainable resource utilization by learning and acquiring best practices.

It is also recommended that some strict and effective protection and conservation measures be implemented to stop the poaching activity in the area. An information and education campaign must be conducted in the nearby communities to educate the people about the importance of birds in forest ecosystem and to explain the potential impact of poaching on avifaunal population. The community must also learn about the contents of the RA 9147 with special citation of penalties for violators. The education campaign must be coupled with strict enforcement of RA 9147 to effectively control the existing poaching activity in the area.

The identified nesting tree species must be prioritized in the conservation and protection strategy to ensure that there will be adequate number of standing nest trees in the future. A forest survey must also be done in the watershed to monitor the regeneration of these nest tree species especially in most parts of the watershed that were exposed to more anthropogenic activities such as the controlled use and multiple use zones. It is also recommended that the nesting tree species be included together with other wildlife food plants in reforestation projects. Conservation of old growth forest must be strictly implemented because these massive and tall trees preferred by cavity dwelling birds were found only in old growth forest.

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Nesting incidence, exploitation and trade dynamics of sea turtles in Balabac Strait Marine Biodiversity Conservation Corridor, Palawan, Philippines

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ABSTRACT

The study assessed the nesting incidence, threats to nesting habitats, exploitation and trade dynamics of sea turtles in the Balabac Strait Marine Biodiversity Conservation Corridor (MBCC). The most number of nests found belonged to the green sea turtle *Chelonia mydas* and only very few were of hawksbill sea turtle *Eretmochelys imbricata.* The shoreline vegetation was the most preferred nesting area, followed by beach forest and open beach. The eggs and meat of sea turtles in Balabac Strait MBCC are exploited for local consumption and trade. Information on trade route and local perception on conservation issues about sea turtles is also presented herein.

Keywords: Balabac Strait, exploitation, nesting incidence, sea turtle, trade dynamics

INTRODUCTION

In 2004, the Conservation International-Philippines (CI-P) declared Balabac Strait as a Conservation Priority Area (CPA) for the presence of several threatened species of vertebrates, particularly sea turtles (Anda and Tabangay-Baldera 2004). In that same year, Torres et al. (2004) together with the Exercise Luzon Sea Team conducted a rapid site assessment in several islands in Balabac Strait including Secam, Roughton and Candaraman Islands. Based on this survey, it was confirmed that there were hawksbill turtle *Eretmochelys imbricata* nests in Roughton Island. Since their assessment was only short, Torres et al. (2004) recommended further studies on sea turtle nesting incidence in other islands in Balabac Strait with suitable nesting areas.

In 2006, the Balabac Island Group was identified as a Key Biodiversity Area (CI-P et al. 2006). As a conservation action, CI-P implemented the Sulu Sulawesi Seascape (SSS) Project, which aimed to manage the resources of Sulu and Sulawesi Seas in partnership with local stakeholders. One of the key project sites of the SSS is the Balabac Strait Marine Biodiversity Conservation Corridor (MBCC). During this project, several surveys were conducted to assess the marine biodiversity of Balabac Strait MBCC. In the May 2006 survey by Matillano et al. (2007), Camiaran Island was reported to have high nesting incidence of sea turtles. Five months later, Ramoso et al. (2006) reported that Balabac is one of the areas in the SSS that acts as a critical marine turtle habitat, with certain islands (Candaraman, Sicsican, Secam, and Camiaran Islands) confirmed to be sea turtle nesting areas. Two marine turtle species, the green sea turtle *Chelonia mydas* and the hawksbill sea turtle *E. imbricata* in their different life stages, were identified and confirmed inhabiting the seas around Balabac Island Group. Ramoso et al. (2006) further identified several threats to sea turtle populations in Balabac Strait, citing occasional butchering for local consumption in traditional festivities, intermittent poaching by foreign nationals and collection of sea turtle eggs.

It is important to note that there are four types of sea turtle habitats within Balabac Island and its vicinities, which include: (1) pelagic sites as migratory corridors (2) coralline beaches for nesting, (3) lagoons and (4) seagrass beds for juvenile development and foraging grounds for adults.

Except for the above-mentioned information, virtually nothing else is known about the sea turtles of Balabac Strait MBCC, particularly on aspects of trade dynamics and exploitation rate. The availability of such information especially on nesting incidence is significant for conservation since Balabac Strait MBCC may be one of the last strongholds of sea turtles in the Philippines. This information can be used as basis in designing conservation strategies and protection measures for sea turtle species in the Balabac Strait MBCC.

Hence, the study aimed to determine the (1) nesting incidence of sea turtles and threats to nesting habitats; (2) local knowledge on nesting areas and nesting season, and (3) exploitation and trade dynamics of sea turtles in Balabac Strait MBCC.

MATERIALS AND METHODS

The study was conducted in selected islands and barangays within Balabac Strait MBCC (Figure 1). Nesting incidents were monitored only in Camiaran and Roughton Islands. The nesting beach in each island was divided into four sectors (north, south, east and west sectors) and was patrolled every sampling event to account for and determine the number of new nests, and to identify the species of nesters. Identification of species was based on the nesting behavior of sea turtles and track marks. To determine nesting preference of sea turtles, the nesting beach in Camiaran was divided into three different habitat zones following Phillips (1992) namely beach forest (consisting primarily of woody and *Pandan* plants), shoreline vegetation (transitional area between the beach forest and open beach composed mostly of small shrubs and salt tolerant vines) and open beach (characterized mostly by open sand area with no vegetation).

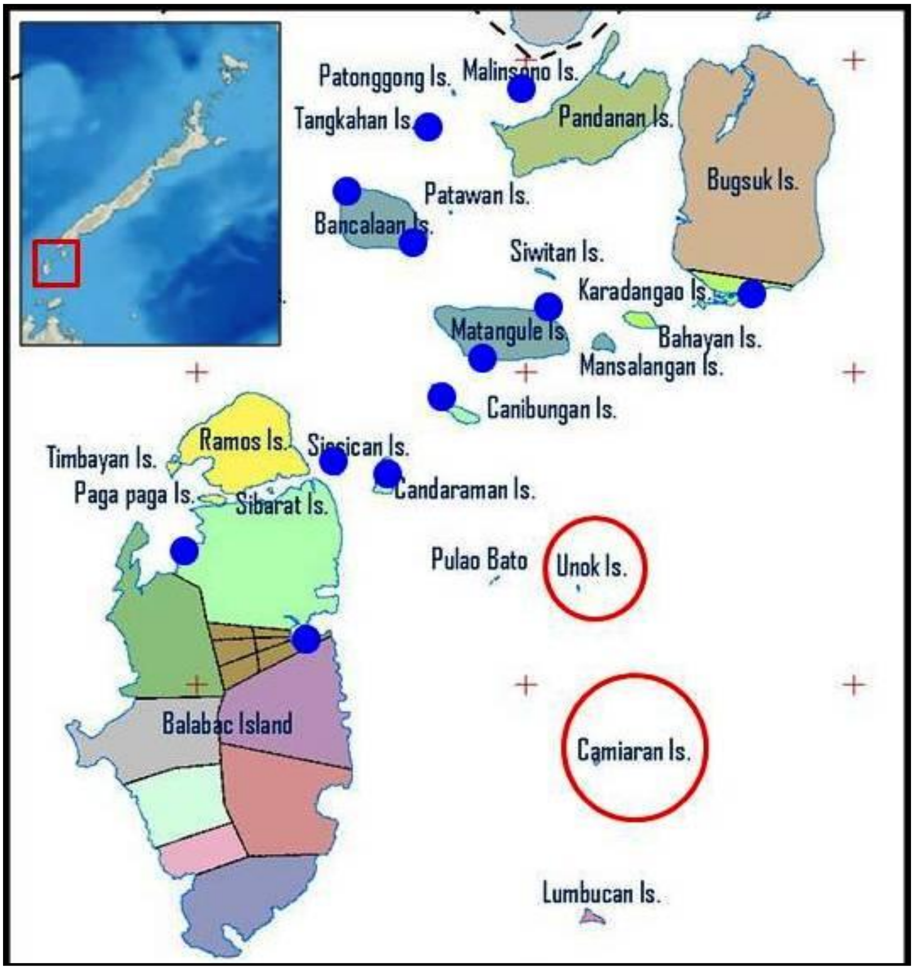

Figure 1. The Balabac Strait Marine Biodiversity Conservation Corridor (red square, top left) and enlarged map of Balabac Strait MBCC showing the two sampling sites (Unok or Roughton and Camiaran Islands) for nesting incidence (red circles) and the islands and barangay (village) visited for field interview (blue circles). (Source: Conservation International (CI); Sulu-Sulawesi Seascape Project and Microsoft Encarta 2006).

Sampling was conducted in July and November 2007. Key informant interviews (KII) with 18 respondents were conducted in May 2008 using structured questionnaire (modified after Pawikan Conservation Program) to gather information on nesting areas, nesting season, trade dynamics, species of sea turtles traded, catch and collection rate of sea turtles and its derivatives. Respondents included locals from selected islands and barangays (villages) of Balabac Island Group. For KIIs, the researchers selected the permanent residents of Balabac with knowledge about sea turtle traders.

RESULTS AND DISCUSSION

Nesting Incidence

Two species of sea turtles were encountered during the surveys, the green sea turtle *C. mydas* and the hawksbill sea turtle *E. imbricata* (Figure 2). During the two survey events, green sea turtles were the most frequently encountered species, especially in Roughton (Unok) Island where they were commonly seen feeding in the shallow seagrass meadows.

Figure 2. The green sea turtle (left) and hawksbill sea turtle (right). The hawksbill sea turtle was rescued from would be butchers at a cost of PhP 250.00 during one of the sampling events. (Photo: R. Antonio).

Nesting incidence surveys at Camiaran and Roughton Islands were conducted from 30 July to 01 August 2007 and 23-24 November 2007 (Table 1). In Camiaran Island, the first survey conducted in July 2007 recorded higher nesting incidence compared to the November sampling. In July, a total number of 60 nests for Camiaran Island; 40 nests in Sector 1 (eastern coast of the island), 11 for Sector 2 (southern coast), and nine in sector 3 (western coast) were encountered (Table1). Apparently, the northern coast of the island was not used as nesting site since the slope was too steep for sea turtles to climb. In November 2007 sampling, the survey team recorded only 35 nests. Thirteen were in sector 1, eight in sector 2 and 14 in sector 3.

Out of the 95 nests recorded in Camiaran Island, 94 nests belonged to green sea turtles while only one was a Hawksbill sea turtle nest. Of these, 12 nests were active, all of which were of green sea turtles. The single nest of hawksbill sea turtle was dug by fishermen who regularly visited the island. Apparently, the eggs were collected for consumption.

Table 1. Total nesting incidence of sea turtles in Camiaran Island during the two sampling periods.

Sampling Period	Location	Species	Nesting Activity	Total No. of Nest(s)	Total Active Nest(s)
First Sampling (30 July to 01 August 2007)	Sector 1	*C. mydas*	Complete	39	3
		E. imbricata	Complete	1	0
	Sector 2	*C. mydas*	Complete	11	4
		E. imbricata	None	0	0
	Sector 3	*C. mydas*	Complete	9	0
		E. imbricata	False nest	0	0
Total				60	7
Second Sampling (23-24 November 2007)	Sector 1	*C. mydas*	Complete	13	2
		E. imbricata	None	0	0
	Sector 2	*C. mydas*	Complete	8	2
		E. imbricata	None	0	0
	Sector 3	*C. mydas*	Complete	14	1
		E. imbricata	None	0	0
Total				35	5
Grand Total				95	12

In Roughton, 12 nests were recorded in July 2007 while in November, only five nests were recorded. No active nests were documented out of the 17 nests encountered. Two of the 17 nests belonged to hawksbill sea turtles while the remaining 15 were of green sea turtles (Table 2). In the course of interview with the island's caretakers, it revealed that the residents immediately collect the eggs in the morning after they are laid, hence there were no active nests recorded.

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Table 2. Total nesting incidence of sea turtles in Roughton Island during the two sampling events.

Sampling Period	Location	Species	Nesting Activity	No. of Nest(s)	No. of Active Nest(s)
First Sampling (30 July to 01 Aug. 2007)	Western Side of the Island	*C. mydas*	Complete	10	0
		E. imbricata	Complete	2	0
Total				**12**	**0**
Second Sampling (23-24 Nov. 2007)	Western Side of the Island	*C. mydas*	Complete	5	0
		E. imbricata	None	0	0
Total				**5**	**0**
Grand Total				**17**	**0**

Of the three nesting habitats, the shoreline vegetation was the most preferred nesting area of sea turtles in Camiaran Island, with a total nest count of 58 (41 during the first sampling and 17 for the second sampling). Thirty nests were recorded in beach forest and eight nests in open beaches. These results suggest that sea turtles prefer to nest in shoreline vegetation in Camiaran. This is maybe because the beach forest of Camiaran Island is elevated, while the open sandy areas were shifting due to constant beach erosion, which makes the nest vulnerable to flooding during high tide. In Setiu, Terengganu, Malaysia, majority of green sea turtles nest in open areas and dunes which provide less hindrance from the vegetation and grassy zone. The dunes are also usually located in flat areas, which are more convenient for digging body pits than in sloping areas (Abd Mutalib et al. 2014).

Threats to Nesting Habitats

The two survey events revealed drastic changes in nesting areas of Camiaran Island. Beach forest conversion is continuous as documented by Matillano et al. (2006). Coconut trees were planted to replace the denuded beach forest. Beach erosion was observed in Sectors 1 and 3 in Camiaran. These sand shifting patterns occur regularly as changes in monsoon winds and wave and current actions erode sand back and forth the northeastern and southwestern coasts of the island. However, the removal of beach forest in this island may aggravate sand shifting patterns as evident on the northeastern portion of Camiaran. Beach conditions, seasons, air temperature and amount of rain are known to influence turtle nesting activities (see Abd Mutalib et al. 2014). In addition to this, drifted garbage from neighboring populated islands including drifted nylon ropes were documented during the second sampling. Plastic cellophane wastes were also common in many areas within Balabac Strait, particularly in South Mangsee generally in the vicinities of highly populated islands. These kinds of waste had long been identified as hazardous for sea turtles as these could be mistaken as food.

Local Knowledge on Nesting Areas, Nesting Season and Trade

Most (83%) of the respondents cited Secam Island as the primary turtle nesting area within Balabac Strait MBCC which corroborate with the report of Ramoso et al. (2006). This was followed by Candaraman, Roughton, Sicsican, Camiaran Island, Patongong Island and Bgy. Sebaring (Table 3).

Table 3. Identified nesting areas and observed nesting seasons within the Balabac Strait MBCC.

Site	Green Sea Turtle	Hawksbill Sea Turtle	Remarks
Bgy. Sebaring	No Information	No Information	Locals stated that turtles nest throughout the year
Camiaran Island	July to November	July to August	High nesting incidence recorded in July 2007
Candaraman Island	June to August	No information	Many sea turtles forage in seagrass meadows in shallow areas of the island
Roughton Island	January to April	January to April	Sea turtles were frequently sighted feeding in seagrass beds around the island.
Sicsican	No information	No information	Locals reported irregular nesting activity in this island.
Patonggong Island	April to June	No information	Locals claimed that many sea turtles were sighted around the island, with high incidence of nesting during northeast monsoon.
Secam Island	No Information	No information	Locals claimed that nesting activity is throughout the year.

For the nesting season, most of the sightings of nesting females were in the months of June to August in Candaraman Island. For other islands however, the respondents provided varying information usually stating that there is no specific month for nesting (Table 3). Interestingly, majority (88%) agreed that there is a pronounced nesting incidence during the northeast monsoon (November to February). The information on nesting season obtained in this study appeared similar to Setiu, Terengganu, Malaysia which occurred from February until September. However, the peak in Setiu occurs between April and August (Abd Mutalib et al. 2014).

Exploitation and Trade Dynamics

Trade (Figure 3) and consumption of sea turtle and its derivatives (eggs, carapace, scutes, and raw meat) persisted within the Balabac Strait MBCC, most especially in the town proper and other remote barangays of the municipality (at least eggs and meat) at the time of this study. The green sea turtles are exploited primarily for their meat and cartilage, and the hawksbill sea turtles are mainly taken for its carapace which is used to make a variety of turtle by-products (e.g. jewelry and combs).

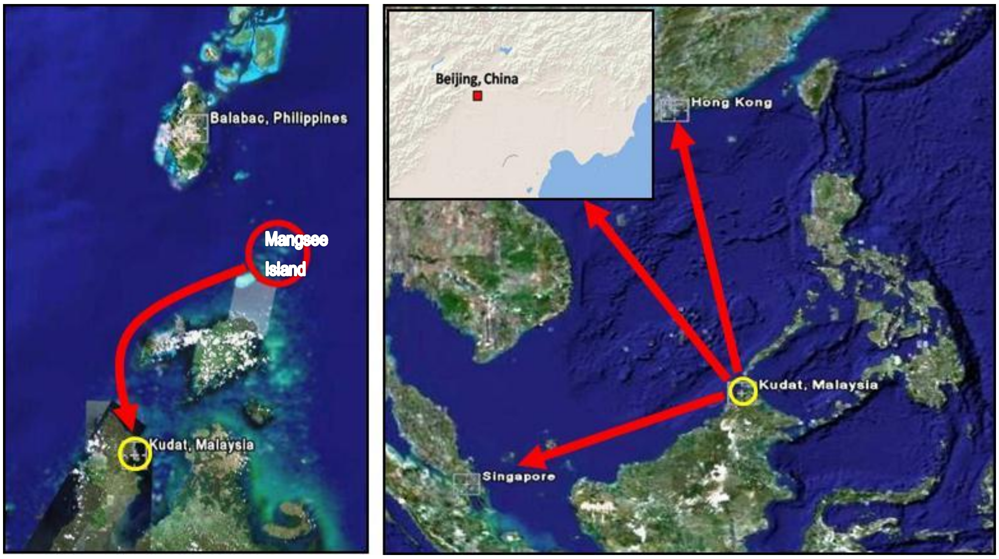

Figure 3. Trade routes of live sea turtles from Mangsee Island (left) to the international market (right). (Map Source: Google Earth 2008 and Microsoft Encarta 2007).

Informants confirmed that live animals were collected in the vicinities of Mangsee Islands, Salinsingan Island, and Simanahan Reef. Gill nets and spear gun were the most common gears used to capture sea turtles within the reef area or seagrass beds. At times, individuals were captured right after laying eggs. Captured sea turtles were then transported from Mangsee Island to Kudat, Malaysia where sea turtle traders kept the animals in pens/net cages for at least a month until there were enough animals (approximately 50 individuals) for one shipment. Reports indicated that Kudat acts as stocking area and transshipment point of sea turtles collected in the waters of Balabac Strait MBCC. By the time when enough numbers of turtles were ready for shipment, the traders transported the animals from

Kudat to Singapore, Hong Kong and mainland China via Chinese fishing vessels (Figure 3).

The individual price of sea turtles depends on size. Turtle traders classify sea turtle size as small (dinner plate size), medium (±50 cm long), and large (>90 cm). For small sea turtles, the traders paid the poachers PhP 200.00, while medium sized costs about PhP 500.00 at the time of this study. A large individual commanded a prize of not lower than PhP 2,500.00. In 2009, the exchange rate between US Dollar and Philippine Peso was 1:46.

Trade of Sea Turtle Derivatives

Eggs. For years, sea turtle's eggs in Balabac Strait MBCC were collected by local fishermen and coastal inhabitants as alternative source of protein, either by just directly taking the eggs from the nests or immediately collecting when the nesters deposit their eggs. Regardless of knowing that it is illegal to collect sea turtle eggs, continuous harvesting still occurs within Balabac Strait MBCC. Data gathered through interviews indicated that there were six islands and one mainland Balabac barangay which serve as the main source of sea turtle eggs that are traded within the area. These are Secam, Sicsican, Candaraman, Roughton, Camiaran, Lumbucan and Bgy. Sebaring.

Collected eggs were sold to pre-contacted buyers from the town proper of Balabac between PhP2.00 and PhP 5.00/egg for green sea turtles eggs and PhP 1.50 per hawksbill sea turtle egg. These buyers then retailed the eggs at the town proper of Balabac at PhP 20.00 for three eggs or usually at PhP 5.00/egg. However, depending on the season and the number bought, the seller might sell for a discounted price of PhP 80.00/150 eggs (Table 4).

Table 4. Collection area, trading hotspots, and prices of sea turtle eggs within Balabac Corridor as of May 2008.

Species	Collection Area/Source	Price of Eggs at source(PhP)	Retail price (PhP)	Trading Area
C. mydas	Secam, Sicsican, Candaraman, Camiaran, Lumbucan Island and Sebaring	2.00 to 5.00 each	3.00 to 5.00 each	Town proper, Bgy. Caguisan and Bgy. Bangkalaan
E. imbricata	Camiaran, Candaraman, and Bgy. Sebaring	1.50 each	4.75 each	Town proper and Bgy. Bangkalaan

The sea turtle eggs that have been collected in Secam Island and Bgy. Sebaring are usually sold in Bgy. Bangkalaan. Newly collected sea turtles eggs from Bgy. Sebaring were found for sale in the island during the last survey, involving both green and hawksbill sea turtle's eggs (Figure 4). Green sea turtle's eggs are commonly sold at a price of PhP 5.00/egg or 4 eggs/20 pesos. Hawksbill sea turtle's eggs are cheaper, sold at PhP 4.75/egg or 5 eggs for PhP 20.00. Traders reported that the eggs are always out of stock, suggesting a very high demand in the area. On the contrary, eggs that have been collected from Roughton Island are mainly intended for consumption by the island's care takers and owner.

Figure 4. Eggs of green (left) and hawksbill (right) sea turtles displayed for sale in Bgy. Bangkalaan, Balabac, Palawan. (Photo: R. Antonio).

It is worth noting that in some areas in Balabac Strait MBCC, collection of sea turtle eggs is more of a daily activity, as in the case of Roughton Island where there are permanent residents. The egg collectors would regularly patrol known nesting beaches and collect the eggs from all nests that they can find. Regardless whether there are only two or ten nests, it seems that the general rule is to immediately collect the eggs, otherwise, other people may find the nest and collect them before one could go back to these active nests. In Camiaran Island however, collection of eggs is usually daily if there are transient fishers that stay for extended period of time, however, in times when fishing is off season, particularly during high monsoon winds, some of the nests survive.

An informant from Bgy. IV reported that the volume of eggs being traded in town proper ranged between 450 and 600 eggs per transaction in 2008, which is usually three times a month. A third of the eggs were supplied by collectors from Sicsican Island while the rest were mainly from collectors in Secam Island.

The sources of eggs sold in Bgy. Bangkalaan are mainly from Secam Island and Bgy. Sebaring. Interviewed egg vendor from Bangkalaan Centro stated that three times a month, an egg collector from Bgy. Sebaring delivered about 500 sea turtle eggs consisted of both green and hawksbill sea turtles. In Sitio Tabudniyayo (part of Bgy. Bangkalaan) about 400 eggs were sold, usually on a monthly basis.

Considering the information gathered, the projected local consumption of turtle eggs in the town proper is from 1,350 to1,800 eggs per month (16,200 to 21,600 eggs per year). In Bgy. Bangkalaan, it ranged between 1,200 and1,680 eggs per month or 14,400- and 20,160 eggs per year (Table 5). This projection only takes into account the volume of eggs for sale and not including those that are collected for household consumption. This projection also did not take into account the seasonality of egg exploitation, i.e. whether there are increases or decreases in the volume of eggs traded in relation to nesting season.

Table 5. Volume of sea turtle eggs traded in the identified areas of the Balabac Strait MBCC.

Species	**Collection Area/Source**	**Market Site**	**Volume of Eggs (pieces)**		
			Per Transaction	**Per Month**	**Annual Projection**
C. mydas	Secam, Sicsican, Candaraman, Roughton, Camiaran, Lumbucan Island and Bgy. Sebaring	Town Proper	450-600	1,350–1,800	16,200-21,600
		Bangkalaan	400-500	1,200-1,500	14,400-18,000
Subtotal			950-1,100	1,550-2,300	18,600-27,600
E. imbricata	Camiaran, Candaraman, Roughton, and Bgy. Sebaring	Town Proper	100-200	300-600	3,600-7,200
		Bangkalaan	100-150	300-450	3,600-5,400
Subtotal			200-250	600-1,050	7,200-12,600
Total			**1,150-1,350**	**2,150-3,350**	25,800-40,200

Scutes. Sea turtle scutes are also a hot commodity in Balabac Strait MBCC. Scutes of hawksbill sea turtles are important for the curio trade (i.e. for making jewelries and combs) and are obtained in a very brutal way. Most scutes collectors do not actually kill the turtle during the process of scutes removal. It takes about an hour to remove complete sets of scutes (13 scutes: 8 marginal and 5 central) from one turtle but not without subjecting the carapace to heat first. Another way of removing the scutes is by grilling the live turtle while lying on its back until the entire carapacial scutes pop up completely. After then, the alive but badly burned turtle is returned back into the sea, where it will eventually die due to the wounds inflicted on it. This method has been previously documented by Van Dijk and Shepherd (2004). The poachers usually return the dying animals to the sea after removing the scutes, in the vain and false hope that it would survive and re-grow its scutes (Van Dijk and Shepherd 2004). These activities were reported not only in Mangsee Island but also in other parts of Balabac Strait particularly in Sitio Palaisan and Sitio Lumbucan. Like live individuals, a complete set of sea turtle scutes (Figure 5) is also categorized as small, medium, and large. A small set of scutes cost PhP 600.00, while medium set sold for PhP 900.00. Large sets command a higher price of PhP 1,500.00 (Figure 5).

Figure 5. A carapace of green sea turtle (Curve Carapace Length: 52 cm) collected from Malinsuno Island in May 2008. This carapace cost the researcher PhP 120.00 (left). To the right are fragments of carapacial scutes of hawksbill sea turtles from Canibungan Island.

Respondents from Malinsuno Island stated that sometime in 2007, about 50 dead hawksbill sea turtles were found drifting in the open water of the northwestern part of the Balabac Strait MBCC. Most of the dead turtles showed evidence of being subjected to excessive heat notably with no carapacial scutes left. The locals claimed that this high concentration of dead turtles came from the fishing vessel of Chinese fishermen which were frequently sighted poaching in the municipal waters of Balabac Strait. It is also worth noting that on April 13, 2008 one of the two Chinese fishing vessels (Quang Mei) sighted near Lumbucan Island was apprehended by local law enforcers. Sea turtles were found in the fishing boat (Anda 2008).

Meat. The meat of both species of sea turtles is utilized in Balabac Strait MBCC. It is a delicacy especially in some religious and traditional festivities within the corridor. This practice is also common in other areas of the Sulu Sea. Based on interviews, four primary sites were identified and confirmed to have slaughtering activities of sea turtles namely Sitio Palaisan, Sitio Malinsuno, Sitio Mariahangin, and Bgy. Sebaring. The turtles were slaughtered either for everyday consumption or for trading purposes but were more commonly butchered during celebrations. Trading of sea turtle meat was more commonly reported in Sitio Palaisan where residents would even sell sea turtle meat at the town proper of Balabac. Sea turtle meat was also available on order basis especially during Christmas season. The local price of sea turtle meat in the town proper of Balabac was PhP 20.00/caltex (about 750 ml). For meat consumers who prefer whole animals, turtle hunters sold a medium sized turtle at around PhP 200.00-250.00. The turtles were made available upon request or placement of orders.

In Sitio Malinsuno, sea turtles were slaughtered either for house hold consumptions or even just as "pulutan" or appetizer during liquor drinking sessions. The first author was able to document one such event during the field survey conducted in May 2008 in Malinsuno, involving a hawksbill sea turtle. The turtle was rescued by the researcher after paying PhP 250.00 for the animal. Respondents from Malinsuno Island also cited that at least ten sea turtles are being slaughtered in Sitio Mariahangin every year during foundation day celebration (June 27th). Interestingly, respondents also claimed that sea turtle meat is more preferred than beef. It was also evident that the locals are the regular hunters and consumers of sea turtle meat in the Balabac Island Group.

CONCLUSION AND RECOMMENDATIONS

All five marine turtle species found in the Philippines (Marine Wildlife Watch of the Philippines 2014) are protected under the Republic Act 9147 otherwise known as Wildlife Protection Act of the Philippines. Under the Wildlife Act, jurisdiction over marine turtles and many other terrestrial and marine flora and fauna is under the DENR and the Palawan Council for Sustainable Development (PCSD) (Marine Wildlife Watch of the Philippines 2014). The two species encountered in this study are in the IUCN Red List with *E. imbricata* categorized as "Critically Endangered" and *C. mydas* categorized as "Endangered" (IUCN 2016).

The recorded nesting incidence in this study Balabac implied the importance of Balabac Strait MBCC as conservation spot for sea turtles not only as foraging area or migratory route from the Sulu Sea but Balabac Strait MBCC also serve as important nesting area of these two globally threatened marine species.

So far, five islands and one island Barangay were identified as important for conservation of the sea turtles within the Balabac Strait MBCC namely; Camiaran Island, Roughton Island, Secam Island, Sicsican Island, Candaraman Island, and Bgy. Sebaring. These islands serve as major nesting sites for sea turtles in the corridor.

For nesting incidence, Camiaran Island have considerably higher occurrence of nesting compared to Roughton Island, however, for other islands, the researcher still has no idea of relative occurrence of nesting. Though the three surveys were not able to document or deduce nesting season, local information suggests a year round nesting incidence, with pronounced frequency during northeast monsoon season.

There is still rampant and continuous hunting of sea turtles within Balabac Strait by foreign poachers and local community members, which may cause significant population decline of sea turtles in the corridor. Egg collection may also significantly cause further population decline as it interferes with recruitment.

Results of the interviews revealed local awareness of laws regarding conservation of sea turtles, however, in the absence of law enforcers, the locals took advantage of this situation, particularly that even in the municipal proper, there seems to be no prosecution of traders who sell turtle meat and eggs. Hence, butchering and collection of sea turtle eggs are still proliferating in the Balabac Strait MBCC.

Marine turtle conservation actions are strongly recommended within the Balabac Strait MBCC. The Balabac LGU under the Municipal Agriculture Office and the DENR officer should conduct regular monitoring of the identified nesting areas. Secondly, it is also recommended that concerned agencies within the corridor regularly check the known egg vendors within the town proper to curb incidents of egg trading. Community managed hatcheries maybe established in several islands to protect the sea turtle eggs from poachers. Marine turtle eggs when transferred to hatcheries have low predation but high hatching rates (Abd Mutalib abd and Fadzly 2015).

Should there be a follow up study on nesting incidence in the area, this should be conducted by someone who resides in Balabac Island at least during the entire duration of the research. This is to minimize delays caused by travel from Puerto Princesa to Balabac, which is often hampered by unfavorable weather conditions. The researcher should be based in Balabac to take advantage of prevailing good weather conditions to sneak into the targeted islands during calm days, making it more plausible to monitor nesting incidence at least once a month for each targeted nesting area. Nesting incidence monitoring should also include the other identified nesting areas within the corridor. For trade related studies, it is strongly recommended to conduct undercover visits to maximize trade data gathering since information is usually withheld by respondents.

Lastly, it is recommended that more information and education campaign activities be conducted to integrate conservation values to the local community for them to support conservation initiatives for sea turtles and other threatened species within the Balabac Strait MBCC.

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Antibacterial potential of crude extracts from sea cucumber *Holothuria fuscoscinerea* Jaeger, 1833

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ABSTRACT

This study was conducted to determine the antibacterial potential of the crude extracts of skin, gonad, Cuvierian tubules, Polian vesicles and intestine of the sea cucumber *Holothuria fuscoscinerea* collected from Rasa Island, Narra, Palawan, Philippines. The antibacterial potential of the extracts was determined against *Escherichia coli* and *Staphylococcus aureus* using filter paper disc diffusion method with Tetracycline as the positive control and distilled water as the negative control. The analysis of variance (ANOVA) of the results showed significant differences in the effects of the treatments when tested against *S. aureus* and *E. coli* ($p<0.05$). Tukey's test further proved that Polian vesicle was significantly highest in terms of antibacterial property among other extracts but not comparable to positive control against *S. aureus*. On the other hand, Tukey's test showed that Cuvierian tubules and Polian vesicles were not significantly different from each other in terms of antibacterial effect but not comparable to tetracycline when tested against *E. coli*. The extracts from skin, gonad and intestine did not show inhibitory effect on the test organisms. T-test showed that *E. coli* and *S. aureus* were not significantly different in terms of susceptibility towards the treatments. Based on the results, extracts from Cuvierian tubules and Polian vesicles of *H. fuscoscinerea* are potential sources of antibacterial compounds.

Keywords: sea cucumber, *Holothuria fuscoscinerea,* antibacterial potential, Cuvierian tubules, Polian vesicles

INTRODUCTION

Bacteria are one of the oldest and simplest organisms but they are more diverse than all other life-forms combined (Stainer et al. 1979). Bacteria are ubiquitous on earth as they play vital roles in many ecosystems. On the other hand, bacteria are also responsible for many serious human diseases that sometimes cause millions of deaths each year (Mitchel 1974). They are also causing problems in aquaculture where infectious diseases

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are always a hazard and may cause significant stock losses and problems in animal welfare (Romero et al. 2012).

Aquaculture is becoming a more concentrated industry in the country. It is still a fast-growing food production sector because it is an increasingly important source of protein for human consumption (Rodgers and Furones 2009). The Food and Agriculture Organization of the United Nation (UN FAO) estimates that half of the world's seafood demands will be met by aquaculture in 2020 (Moriarty 1999). In spite of this, aquaculture faces many problems that may hinder its increasing production. Diseases caused by bacteria and parasites are major factors that influence the production and quality of stocks (Frappado and Guest 1986). To counteract this problem, aquaculture industries use antibiotics to combat these fish diseases and parasites. Administration of low dose of antibiotics for growth promotion has also led to the increased use of antibiotics in aquaculture. However, the potential consequences of antibiotics use are the development of antibiotic-resistant microorganisms, multiple antibiotic resistance, and resistance transfer to pathogenic bacteria and reduced efficacy of antibiotic treatment for diseases caused by resistant pathogens (Khan 2008).

Antibiotic resistance of bacteria has led to the search for potential sources of bioactive compounds with antimicrobial properties. Some marine invertebrates successfully thrive in environments full of pathogenic microorganisms which develop their defense mechanisms and have made them prime candidates for extraction of antimicrobial compounds (Mydlarz et al. 2006). Sea cucumbers are presented as potential marine sources of antimicrobial compounds (Mokhlesi et al. 2012). They can be found throughout the world and have been used medicinally in Asian countries for years (Boadbar et al. 2011). The bioactive compounds in sea cucumbers make it a potent therapeutic food source. This echinoderm lives in the benthic areas and is a treasure chest of many vitamins and minerals, and contains anti- inflammatory, antimicrobial, antioxidant and anti- angiogenic properties (Mokhlesi et al. 2012). Some species of Family Holothuriidae exhibit antimicrobial activity like *Holothuria atra* (Boadbar et al. 2011), *H. leucospilota* (Mokhlesi et al. 2012), and *H. scabra* (Althunibat et al. 2009).

Holothuria fuscoscinerea is one among the sea cucumber species found in the Philippines (Kerr et al. 2006). It is usually found under rubble on the reef flat during the day and venturing out only at night. This species grows up to 30 cm long (Purcell et al. 2012). It readily releases large translucent Cuvierian tubules when disturbed (Kerr et al. 2006). However, this species has low commercial value in Western Central Pacific, though it is of commercial importance in China, Malaysia and Philippines (Purcell et al. 2012). According to Schoppe (2000), *H. fuscoscinerea* has a value of about PhP55 kg^{-1} or 1.38 US$ kg^{-1}. Since *H. fuscoscinerea* occurs in Rasa Island

Wildlife Sanctuary in Narra, Palawan, Philippines, looking for other potential use of this organism such as antibacterial resource is deemed important.

This study aimed to determine the antibacterial potential of crude extracts from sea cucumber *Holothuria fuscocinerea*. Specifically, this study aimed to (1) determine the antibacterial effect of the crude extracts from gonad, intestine, Cuvierian tubules, Polian vesicle and skin of the sea cucumber against *Escherichia coli* and *Staphylococcus aureus*; (2) if antibacterial effect is present, determine which of the five extracts (gonad, intestine, Cuvierian tubules, Polian vesicle, skin) of sea cucumber would show the highest antibacterial potential and (3) determine which of the two test bacteria (*Escherichia coli* and *Staphylococcus aureus*) is more susceptible to the treatments if antibacterial potential is present.

MATERIALS AND METHODS

Sample Collection and Locale of the Study

This study was conducted from October to November 2015. Samples of *H. fuscoscinerea* were collected from Rasa Island, Narra, Palawan (Figure 1). The Island is characterized by having coastal forest, mangrove area, coconut plantation, barren areas and coral outcrops. Samples were collected during low tide in the reef areas. The collected samples were cleaned by rinsing with seawater, placed in a cooler box (with seawater) and immediately transported to the laboratory for analyses.

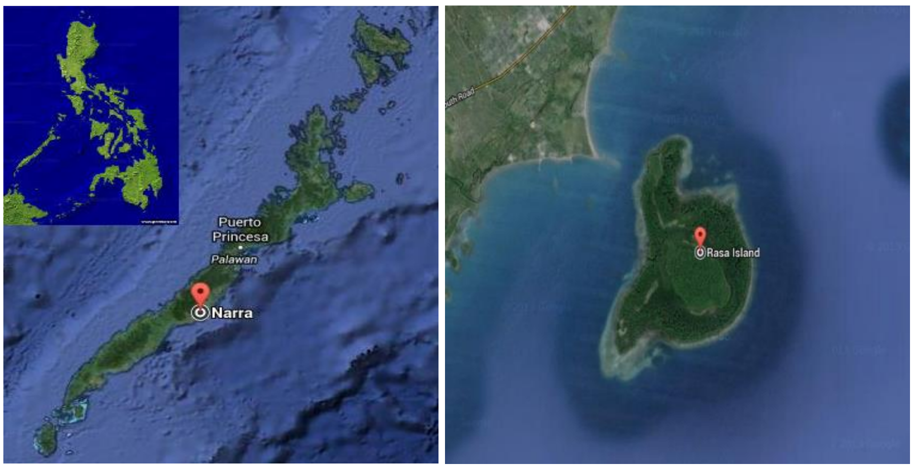

Figure 1. Map of Palawan, Philippines (left); location of Rasa Island, Narra, Palawan, Philippines (right) (Source: Google map).

The intestine, gonads, Cuvierian tubules, Polian vesicles and skin were separated in the Microbiology Laboratory Room of Western Philippines University - Puerto Princesa Campus, Sta. Monica, Puerto Princesa City. These were placed in separated pre-labeled sterile containers and extraction immediately followed.

Inducement of Cuvierian Tubules

The expulsion of Cuvierian tubules was induced mechanically by pinching the dorsal integument of the sea cucumber with forceps. Five individuals were stimulated twice to expel about 10 tubules (Vandenspiegel et al. 1999). After tubule expulsion, Cuvierian tubules were placed in a sterile pre-labelled container.

Culture Media Preparation

The nutrient agar was prepared by dissolving 23 g of nutrient agar in 1000 ml distilled water while the nutrient broth was prepared by dissolving 13 g of nutrient broth in 1000 ml distilled water as prescribed by the manufacturers. Both preparations were done in Erlenmeyer Flasks aided by hot water bath to hasten the dissolution with constant stirring until the liquid became transparent. Sterilization at 121°C, 15 psi for 15 minutes then followed (Mabuhay 2015).

Preparation of Microorganisms

Pure cultures of *E. coli* and *S. aureus* were obtained from the culture collection of DOST-Palawan, Sta. Monica, Puerto Princesa City, Palawan. These were the bacteria used in the study because they were readily available to represent the two physiologic groups of bacteria, the Gram-positive and the Gram-negative bacteria.

Extraction of Samples

Each of the samples of intestines, gonads, Cuvierian tubules, Polian vesicles and skin was soaked in 10 ml distilled water for 3 min and then the water was discarded. This procedure was repeated five times to remove the salt content of the samples. After soaking, the samples were crushed separately using a mortar and pestle with the ratio of 4 g of sample is to 1 ml distilled water. Four replicates were prepared for each sample. The extracted samples were then placed in pre-labelled sterile containers. The samples were stored at refrigerator for at 0°C until usage (Mokhlesi et al. 2012).

Antibacterial Assay

The filter paper disc diffusion method was used to determine the antibacterial potential of the extracts from sea cucumber. For the positive control, a 500 mg Tetracycline, which was dissolved in 10 ml sterile distilled water, was used. Tetracycline is one of the popular antibiotics, which are most extensively used in aquaculture (Neela et al. 2008). On the other hand, 10 ml sterile distilled water was used as the negative control. The treatment designations were the following; T1 (Gonad), T2 (Skin), T3 (Cuvierian Tubules), T4 (Polian vesicle), T5 (Intestine), T6 (Positive control) and T7 (Negative control).

A loopful of test organisms, *E. coli* and *S. aureus*, from the subcultures were inoculated into the sterile nutrient agar (20 ml) by direct seeding before pouring it into Petri dishes and allowed to solidify. The previously sterilized filter paper discs (cut by paper puncher to 6mm diameter) were soaked to saturation in different extracts (gonad, intestine, Cuvierian tubules, Polian vesicle, skin) and in the positive and negative control using sterile forceps. These impregnated discs were placed on the designated areas (3 discs on 1 plate) (Figure 2). Four replicates for each treatment were prepared. The Petri plates were incubated for 24 hours to allow bacterial growth. After 24 hours, the plates were examined and zones of inhibition were measured using standardized transparent ruler (in mm).

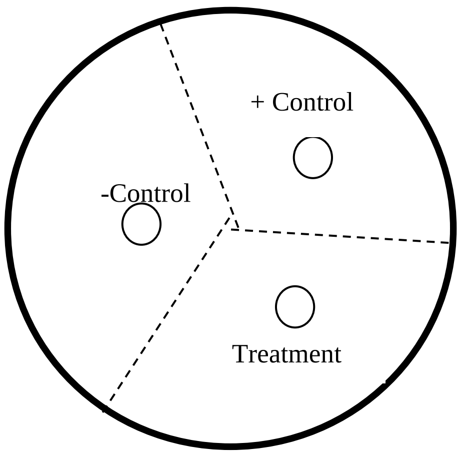

Figure 2. Illustration of antibacterial assay.

Statistical Analyses

The data on the zones of inhibition of the treatments against *Escherichia coli* and *Staphylococcus aureus* were analyzed using one- way analysis of variance (ANOVA) to test the significant differences. The data were subjected to Post Hoc test (Tukey's Test) to compare the means. T-test was used to test the significant difference in the susceptibility between the two test organisms.

RESULTS

Antibacterial Effect of Treatments against *E. coli*

In this study, extracts from the skin, Cuvierian tubules and Polian vesicle showed zones of inhibition when tested against *E. coli* while gonad and intestine did not (Table 1). ANOVA proved that there were significant differences in the effects of the treatments when tested against *E. coli.* Tukey's test showed that the antibacterial property of Cuvierian tubules (Figure 3) and Polian vesicles (Figure 4) did not differ significantly from each other but significantly higher compared to other treatments. However, positive control is still significantly higher compared to Cuvierian tubules and Polian vesicles (Table 1). Although extract from skin showed zones of inhibition in 2 out of 4 replicates against *E. coli*, it was not significantly different from negative control.

Table 1. Mean zones of Inhibition (in mm) of the treatments against *E. coli*, its ANOVA and Tukey's test.

TREATMENTS	**MEAN (mm)**	**Tukey's test (α=0.05)**	**ANOVA**
T1 (Gonad)	**0**	**A**	**F=** 63.49** *p*=**.**000
T2 (Skin)	**6.5**	**A**	
T3 (Cuvierian Tubules)	**18.25**	**B**	
T4 (Polian vesicle)	**21.75**	**B**	
T5 (Intestine)	**0**	**A**	
T6 (Positive control)	**32.95**	**C**	
T7 (Negative control)	**0**	**A**	

**highly significant at α=0.01; Different letters signify significant differences at α=0.05

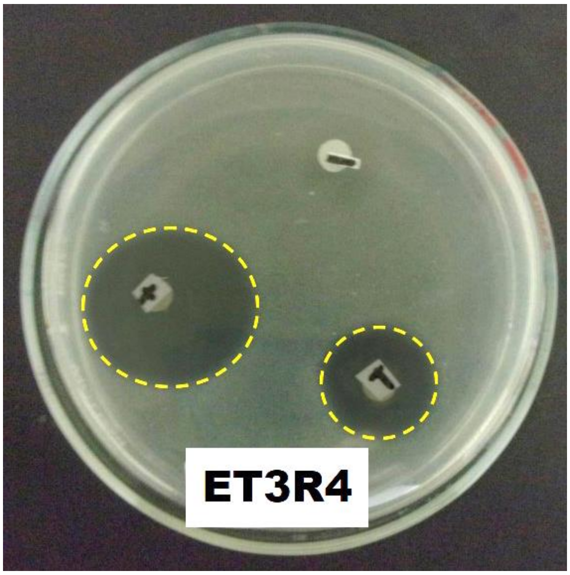

Figure 3. A representative replicate of Cuvierian Tubules extract against *E. coli*. Circles highlight the zones of inhibition. T for treatment, + for positive control, - for negative control.

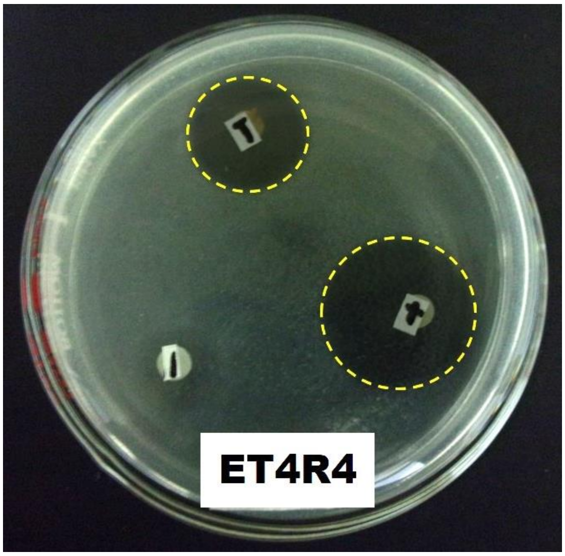

Figure 4. A representative replicate of Polian vesicle extract against *E. coli*. Circles highlight the zones of inhibition. T for treatment, + for positive control, - for negative control.

Antibacterial Effect of Treatments against *S. aureus*

The gonads, Cuvierian tubules and Polian vesicle extracts showed zones of inhibition when tested against *S. aureus* while the skin and intestine did not (Table 2). ANOVA showed that there were significant differences in the effects of the treatments when tested against *S. aureus.* Tukey's test further proved that Polian vesicle (Figure 5) was significantly highest in terms of antibacterial property among other treatments next to positive control as shown in Table 2. Although the extracts of Cuverian tubules and gonad showed zones of inhibition in 2 of 4 replicates against *S. aureus*, they were not significantly different from negative control.

Table 2. Mean zones of inhibition (in mm) of the treatments against *S. aureus*, its ANOVA and Tukey's test.

TREATMENTS	MEAN (mm)	Tukey's test (α=0.05)	ANOVA
T1 (Gonad)	**5.5**	**A**	F= 38.204** *p*=.000
T2 (Skin)	**0**	**A**	
T3 (Cuvierian Tubules)	**5.75**	**A**	
T4 (Polian vesicle)	**16.25**	**B**	
T5 (Intestine)	**0**	**A**	
T6 (Positive control)	**35.15**	**C**	
T7 (Negative control)	**0**	**A**	

** highly significant at α=0.01, Different letters signify significant differences at α=0.05

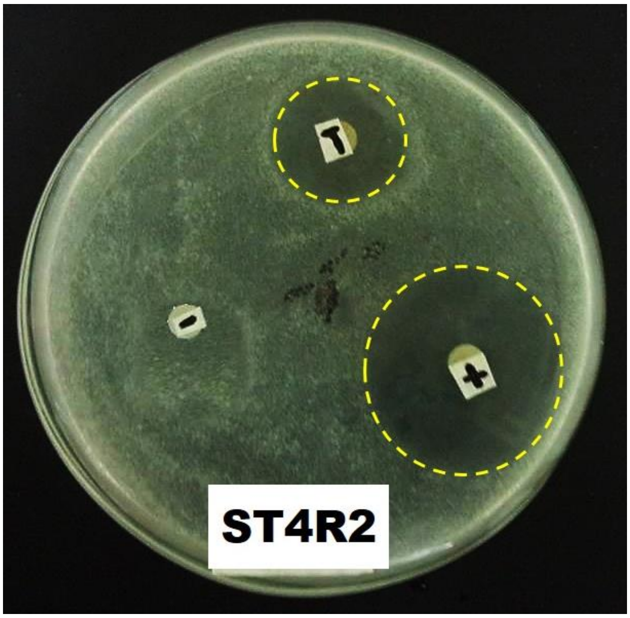

Figure 5. A representative replicate of Polian vesicle extract against *S. aureus.* Circles highlight the zones of inhibition. T for treatment, + for positive control, - for negative control.

Susceptibility of *E. coli* and *S. aureus* towards the Different Treatments

The T-test showed that *E. coli* and *S. aureus* were not significantly different in terms of susceptibility towards the treatments (Table 3).

Table 3. T- test on the susceptibility of *E. coli* and *S. aureus* towards the different treatments.

Pair	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
E. coli – S. aureus	2.40	7.215	1.363	-.397	5.197	1.76	27	.090^{ns}

ns – not significant

DISCUSSION

Among the five extracts tested, Polian vesicles showed the highest inhibitory effects against *E. coli* and *S. aureus*. According to Smith (1978) as cited by Boadbar et al. (2011), Polian vesicles of *Holothuria cinerascens* are known to be the organ attributing inflammatory (including immunologic) receptiveness of this species. Polian vesicles are “muscular” sacs arising interradially from the water ring. They accept excess water-vascular fluid when the animal contracts in an emergency and maintain pressure in the system (Baccetti and Rosati 1968). Echinoderms, like sea cucumbers, are able to give a cell- mediated response against pathogens and parasites (Canicattì et al. 2009). It is due to the presence of humoral substances such as agglutinins, lysins and other compounds that exert antibiotic effects. In some holothurians, Coelomocyte aggregates form around the foreign materials. They are referred to as brown bodies and represent an efficient structure related to the defense mechanism of the host (Canicattì et al. 2009). It is mainly produced in the coelomic cavity but they are also produced by Polian vesicles (Canicattì et al. 2009). Brown bodies from both coelomic cavity and Polian vesicles are composed of phagocytes, cells (as a white blood cell) that engulf and consume foreign material such as microorganisms and debris (Canicattì et al. 2009). They are probably elicited in response to natural invaders.

In this study, Cuvierian tubules extract showed inhibitory effect on *E. coli* but not to *S. aureus*. These results were different from the study conducted by Mokhlesi et al. (2012) wherein Cuvierian extracts of *Holothuria leucospilota* did not show antibacterial activity against *E. coli* and *S. aureus* (Table 4). However, it showed antifungal activity against *Candida albicans* and *Aspergillus niger*. Adhesion plays an important role in many invertebrates for a variety of different functions. Some species of holothuroids possess a special defense system involving adhesion, called the Cuverian tubules, which are mainly activated when mechanically stimulated. These white sticky filaments can entangle and immobilize potential predators (Baranowska et al. 2011). They are present in several species of order Aspidochirotida. Cuverian tubules are attached to the base of the respiratory trees and can be expelled in some *Holothuria* and *Bohadschia* species as a response to irritation (Mokhlesi et al. 2012). Cuverian tubules are made up of 60% protein and 40% carbohydrates. They are considered to be highly insoluble.

Numerous chemical and pharmacological studies were done on several species of sea cucumbers indicated that they contain triterpene glycoside with antifungal, antibacterial and cytotoxic properties (Mokhlesi et al. 2012; Aminin et al. 2015). A lot of these organisms produce their antibacterial factors as a first line of defense against pathogenic microorganisms (Ibrahim 2012). Huag et al. (2002) studied the antibacterial activity of different parts of sea cucumber *Cucumaria frondosa*. They found that antibacterial activity was detected but mainly on coelomycetes and body wall of the organism. According to Layson et al. (2014), *Holothuria nobilis, Bohadschia marmorata* and *Stichopus chloronotus* have antibacterial activity in all extracts (aqueous, chloroform and hexane), except in methanol. The body wall of *Stichopus hermanii* showed high antifungal activity when tested against the fungus *Aspergilus niger* but it did not show antibacterial property against *E. coli* and *S. aureus* (Sarhadizadeh et al. 2014) (Table 4).

Table 4. List of sea cucumber species and their body parts extracts tested against *E. coli* and *S. aureus.*

Sea Cucumber Species	**Author/s**	**Body Parts**	***E. coli***	***S. aureus***
Holothuria. leucospilota	Mokhlesi et al. 2012	Coelomic fluid	-	-
		Cuvierian organ	-	-
		Body wall	-	-
	Ibrahim HA, 2012	Coelomic fluid	-	+
		flesh	-	+
	Shakouri et al. 2014	Body wall	+	+
		Guts	+	+
		White strings	+	+
	Adibpour et al. 2014	Body wall	+	+
		Cuvierian organ	+	+
		Coelomic fluid	+	+
H. scabra	Ibrahim HA, 2012	Coelomic fluid	-	+
		flesh	-	+
H. atra	Ibrahim HA, 2012	Coelomic fluid	-	+
		flesh	-	+
Stichopus hermanni	Sarhadizadeh et al. 2014	Gonad	-	-
		Respiratory tree	-	-
		Cuvierian organ	-	-
		Body wall	-	-

+ = presence of antibacterial property: **-** = absence of antibacterial property

This study proved that extracts from Cuvierian tubules and Polian vesicles of sea cucumber *Holothuria fuscoscinerea* have antibacterial property. This species contains triterpene glycosides (saponin) bioactive compound (Boadbar et al. 2011) which could probably be present in Cuvierian tubules and Polian vesicles which explains their antibacterial effects.

The susceptibility or resistance of the test microorganisms representing two physiologic groups, the Gram positive and Gram negative bacteria, gives a picture of the spectrum of the antibacterial potential of the treatments. Since the two test organisms did not vary in their susceptibility, the antibacterial potential of the treatments, especially Polian vesicle, can be applied to both Gram positive and Gram negative bacteria.

Due to emergence of a number of drug- resistant bacteria, fungi and viruses, extraction of different sea cucumber species and other potential sources of antimicrobial compounds were conducted. *Holothuria fuscoscinerea* is one of the potential species of sea cucumber to be a good source of antibacterial compounds. In this study, its Polian vesicles extract showed highest antibacterial property when tested against *E. coli* and *S. aureus*. Cuvierian tubules extracts showed only antibacterial property against *E. coli*. *S. aureus* and *E. coli* have similar susceptibility towards the treatments. These findings are significant in medical, pharmacological and allied health fields as *Holothuria fuscoscinerea* can be a source of potent antimicrobial drugs within the reach.

With the findings of the study, it is recommended to isolate and identify bioactive compounds from the active extracts (Cuvierian tubules and Polian vesicles) of sea cucumber *Holothuria fuscoscinerea*. Also, a similar study can be conducted using the same species but will be collected from other areas of Palawan and use other parts of the sea cucumber such as Coelomic fluid and respiratory tree for antibacterial property. The antibacterial potential of the sea cucumber extracts was proven in this study through the use of Filter Paper Disc Diffusion Method but is recommended to countercheck results by using other method such as Minimum Inhibitory Concentration and viability assays.

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Research Notes

First record of the elusive Freshwater snapper *Lutjanus fuscescens* (Valenciennes, 1830) in Palawan, Philippines

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The Freshwater snapper (*Lutjanus fuscescens*) is a large species of snapper that inhabits freshwater and brackish water habitats (Martinez-Andrade, 2003). Unlike other snapper species, the complete lifecycle of the species takes place in freshwater habitats. The Freshwater snapper is known to grow up to 40 cm, making it one of the largest species of snapper (Allen 1985), and it is thought to act as a top predator feeding on different smaller fishes. Despite its large size, the species remains understudied and little is known about it. The species is known from Southeast Asia where it has been found in Papua New Guinea, Indonesia, the Solomon Island, Timor-Leste and the Philippines (Miller and Cribb 2007; Polhemus et al. 2008; Larson et al. 2007). Within the Philippines, the species is mainly known from the island of Mindanao, which is in the southeast of the archipelago.

As part of their work for a proposed conservation project in Cleopatra's Needle mountain range, two local biologists trekking upstream along the Langogan River on the northern border of Puerto Princesa City encountered the Batak Tribe on 24 February 2015 with their newly caught fishes from the river. The Langogan River is one of the largest rivers which originated from the Cleopatra's Needle forest (Figure 1) in the north eastern part of Puerto Princesa City (van Beijnen and Jose in press).

Five large fish locally called "Magaragan" caught by the Batak Tribe in Langogan River were noted among others. They described the behavior of the fish as aggressive and none of the hunters dared to spearfish in its known habitat. They explained further that ever since they have not caught this species in the lower downstream of the river.

Figure 1. View of Cleopatra's Needle from the west coast of Palawan (left) and aerial shot of the upper portion of the Langogan River (Photo credits: Jonah van Beijnen).

The species approximately weighs between 5-25 kg and the total lengths ranged between 30 and 90 cm (Figure 2). Besides being a freshwater species, the fish has a truncated caudal fin with a prominent dark spot on the body right below the second dorsal fin, greyish-brown back and side colors, whitish on belly and bisected by lateral line below its anterior soft dorsal rays which are characteristics resembling that of *Lutjanus fuscesens*. The coloration of the freshwater snapper is lighter compared to Mangrove Jack (*Lutjanus argentimaculatus*) which is likewise known to reach lower freshwater habitats (Allen 1985). The identification of the species was further confirmed by fish experts in the Philippines. Lastly the Batak noted that their snapper species catch in this trip were medium sizes, as fully grown individuals can reach the same size as the stretch length of their two arms (approximately 120 – 140 cm), much higher that the reported 40 cm maximum length (Allen 1985). This was an opportunistic observation of the two local biologists so no actual measurements were made and no specimens were collected.

The species status has not yet been assessed by IUCN while there are no official records in the province of Palawan. The fish could be easily overexploited by outsiders because of its limited distribution and large size. Additional threats to the population in Langogan River include illegal gold mining activities in the area and a proposed paved provincial road along the river to cross the island from the east coast to the west coast, which most probably would mean the end of this local fish population. Additionally a hydropower project is proposed at the upstream portion of the Langogan River which needs to be planned well as a recently published study shows

Figure 2. Lateral view of the Freshwater snapper (left) and a local biologist holding an individual which approximately measures 90 cm total length (right).

that about 1/3 of the world's freshwater fish species are under threat due to hydropower development (Nam et al. 2016). Furthermore, there have been unconfirmed reports of Freshwater snapper in the upper portions of the Iwahig River in central Puerto Princesa that should be further investigated to get more clarity on the distribution of the species within Palawan. To further understand its biology and ecology, as well as its economic potential for fisheries in the province, it is highly recommended to obtain specimen/s of the species for further scientific studies and purposes while further researches and assessments of the species in this newly recorded geographical range and in the whole province of Palawan are necessary. Lastly, this new record of the species together with several new other discoveries for the area should provide sufficient arguments to enthuse the local government to provide the whole Cleopatra's Needle Mountain Range with an official protective status.

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ABSTRACT	**ABSTRACT**
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METHODS or MATERIALS AND METHODS	**METHODS or MATERIALS AND METHODS**
RESULTS AND DISCUSSION	**RESULTS**
CONCLUSION AND RECOMMENDATIONS	**DISCUSSION**
ACKNOWLEDGMENTS	**ACKNOWLEDGMENTS**
REFERENCES	**REFERENCES**

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    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.

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